University of Alberta

Effects of porcine luteinizing hormone on synchronization of ovulation and corpus luteum development in beef and dairy cows

by

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DEDICATION

Scientific research is inspired by a need and/or desire to provide an answer to one or more questions. As a student one hopes to add to the existing body of knowledge by finding an answer to the original question and also, through the process, to generate several new questions that will someday be answered by further scientific research. During my time as a student, I have asked many questions of many people, all of whom have been very patient and extremely helpful to me. It is my hope that, with the completion of this thesis, I will provide useful information to the existing body of knowledge.

Although much of my time as a student has been consumed by my search for answers, I have been extremely fortunate to act as the "provider of information" to a pair of eager, growing, little minds. This work is dedicated to my children, Samuel Owen K Ree and Caleb Edwin K Ree who constantly bombard me with a litany of questions. For them life is a constant search for answers, created by an insatiable thirst for understanding. May I always have the patience to answer their questions carefully, and may I always remember that my favorite questions begin with "*Daddy, why does.....?*"

ABSTRACT

Synchronization of ovulation is essential for successful timed artificial insemination in cows. Previous studies using porcine luteinizing hormone (pLH) have demonstrated its effectiveness in inducing ovulation in cows and increasing the productivity of the corpus luteum (CL). This study aimed to compare the ovulatory response, and CL development and function in nonlactating cows treated with 8, 12.5, or 25 mg pLH (Lutropin-V, Bioniche) or 100 μg gonadotropin releasing hormone (GnRH; Fertiline, Vetoquinol). Cows given 25 mg pLH or 100 μg GnRH had a greater ovulatory response, CL area, and plasma progesterone than those treated with 8 mg pLH, whereas 12.5 mg pLH gave an intermediate response. Cows treated with 25 mg pLH also had higher mean plasma LH concentrations than those given100 μg GnRH, 12.5 mg pLH, or 8 mg pLH. A dose of 8 mg pLH was not consistently effective for synchronizing ovulation in mature cows.

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And finally, to my parents, Owen and Patricia thank you for pushing me to the end. Look mom, it's done!

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LIST OF ABBREVIATIONS

AI	= artificial insemination
ANOVA	= analysis of variance
CIDR	= controlled internal drug release
CL	= corpus luteum
FSH	= follicle stimulating hormone
GnRH	= gonadotropin releasing hormone
LH	= luteinizing hormone
LHRH-antagonist	= luteinizing hormone releasing hormone antagonist
LLC	= large luteal cells
PGF	= prostaglandin F2 alpha
pLH	= porcine luteinizing hormone
SLC	= small luteal cells
TAI	= timed artificial insemination

CHAPTER 1
GENERAL INTRODUCTION

1.1 Introduction

Dairy herd profitability is highly correlated to milk production and reproductive efficiency. A 13-month calving interval is considered optimum (Holmann et al., 1984; Schmidt, 1989). Herds that have poor reproductive efficiency will have longer calving intervals, produce fewer replacement heifers, have an increased number of services per conception, and a higher rate of involuntary culling. Inefficient estrus detection is one of the primary limiting factors to getting dairy cows pregnant, because artificial insemination (AI) is commonly used in the dairy industry. Controlled breeding protocols that allow synchronization of ovulation and timed artificial insemination (TAI) can eliminate the requirement for estrus detection.

Early embryonic loss constitutes a large portion of reproductive inefficiency in cattle (Ayalon, 1978) and has been demonstrated to be of greater importance in cattle following the use of a controlled breeding program. This may be due, in part, to inadequate function of the corpus luteum (CL). Development and function of the CL may be positively affected by a strong luteinizing hormone (LH) surge and therefore decrease the likelihood of early embryonic loss (Wolfenson, 2006). A commonly used TAI protocol referred to as Ovsynch (Pursley et al., 1995) uses gonadotropin releasing hormone (GnRH) to initiate follicular growth and to synchronize ovulation. Recent research using porcine luteinizing hormone (pLH) instead of GnRH has demonstrated an improved ovulatory response in both cows and heifers. Whether the use of pLH to induce ovulation will result in a more robust CL is not known. It is also not well established if GnRH treatment

during a specific phase of the estrous cycle will influence the magnitude of the LH surge. Also, will CL function differ following ovulation induced by GnRH or various doses of pLH?

In the work described here, two experiments were conducted to evaluate the use of various doses of pLH *versus* GnRH in a TAI protocol. Their effects on ovulatory response, CL size, and CL function in beef and dairy cattle were determined. Overall, this thesis aimed to determine a cost-effective dose of porcine LH that would more reliably synchronize ovulation and establish a more productive CL than one induced following GnRH treatment.

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CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

The use of AI by livestock producers is widespread. Artificial insemination provides rapid genetic improvement through the use of progeny tested sires, leading to improved animal production and conformation (Overton, 2005). It also minimizes the risk of transmitting venereal disease, reduces dystocia in young females by utilizing semen from bulls that produce calves with lower birth weights, enables management of breeding and parturition dates, and of considerable importance, eliminates the need to house and manage breeding males (Vishwanath, 2003). Although the benefits of employing AI are numerous, several challenges do exist. The use of AI requires specialized training, skills, and practice to inseminate females properly, appropriate handling facilities, and better management of the health and condition of females (Overton, 2005). The most important challenge faced by producers that use AI is estrus detection (Senger, 1994). Human involvement in the detection of estrus can be expensive because it requires considerable time, labour and skill. Without proper estrus detection, reproductive efficiency rates will be very low, leading to poor economic returns for the producer.

Patterson et al (2003) described several protocols that enable the synchronization of the estrous cycle. The Ovsynch protocol (Pursley et al., 1995) is a method of eliminating the challenge of estrus detection by treating cows with exogenous hormones to induce the manipulation of luteal and follicular functions, ultimately leading to the synchronization of ovulation. The Ovsynch protocol employs the treatment of cows at any time in the estrous cycle with 2 treatments of GnRH, 9 d apart. A prostaglandin F2α (PGF) treatment

is given 7 d after the first GnRH treatment, and cows are time-inseminated 16 to 20 h after the second GnRH treatment.

The first GnRH treatment is administered at a random stage of the estrous cycle. The purpose of the first GnRH treatment is to ovulate any large functional follicles, and initiate a new follicular wave. This first GnRH treatment will also increase the proportion of animals that would respond to the PGF treatment. The PGF treatment 7 d later will cause regression of the CL. The regression of the CL will remove the inhibitory action of progesterone, allowing the follicle to grow and mature in preparation for ovulation. The second treatment of GnRH increases the synchrony of ovulation by inducing the ovulation of the dominant, preovulatory follicle following endogenous LH release; cattle can be inseminated 16 to 20 h after the second GnRH treatment.

The obvious advantage of using the Ovsynch protocol is eliminating the need for estrus detection, but the challenge is that it may not entirely mimic the necessary endogenous hormone profiles, leading to a potential increase in early embryonic loss, compared to AI after estrus detection (Vasconcelos et al., 1999; Lucy, 2001). This may be due, in part, to reduced progesterone concentrations as a result of inadequate LH support during CL development.

2.2 The corpus luteum

The CL is a temporary endocrine gland that forms at the site of the postovulatory follicle on the female ovary. Its primary function is to synthesize and release progesterone, a steroid hormone, during the luteal phase of the female reproductive cycle and during pregnancy. The CL develops from the granulosa and theca interna cells of the former follicular wall. These cells undergo a transformation in a process called luteinization,

where they are transformed into progesterone-producing luteal cells. The CL will continue to release progesterone until the hormone $PGF2_{\alpha}$, produced by the bovine uterus, initiates a decrease in the production of progesterone and the eventual breakdown of the luteal cells of the CL. The process whereby the CL ceases to produce progesterone and regresses is called luteolysis. Luteolysis occurs when the uterus recognizes that there is no functional zygote present at the end of the estrous cycle to warrant the continued maintenance of the uterine environment for pregnancy and embryonic development.

2.3 Ovulation and luteinization

Throughout the estrous cycle, there is a continual cycle of recruitment, selection, and dominance of follicles. During the luteal phase, the dominant follicle is unable to ovulate spontaneously and release the ova because progesterone concentrations are too high, effectively blocking an endogenous LH surge. These follicles will become atretic and regress. After luteolysis, progesterone concentrations begin to decrease, allowing the dominant follicle to continue developing. While the preovulatory follicle is growing, LH released from the anterior pituitary will bind to specific receptor sites on the theca interna cells of the follicle wall. The net effect of this binding action will result in the conversion of cholesterol to testosterone, which then diffuses out of the theca interna cells and migrates into the granulosa cells. These cells have been exposed to increasing concentrations of follicle stimulating hormone (FSH), which bind to specific receptor sites on the granulosa cells, causing testosterone to be converted to estradiol. At the same time, inhibin is produced by the granulosa cells in response to high FSH concentrations. Increasing inhibin concentrations will decrease the synthesis and release of FSH, preventing additional follicles from developing. As the follicle continues to grow,

estradiol production increases, stimulating a GnRH surge from the hypothalamus, which in turn stimulates an LH surge from the surge centre of the anterior pituitary. This preovulatory LH surge initiates a number of events in the Graafian follicle that will eventually enable the formation of the CL after ovulation. The LH surge followed, by ovulation, induces a rapid decrease in estradiol production and an increase in progesterone concentration (Wolfenson, 2006). Both granulosa and theca cells undergo a morphological change resulting in the luteinization of the cells, the granulose cells becoming large luteal cells (LLC) and the theca cells becoming small luteal cells (SLC). The decrease in estradiol production is as a result of the decreased production of P-450 aromatase and increased production of P-450-scc and 3β-HSD in the granulosa cells (Wolfenson, 2006). This same activity will increase the progesterone production in the ovulatory follicle. Wolfenson (2006) suggested that a low LH surge could result in low progesterone because of poor luteinization of the growing CL.

When a Graafian follicle has reached its mature state, the granulosa cells have developed receptors for LH by the combined effects of FSH and estradiol (Keyes and Wiltbank, 1988). The preovulatory LH surge stimulates these granulosa cells to differentiate into steroidogenic luteal cells. This evolution involves cell hypertrophy, and a number of changes that enable the cells to produce progesterone. In cattle, the newly formed luteal cell loses its ability to produce estradiol shortly after luteinization, whereas other species, e.g. humans and rats, retain this ability (Keyes and Wiltbank, 1988).

During the process of luteinization, although the number of large luteal cells remains constant in the CL, they increase to nearly double the size of the original granulosa cells. Concurrently (from days 4 – 16) the small luteal cell (SLC) numbers increase 5-fold,

fibroblasts double, and endothelial cells increase approximately 6.5-fold in number (Niswender et al., 2000). The rate of growth of the CL during this period is so rapid that Jablonka-Shariff et al. (1993) compared it to the growth rate of rapidly growing tumors. Although the proportion of SLC to LLC becomes quite large in the CL, the majority of progesterone is produced (approximately 80%) by the LLC (Niswender et al., 1985) indicating the importance of the proportion of LLC in the CL.

Progesterone production by both LLC and SLC is distinctly different (Smith et al., 1994) depending on the presence or absence of LH. Basal rates of progesterone secretion by LLC is 2 to 40-fold that of SLC (Stocco et al., 2007) except in the presence of LH, where small luteal cells (in vitro) produce a greater amount of progesterone (Grazul-Bilska et al., 1991; Niswender et al., 2000). This demonstrates the importance of LH to the function of the CL in the post-ovulatory period.

Along with the development of luteal cells, there is a dramatic change in the extracellular matrix. Before ovulation, granulosa and theca cells are avascular, whereas after luteinization, each cell is in contact with vascular tissue. This is critically important, because it is necessary to provide the steroidogenic cells with the precursors to produce progesterone, as well as providing efficient delivery of the progesterone to the necessary site. Blood flow to the CL exceeds that of other tissues (Niswender et al., 2000) and oxygen consumption is 2- to 6-fold that per unit weight of the liver, kidney, and heart (Swann and Bruce, 1987). In the early developing CL, it appears that most of the dividing cells (95%) are vascular endothelial cells (Stouffer, 2006). In the mature CL, a large portion of the cells (52%) is associated with the vascular system in the CL (Wiltbank, 1994). Although critically important to the function of the CL, the growth and

development of the capillary network of the CL is not completely understood (Stouffer, 2006).

As previously discussed, the preovulatory LH surge initiates the luteinization of the granulosa and theca cells that become the progesterone synthesizing cells of the CL. Quintal-Franco et al. (1999) demonstrated the importance of preovulatory LH pulses on the development of the CL in cattle. Treating cows with a LH-releasing hormone antagonist (LHRH-antagonist) for 48 hours before the preovulatory LH surge compared to untreated cows, cows treated at the initiation of, or 48 hours after the initiation of the preovulatory LH surge, established CL with a smaller diameter. The authors suggested that the preovulatory LH surge is necessary for the maturation of the follicle by preparing the theca interna and granulosa cells for luteinization. Also, in order for normal CL development to occur, the mature follicle must have a normal population of granulosa cells that, after luteinization, will produce the majority of the progesterone. These granulosa cells must also possess an adequate number of LH receptor cells that are created during the preovulatory period as a result of the pulsatile LH surges (Quintal-Franco et al., 1999).

Suppression of LH in the preovulatory and post-ovulatory period also inhibited progesterone secretion, indicating not only an effect on the size of the CL, but also an effect on the function of the CL. Quintal-Franco et al. (1999) reported that the CL produced by animals treated with the LHRH antagonist in the 48 hr preovulation period produced only 25% of the progesterone compared to the untreated group and the cows treated with the LHRH antagonist from Days 2 to 12 of the estrous cycle produced approximately 50% of the progesterone compared to the untreated group.

Wolfenson (2006) speculated that a low LH surge would lead to inadequate luteinization of the CL, resulting in low progesterone profiles. This suggestion was supported by research that a low LH surge results in poor CL development and therefore low progesterone concentrations (Rajamahendran et al., 1998).

In more recent research, Ambrose et al. (2005) compared the peripheral plasma LH profiles of animals treated with 100 μg GnRH, or 12.5 or 25 mg pLH. Treatment with 100 μg GnRH resulted in an acute LH surge that rapidly returned to basal levels in 6 to 8 h after treatment, whereas in animals treated with either 12.5 or 25 mg pLH, LH concentrations were increased and remained at higher-than-basal concentrations for up to 24 hours after treatment. Schmitt et al. (1996) suggested that the inclusion of treatments that extend the release period of LH will produce a larger, more steroidogenic CL. Although progesterone concentrations measured on Days 9, 12 and 14 postovulation were numerically higher in heifers given 25 mg pLH, progesterone concentrations were not significantly different from that of heifers treated with GnRH. Though results are inconclusive, these studies (Schmitt et al., 1996; Rajamahendran et al., 1998; Ambrose et al., 2005) suggest that the pre- and post-ovulatory LH surge can influence the development and function of the CL.

2.4 The function of progesterone

Progesterone is commonly referred to as the "hormone of pregnancy". Its primary function is to prepare the female reproductive tract for the commencement and maintenance of pregnancy. During the luteal phase of the reproductive cycle, progesterone is synthesized and released by the luteal cells of the CL. The synthesis and release of progesterone affects both the female reproductive tract and the hypothalamic-

hypophysial-ovarian axis. The presence of estradiol, produced by the growing follicle, is required to stimulate the development of receptor cells for progesterone in the reproductive tract. Estradiol will also stimulate the growth and development of the endometrium and initiate and promote the growth of ciliated cells in the oviduct. Progesterone will, in turn, down regulate the receptors for estradiol in the same area. Together, estradiol and progesterone will work to manage the contractions of the oviduct and therefore control the rate at which the ovum/zygote is transported through the oviduct to the uterus. Progesterone influences the epithelium of the oviduct by stimulating it to produce secretions necessary for the survival and development of the newly formed embryo (Geisert et al., 1992). This coupled with the action provided by the presence of progesterone on the endometrial cells of the uterine mucosa offer a nutrient rich environment for a newly formed zygote to support itself until implantation.

During the luteal phase of estrous and pregnancy, high progesterone concentrations produced by the CL and placenta prevent uterine myometrial contractions. This is due, in part, to the blocking of electrical signals between myometrial cells, reducing the uptake of calcium, and blocking estradiol from inducing α-adrenergic receptors that are essential for the initiation of myometrial contractions. This period of calm creates an environment much more likely to enable the implantation of a newly formed zygote.

High systemic progesterone concentrations influence the hypothalamic-hypophysial-ovarian axis by down-regulating the release of GnRH from the hypothalamus (Kasa-Vubu et al., 1992) and decreasing the number of receptors for GnRH in the anterior pituitary (Laws et al., 1990). The combination of these effects results in high amplitude,

low frequency LH pulses preventing ovulation of dominant follicles during diestrous phase.

2.5 Progesterone concentrations and their effect on embryo development and growth

Successful pregnancy in the cow is dependent on the ability of the embryo to develop and produce adequate quantities of interferon-τ by Day 15 post-ovulation, such that it will inhibit luteolysis (Mann and Lamming, 1999). Progesterone is the primary hormone responsible for the creating a uterine environment with the appropriate secretions for the successful development and implantation of the embryo. Inadequate CL development can lead to reduced progesterone production, which can result in poor embryo development. Mann and Lamming (2001) clearly demonstrated a relationship between maternal progesterone concentrations and embryo development. They found that cows that had a delayed increase in progesterone concentration and lower progesterone profiles had poorly developed embryos on Day 16. These embryos produced inadequate quantities of interferon-τ, leading to luteolysis. In another study, Mann et al. (2006) were able to establish a 4-fold increase in embryo size and 6-fold increase in interferon-τ concentration in the uterus by supplementing progesterone in the early luteal phase (days 5-9). Therefore a robust, well-developed, high-progesterone producing CL should decrease the number of early embryonic mortalities in high producing dairy cows.

2.6 Morphology of the CL (histology & ultrasonography)

The preovulatory LH surge initiates the transformation of theca interna and granulosa cells of the preovulatory follicle into luteal cells of the CL. The term luteinization is used

to describe the biochemical and morphological changes of the cells of the preovulatory follicle cell. Prior to ovulation, granulosa cells increase in size and undergo nuclear activation. Concurrently, the basement membrane begins to break down, partially removing the barrier that existed between the granulosa cells and the theca interna cells. During ovulation, small capillaries present in the follicle wall rupture, forming the corpus hemorrhagicum. The wall of the follicle collapses in on itself, forming many internal folds. The folds allow the granulosa cells and theca interna cells to create a uniform mix. The former basement membrane creates the connective tissue substructure of the newly forming CL. It is generally recognized that the granulosa cells form the LLC whereas the theca interna cells develop into SLC, although Alila and Hansel (1984) used monoclonal antibodies specific to either theca internal or granulosa cells and found that in mid-estrous, cells of theca interna origin can develop into LLC.

After undergoing luteinization, the steroidogenic pathways of the cells are altered so that both cell types produce progesterone (Niswender et al., 2000).

In addition to SLC and LLC, the CL contains two other forms of cells. These are capillary endothelial cells, and fibroblasts. Wiltbank (1994) summarized the composition of the mid-cycle bovine CL. Large luteal cells comprised about 40% of the volume of the CL, whereas SLC, capillary endothelial cells and fibroblasts made up 28%, 13%, and 6% respectively. In cows, the LLC are considerably larger than the SLC, measuring 38 μm as compared to 17 μm. Endothelial cell and fibroblasts measured 11 and 15 μm respectively. As a proportion of overall cell numbers in the CL, LLC are 3.5%, SLC are 27%, endothelial cells are 52%, and fibroblasts are 10%.

Wiltbank (1994) summarized the characteristics of each of the major cell components of the CL. Large luteal cells are described as spherical to polyhedral with a large spherical nucleus, containing abundant stacks of rough endoplasmic reticulum and an abundance of smooth endoplasmic reticulum. Mitochondria are abundant and occupy about 20% of the volume of the cell, while secretory granules are also abundant. Small luteal cells are spindle shaped with an irregular nucleus containing cytoplasmic inclusions. Small luteal cells have an abundant smooth endoplasmic reticulum and mitochondria (15% of volume), but lack rough endoplasmic reticulum and secretory granules. Endothelial cells are elongated and present in the lumen of and around blood vessels of the CL. The nucleus of the endothelial cells is irregularly shaped, contains prominent aggregates of heterochromatin. The nucleus is large in relation to the cytoplasmic volume. Fibroblasts are elongated with an elongated nucleus containing large amounts of heterochromatin. Rough endoplasmic reticulum is present but smooth endoplasmic reticulum is rare.

Ultrasonography has been used to establish CL size by many researchers and offers a noninvasive method of obtaining real-time images of CL for evaluation. Corpus luteum characteristics, as measured by ultrasonography, have been demonstrated to have a high correlation to peripheral plasma progesterone concentrations (Kastelic et al., 1990; Assey et al., 1993; Davies et al., 2006). Kastelic et al. (1990) found that as the CL increased in size from Day 2 to 11 there was a corresponding increase in plasma progesterone concentrations. Assey et al. (1993) demonstrated that as the CL regressed following luteolysis, there was a strong correlation to the reduction in peripheral plasma progesterone concentrations. One of the challenges of using only morphometric analyses in determining the functioning of the CL is that the functional regression of the CL

precedes the physical breakdown by 1-2 days (Assey et al., 1993). This challenge can be overcome by analyzing the mean pixel density of ultrasonic images taken of the CL during growth, maximum function, and regression (Tom et al., 1998). Pixel intensity is a measure of the brightness in a grey scale, varying from black to white. Pure black images are created by structures that do not echo (reflect) the ultrasonic waves. As the structure increases its reflectivity the pixel becomes whiter. The measure of intensity as represented by the grey colour can be given a numeric value and can then be analyzed objectively. Tom et al. (1998) correlated the pixel density of ultrasonic images of CL with plasma progesterone concentrations in cattle. They found that as the CL grew, it decreased in pixel intensity and remained low during the period of maximal luteal function. At the onset of luteolysis the pixel intensity of the CL dropped dramatically but then began to increase again after Day 17 (Tom et al., 1998).

Colour Doppler ultrasonography is also being used to evaluate the vasculature of ovulation, CL development and regression (Acosta and Miyamoto, 2004). The images created by colour Doppler ultrasonography can be analyzed quantitatively.

2.7 Luteolysis

Luteolysis is defined as the functional and structural breakdown of the CL (Stocco et al., 2007). This process occurs at either one of two stages in cattle. The first is at the end of the luteal phase, when the uterus recognizes the absence of a developing zygote. The second is at parturition, when progesterone is no longer necessary to establish the quiescence of the uterus. Luteolysis is an important stage of the reproductive cycle because the cessation of progesterone production is necessary to allow the female to

return to the follicular phase that, in turn, allows for another opportunity for fertilization and pregnancy.

Luteolysis affects both the ability of the CL to synthesize and release progesterone, and the physical breakdown of the cells that make up the CL. As the CL loses function, there is a rapid decrease in plasma progesterone concentrations, followed by a gradual programmed death of the cells comprising the CL and the eventual formation of the corpus albicans. During the process of luteolysis, the CL undergoes a dramatic transformation in steroidogenic capacity, vasculature, and structural remodeling (Stocco et al., 2007). The primary luteolytic agent is PGF that is produced by the uterine endometrium, under the collective effects of oxytocin, estradiol and progesterone that are produced by the ovary. During the early portion of the luteal phase, progesterone inhibits the development of oxytocin receptors in the uterus. The lack of oxytocin receptors in the uterus blocks the endometrium from producing PGF.

In the late luteal phase, after day 10 – 12, progesterone loses its ability to block the formation of oxytocin receptors. The CL will continue to produce both progesterone and oxytocin. Oxytocin receptors in the uterine endometrium will develop over the last few days of the luteal phase under the estrogenic influence of the dominant follicle. As the number of oxytocin receptors increase in the endometrium, the amplitude and frequency of PGF secretion also increases. It has been established that approximately 5 pulses of PGF within a 24 h period are necessary to cause luteolysis. The treatment of cows with exogenous PGF does not require a pulsatile release pattern. This treatment will almost always cause luteolysis if administered after day 6, but will likely have no effect in the first 2 to 4 days after ovulation.

Prostaglandin $F_{2\alpha}$ that is produced and released by the endometrium is transported to the ipsilateral ovary through a vascular countercurrent exchange mechanism; PGF is released in high concentrations in the uterus and is then carried into the uterine vein. The ovarian artery is in very close proximity to the uterine vein and PGF crosses from the uterine vein to the ovarian artery by way of the vascular countercurrent exchange mechanism. If PGF were released into general circulation, 90% of it would be denatured in the bovine lung. Once in the ovarian artery, the PGF, now present in high concentrations, is carried directly to the ovary where it can act on the CL.

Once the CL is exposed to the PGF, a series of events takes place leading to the reduction in progesterone production and the eventual destruction of the CL cells. The exact mechanism and sequence of events are not yet clearly understood but a number of events have been identified and reviewed by Knickerbocker et al. (1988). The first event appears to be an overall decrease in luteal blood flow. More recent research using colour Doppler ultrasonography (Acosta and Miyamoto, 2004) suggest that after treatment with PGF, there is an acute increase in blood flow, followed by a decrease in the mid-cycle CL in cows. Acosta and Miyamoto (2004) suggest that the increase may trigger the onset of luteolysis. The second event is characterized by morphological changes in the cells of the CL and includes lipid droplet accumulation and a decrease in protein-containing secretory granules. Changes to the capillary structure also become evident leading to the eventual disintegration of the capillaries. Both LLC and SLC have receptors for PGF. These receptor sites enable the attachment of PGF, which in turn leads to apoptosis or programmed death of luteal cells and the eventual regression of the entire CL and formation of the corpus albicans.

2.8 Conclusions

As reviewed, ovulation synchrony is essential for the successful implementation of a TAI protocol. The current use of exogenous hormone treatments in TAI protocols allows the exclusion of estrus detection, but appears to increase the risk of early embryonic loss as compared to AI with estrus detection. This is likely because of inadequate LH concentrations during the development of the CL. In Canada, there is a pLH product available for use in cows that may increase ovulatory response and CL function when used instead of GnRH in an Ovsynch protocol. With this in mind, the objectives of this research are to establish if pLH, even at reduced doses, will increase ovulatory response *versus* GnRH, and increase the size and function of the CL in mature cows.

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CHAPTER 3

THE EFFECTS OF PORCINE LUTEINIZING HORMONE ON SYNCHRONIZATION OF OVULATION AND CORPUS LUTEUM DEVELOPMENT IN BEEF AND DAIRY COWS

3.1 Introduction

One of the primary limiting factors to achieving successful artificial insemination (AI) in cattle is estrus detection. It is generally acknowledged that estrus detection efficiency in high-producing dairy herds is less than 50% (Stevenson and Britt, 1977; Senger, 1994; Leblanc, 2005; Ambrose and Colazo, 2007). Employing a timed artificial insemination (TAI) protocol, through synchronization of ovulation can eliminate the requirement for estrus detection and increase reproductive efficiency (Leblanc, 2005).

Several protocols have been described to synchronize estrus in cattle (Odde, 1990; Larson and Ball, 1992). Dairy producers prefer the Ovsynch program for TAI because it enables insemination without estrus detection. This protocol consists of two treatments of GnRH given 9 d apart, and GF given 7 d after the first GnRH treatment (Pursley et al., 1995), followed by TAI 16 to 20 h after the 2nd GnRH treatment. Conception rate to TAI following Ovsynch is approximately 40% (Pursley et al., 1997). In recent studies in lactating beef cows, there was a positive relationship between ovulatory response to first GnRH treatment and pregnancy rate (Colazo et al., 2004; 2006). Approximately 50% of dairy heifers given GnRH ovulated to the treatment (Pursley et al., 1995; Ambrose et al., 2005), whereas approximately 80% of beef heifers given a pLH preparation ovulated (Martinez et al., 1999). Therefore using pLH instead of GnRH may increase ovulation rate to the first treatment and thereby pregnancy rates. Currently there is only one pLH product available on the Canadian market (Lutropin-V; Bioniche Animal Health,

Belleville, ON), and it is rarely used for ovulation synchronization because of its high cost. Doses lower than the recommended 25 mg of Lutropin-V have been used for inducing ovulation (Oswald et al., 2000; Ambrose et al., 2005), but results were inconsistent. More studies are essential before establishing the most dependable, yet cost-effective dose of pLH for inducing ovulations during diestrus and proestrus as commonly done during Ovsynch/TAI programs.

Many studies (Vasconcelos et al., 1999; Lucy, 2001) have reported that the incidence of pregnancy losses in dairy cattle were higher after TAI using a GnRH-based Ovsynch protocol than after insemination at detected estrus. In a recent study at the University of Alberta (Ambrose et al., 2006), pregnancy rate was lower and embryonic loss higher in dairy cows subjected to an Ovsynch program (using GnRH) than those inseminated after estrus detection. Ambrose et al. (2005) found increased progesterone concentrations late in the luteal phase in heifers induced to ovulate with 25 mg of pLH versus 100 μg of GnRH, suggesting enhanced CL function. Reasons for the difference were not clear. A robust LH surge may affect CL development and function and therefore decrease the likelihood of early embryonic loss (Wolfenson, 2006).

According to LeBlanc (2005), current average insemination rates, conception rates, and pregnancy rates in Canada are 35, 38 and 13%, respectively. By implementing a TAI program, it is reasonable to expect a pregnancy rate of approximately 25%. However, because the risk of pregnancy loss seems to be higher with GnRH-based TAI programs, it is important to find cost-effective ways of reducing the risk.

The general hypothesis for this work is that pLH, even at reduced dosages, will stimulate greater ovulatory response and establish a more functional CL in TAI protocols when

compared to GnRH. Therefore, the objectives of this research were to compare ovulatory response, and CL development and function in nonlactating cows treated with 8, 12.5, or 25 mg pLH, or 100 μg GnRH during diestrus and proestrus. Plasma LH concentrations in response to the above treatments were also determined during diestrus.

3.2 Materials and methods

3.2.1 Experiment 1

This study was conducted at the Oscar Peterson Artificial Insemination Centre of Lakeland College (Vermilion, AB, Canada; 53° 20' N, 110° 52' W) between May and August 2006. All experimental procedures were approved by the Lakeland College Animal Care Committee and by the Animal Policy and Welfare Committee, University of Alberta (protocol no. AMBR-2006-31). Eighty-five cyclic, nonlactating cattle (34 dairy cows and 51 beef cows), were treated with a 1.9 g progesterone intravaginal device (CIDR, Pfizer Animal Health, Kirkland, QC, Canada) for 10 d and 500 μg cloprostenol (PGF, Estrumate, Schering Canada Inc., Pointe-Claire, QC, Canada) at CIDR removal (Figure 3.1).Ten cows (all dairy) were removed from the study for either health-related reasons or not coming into estrus. Estrus detection was performed 3 times daily (for 30-60 min) for 5 d by visual observation with the aid of Kamar® heat detection patches (Kamar Inc., Steamboat Springs, CO, USA). On d 5 (estrus = d 0), transvaginal ultrasound-guided follicular ablation (Aloka-SSD-500 scanner, equipped with a 7.5 MHz linear-array transducer, Aloka Co., Tokyo, Japan) was performed on all ovarian follicles ≥8 mm in all cattle to synchronize follicular wave emergence. On d 12, when a 5- or 6 d-old growing dominant follicle was expected, all cattle were allocated to 1 of 4 groups to receive: 8, 12.5, or 25 mg pLH (Lutropin-V, Bioniche Animal Health, Beltsville, ON,

Canada) or 100 μg GnRH (gonadorelin acetate, Fertiline, Vetoquinol Inc., Lavaltrie, QC, Canada).

In a subset of 6 cows from each treatment group, blood samples were collected (by jugular catheter), to determine LH concentrations, according to the following schedule: 15 min before treatment, immediately preceding treatment, every 15 min for the first 1 h following treatment, and then every 30 min for the next 9 h. Samples were collected in 10 mL heparinized tubes (Vacutainer, Beckton Dickson, Franklin Lakes, NJ, USA), stored at 4 °C for up to 1 h, and centrifuged (1500 x *g*) for 20 min. Plasma was stored at -20°C until assayed. Basal LH concentrations were established by calculating the mean of samples collected before treatment. Plasma progesterone concentration was also quantified in the first sample. Ovarian transrectal ultrasonography was performed at 27, 48 and 72 h post treatment to determine ovulation.

3.2.2 Experiment 2

Seventy-six cyclic nonlactating cows (24 dairy and 52 beef) were treated with PGF at the termination of Experiment 1 and allowed to rest for 10 d (Figure 3.2). Following the rest period, a second PGF treatment was given to induce estrus. Estrus detection was performed 3 times daily for 3 d (estrus = d 0), as described in Experiment 1. Eight cows were removed from the study (2 dairy and 6 beef) because they did not respond to the PGF treatment. On d 7, cattle were treated with PGF and 36 h later they were allocated to 1 of 4 groups to receive: 8, 12.5 or 25 mg pLH, or 100 μg GnRH. Transrectal ultrasonography was done at 27, 36, 48, and 72 h to confirm ovulation.

The ovaries of a subset of 17 dairy cows were monitored ultrasonically every 2 d, from ovulation to 14 d after ovulation to assess the CL. Video images of the CL were captured

from the ultrasound machine using a computer software program (Pinnacle Studio, Version 8.4.5, Pinnacle Systems, Smart Sound® Technology by Sonic Desktop, Real Producer SDK © 1995-2002 Real Networks, Inc, Mountainview, CA, USA.) on d 4, 6, and 12 (post-ovulation) to measure pixel intensity. A single frame image from the video file (Figure 3.3) was then captured using AVS Video Converter 5.6 (Online Media Technologies Ltd., London, UK). The still images were analyzed for pixel intensity (Scion Image for Windows, Frederick, MD, USA). Pixel intensity values are reported as a grey-scale value on a scale from 0-255, where 0 is black and 255 is white. Results were an average of the total area measured of the CL. Furthermore, the area of the CL was calculated as described by Kastelic et al. (1990b).

Blood samples for progesterone concentration were collected on the same days as ultrasonography by coccygeal venipuncture into 10 mL heparinized tubes (Vacutainer) and handled as in Experiment 1 until assayed.

3.2.3 Hormone assays

Plasma progesterone concentration was determined with a modified, solid-phase ^{125}I-radioimmunoassay kit for progesterone (Coat-A-Count Progesterone; Diagnostic Products Corporation, Los Angeles, CA, USA). The sensitivity of the assay was 0.1 ng/mL and the inter- and intra-assay coefficients of variation measured in this experiment were 7.5 and 4.9%, respectively. Plasma concentrations of LH from both pLH and GnRH treated cows were determined by radioimmunoassay using 518B7 anti-bovine LH MAB (Quidel Corporation, San Diego, CA 92121, JF Roser, Dept Animal Science, University of California, Davis) which has a high cross reactivity with both porcine and bovine LH

(Matteri et al., 1987). Inter- and intra-assay coefficients of variation measured in this experiment were 1.5 and 8.1%, respectively.

3.2.4 Statistical analyses

Throughout this manuscript, data are reported as mean ± SEM. All probabilities ≤ 0.05 were considered significant whereas $0.05 > P \leq 0.10$ were considered trends approaching significance. All data were analyzed using a computerized statistical analysis program (SAS Version 9.0 for windows; SAS Institute, Cary, NC, USA). Plasma hormone concentrations, CL area, and pixel intensity were analyzed by the MIXED procedure for repeated measures. Autoregressive-1 (AR-1) was used as the covariate structure for repeated measurements. The statistical model included treatment (8, 12.5, or 25 mg pLH, and GnRH) and time as the main effects and the treatment by time interactions. In Experiment 1, ovulation rate was analyzed with Chi square. In Experiment 2, ovulation interval was analyzed by one-way ANOVA. All data were analyzed for normality using PROC univariate and Bartlett's test was used to establish equality of variance.

3.3 Results

3.3.1 Experiment 1

The mean plasma progesterone concentration for all cows at the time of treatment was 5.6 ± 0.2 ng/mL.

Treatment, time, and treatment by time interactions for plasma LH concentrations in a serial blood collection from 15 min before to 10 h after treatment were different ($P < 0.01$). The mean plasma LH concentration was higher ($P < 0.001$) for cows treated with 25 mg pLH (4.3 ± 0.4) than for cows treated with either 12.5 (2.1 ± 0.4), or 8 mg pLH

(1.9 ± 0.4), or 100 μg GnRH (1.8 ± 0.4; Figure 3.4). Plasma LH concentration in cows treated with 25 mg pLH were higher than cows treated with GnRH at 1.5 h after treatment and not different at 2 h after treatment. Plasma LH concentration peaked in the 25 mg pLH group 2.5 h after treatment and was higher than the GnRH treatment group from 2.5 h post treatment to the conclusion of collections, (10 h after treatment). Cows given 25 mg pLH had higher plasma LH concentrations from 1.5 h after treatment to 9 h after treatment than those given 12.5 mg pLH, and higher than those given 8 mg pLH (from 1 h after treatment until 9.5 h after treatment). The GnRH treatment group had plasma LH concentrations higher than the 12.5 mg pLH group 30 and 45 min post treatment, again at 2 h post treatment, and from 4.5-5.5 h after treatment. The 8 mg pLH treatment group had plasma LH concentrations lower than the GnRH treatment group at 30 min and 2 h after treatment, but was not different for the remainder of the collection period. The plasma LH concentrations of the 12.5 mg pLH group and the 8 mg pLH treatment group did not differ.

The ovulatory response, as determined by ultrasonography, differed among treatment groups (Figure 3.5). The ovulatory response to 25 mg pLH (84%) or GnRH (72%) was higher ($P < 0.05$) than to 8 mg pLH (32%); ovulatory response to 12.5 mg pLH tended to be lower ($P < 0.07$; 58%) than to 25 mg pLH.

3.3.2 Experiment 2

The mean plasma progesterone concentration for all cows at the time of treatment was 0.3 ± 0.05 ng/mL.

All cows ovulated. The mean interval from the time of treatment to ovulation did not differ among treatments, but was most variable ($P < 0.01$) in cows given 8 mg pLH (28.1

± 0.7, 29.1 ± 1.0, 33.5 ± 2.8, and 29.1 ± 1.0 h for 25, 12.5, or 8 mg pLH and GnRH, respectively; Figure 3.6).

Although CL area (mm^2) was larger ($P < 0.02$) in cows treated with 25 mg pLH (301.5 ± 22.6 mm^2) or GnRH (305.2 ± 22.6 mm^2) than cows treated with 8 mg pLH (222.1 ± 22.7 mm^2), it did not differ from cows treated with 12.5 mg pLH (260.8 ± 20.3 mm^2; Figure 3.7). Mean plasma progesterone concentrations were higher ($P < 0.04$) in cows given 25 mg pLH (3.5 ± 0.3 ng/mL) or GnRH (3.8 ± 0.3 ng/mL) than cows treated with 8 mg pLH (2.4 ± 0.3 ng/mL). Progesterone concentration was lower in cows treated with 12.5 mg pLH (2.6 ± 0.3 ng/mL) than in cows treated with GnRH ($P < 0.02$), and tended ($P < 0.07$) to be lower than cows treated with 25 mg pLH (Figure 3.8). A positive correlation was found between progesterone concentration and CL area ($r = 0.73$, $P < 0.01$; Figure 3.9).

An example of a CL image captured for pixel intensity analysis is shown (Figure 3.3). Mean pixel intensity measurements (greyscale) were 173 ± 4.8, 165.7 ± 4.4, 168.9 ± 4.8, and 161.8 ± 4.8, for 25, 12.5, and 8 mg pLH and 100 μg GnRH. Mean pixel intensity did not differ between treatments, but pixel intensity tended ($P < 0.08$) to be affected by time. There was no correlation ($r = 0.18$, $P > 0.20$) between pixel intensity and CL area (Figure 3.10) but pixel intensity tended ($P = 0.10$) to have a positive correlation ($r = 0.23$) with progesterone concentration (Figure 3.11).

3.4 Discussion

In the present studies, the hypothesis that cows treated with pLH *versus* GnRH will have improved ovulatory response and CL function, even at reduced dosages, was not supported. In diestrus, high peripheral progesterone concentrations affect the anterior

pituitary and inhibit the effect of GnRH resulting in a suppression in the LH surge and ovulation (Ambrose et al., 2008; Colazo et al., 2008a). The age of the dominant follicle being approximately the same between diestrus (7 d after ablation) and proestrus (7 d after ovulation) treatments, in the present report, the ovulatory response of cows treated with pLH should not have been affected by the high progesterone concentrations during diestrus, because pLH acts directly at the ovarian level. However, cows treated with GnRH during diestrus were expected to have a reduced ovulatory response because GnRH acts at the pituitary level to trigger LH release, which in turn, acts at the ovarian level to induce ovulation. As high progesterone concentrations can have a negative effect on pituitary LH release, GnRH treatment was expected to be less effective than pLH in inducing ovulations during diestrus. During proestrus, progesterone concentrations are rapidly declining, resulting in a greater response of the anterior pituitary to GnRH. Thus, no differences in ovulation were expected among treatments during the proestrous phase. Although Oswald et al. (2000) reported that treatments with 5 mg pLH induced a satisfactory ovulation rate (6 of 12 ovulated) comparable to 10 mg pLH (8 of 12 ovulated), or 25 mg pLH (7 of 12 ovulated) in beef heifers, 5 mg pLH was unsatisfactory for synchronizing ovulation in dairy heifers in another study (Ambrose et al., 2005). In the present study, ovulatory response of diestrous cows given 12.5 or 25 mg pLH did not differ from cows given 100 μg GnRH, but both GnRH and 25 mg pLH resulted in significantly higher ovulation rates than 8 mg pLH. Thus, based on previous findings (Oswald et al., 2000; Ambrose et al., 2005) and that of the present study, it is evident that low doses of 5 to 8 mg pLH do not yield consistent ovulatory responses. Furthermore, based on ovulation rates, pLH was not statistically better ($P > 0.05$) than GnRH, even at

the highest dose (25 mg). If more animals were included in the study, the difference in ovulation rate of 84% for 25 mg pLH *versus* 72% for 100 μg GnRH may have been statistically significant.

Cows given 25 mg pLH during diestrous had a higher mean plasma LH concentration than those given 100 μg GnRH, or 12.5 or 8 mg pLH. The LH peak in cows given the 25 mg pLH treatment was higher and persisted longer than that in all other treatments from 2.5 h after treatment to at least 9 h post treatment, and had not returned to basal concentrations at the conclusion of the collection period. Even with lower doses of pLH (12.5 or 8.0 mg), plasma LH concentrations remained elevated for a considerably longer interval than following GnRH treatment. Ambrose et al. (2005) described LH profiles similar to that of this study, with plasma LH concentrations following treatment with either 12.5 or 25 mg pLH having a significantly greater area under the curve and LH remaining elevated for a longer interval compared to GnRH-treated animals. Cows treated with 100 μg GnRH had an LH peak at 2 h, but LH concentrations were basal by 4.5 h after treatment. In contrast, the 25 mg pLH treatment established an LH profile that seemed similar to a spontaneous LH surge, with a higher peak that was sustained for a longer interval (Thatcher and Chenault, 1976). Plasma LH concentration measured during the serial blood collection is assumed to be present as a result of the treatment (pLH or GnRH) and not occurring spontaneously.

To maximize fertility in a TAI protocol, timing of ovulation is important. Dalton et al (2001) found that the timing of insemination in relation to estrus and ovulation was crucial. When cows were inseminated 12 h after the onset of estrus, acceptable fertilization rates, number of accessory sperm, and embryo quality optimized fertility

rates in cattle (Dalton et al., 2001). Therefore, the ability to establish a consistent and predictable interval between the last treatment and ovulation in an Ovsynch protocol will be very important in a TAI protocol. The synchrony of ovulation for cows in proestrus did not differ among cows treated with 25 or 12.5 mg pLH or GnRH. However, cows given 8 mg pLH had the longest ovulation interval and differed from the other treatment groups. Twelve percent of cows treated with 8 mg pLH ovulated after 36 h from the time of treatment. This represents a large number of cows that did not respond well to the treatment and would not ovulate synchronously, to successfully implement a TAI protocol.

All cows in Experiment 2 ovulated, as would be expected of cows in proestrus. However, cows given 8 mg pLH had the most variable interval from treatment to ovulation, reconfirming that 8 mg of pLH was inadequate to synchronize ovulation. Clearly, 8 mg pLH cannot be recommended for synchronizing ovulation.

In previous studies, Colazo et al (2008a) demonstrated that in cows given GnRH, plasma LH concentrations were inversely related to plasma progesterone concentrations. In Experiment 2 all cows were treated during proestrus, when progesterone concentrations were low. Although LH concentrations were not measured following the proestrous treatment, we speculate that LH release in GnRH-treated cows were higher in this experiment than in Experiment 1, whereas LH concentrations in pLH-treated cows would have been similar to those in Experiment 1. We should then expect that the CL in GnRH treated cows in proestrus will increase in size and progesterone production relative to the expected increase in the height of the LH surge (Wolfenson, 2006). Based on the LH concentrations in Experiment 1 (Figure 3.4) we assume that the amplitude of the LH

surge during proestrus would have been greater, relative to that of diestrus, yet returned to basal levels approximately 4 h after treatment. Considering that postovulatory progesterone concentrations after treatments with either pLH (25 mg) or GnRH (100 μg) were similar but higher than that following lower doses of pLH, it appears that high LH concentrations during the first few hours of the LH surge is important for higher progesterone production by the newly formed CL.

Several studies have reported a correlation between CL size and peripheral progesterone concentrations. In one study, Kastelic et al. (1990a) established a high correlation between the cross-sectional area of CL using ultrasonography and plasma progesterone concentration during luteal development. In another study, there was a linear relationship between CL size (as determined by transrectal palpation) and plasma progesterone concentrations (Ambrose et al., 1999). More recently, Gonzalez de Bulnes et al. (2000) also established, in ewes, a strong relationship between the size of the CL and its ability to produce progesterone, which then leads to the suggestion that as the size of the CL increases so will the progesterone concentration. Our results supported these findings.

The CL area of cows treated with 100 μg GnRH, 25 mg pLH, or 12.5 mg pLH did not differ, but were all greater than that in those treated with 8 mg pLH. Our hypothesis that pLH will enhance CL function, even at reduced doses, was not supported. Furthermore, although the 25 mg pLH treatment was clearly effective in inducing greater LH concentrations (Experiment 1) and an LH profile that was sustained over a prolonged interval, this treatment did not increase the size or progesterone production capacity of the CL relative to that in cows given 100 μg GnRH. These results were in partial agreement with that of Ambrose et al. (2005) who reported that heifers induced to ovulate

with either 25 mg pLH or 100 μg GnRH, given 48 h after CIDR removal and PGF treatment, had similar progesterone concentrations in late diestrus. However, progesterone was higher on day 9 of the cycle in heifers treated with 25 mg pLH. Although progesterone was not measured prior to day 9 of the cycle in that study, the authors suggested that heifers treated with 25 mg pLH may have had an earlier rise in progesterone. In the same study, heifers treated with 12.5 mg of pLH produced less progesterone than those treated with 25 mg pLH on d 9, 12 and 14 of the cycle.

Also in that study, pregnancy rates were numerically lower (45.9%) when heifers were subjected to TAI after treatment with a modified Ovsynch protocol wherein both the GnRH treatments were replaced by 12.5 mg pLH compared to a standard Ovsynch protocol using GnRH (58.7%). More recently, in a study involving 495 dairy cows, Colazo et al (2008b) reported that replacing the second GnRH treatment in an Ovsynch/TAI protocol with 25 mg pLH improved pregnancy rates significantly without increasing postovulatory progesterone concentrations. Although there may be an effect of an extended LH surge on oocyte maturation, fertilization, and embryo survival, more research is necessary to investigate these effects.

Previous research has demonstrated that pixel intensity as a measure of ultrasonographic echotexture can be used to evaluate the function of the CL (Tom et al., 1998). In the present study, there was no significant difference among groups in pixel intensity, indicating that the structure of the CL was not different among treatments, but there was a tendency ($P < 0.08$) for the pixel intensity to change over time. This change over time would be expected because the cellular composition and structure of the CL also changes over time.

In summary, cows treated with 25 mg pLH had higher mean plasma LH concentrations than those given 100 μg GnRH or 12.5 or 8 mg pLH (Experiment 1). Cows given 25 mg pLH or 100 μg GnRH had greater ovulatory response (Experiment 1), larger CL area (Experiment 2), and higher plasma progesterone concentrations than those treated with 8 mg pLH (Experiment 2), whereas 12.5 mg pLH gave intermediate responses. The lowest dose of pLH (8 mg) was not consistently effective for synchronizing ovulation in mature cows.

Overall, both 25 mg pLH and 100 μg GnRH were equally effective in synchronizing ovulations and inducing a CL with physiologic progesterone concentration. However, reduced doses (12.5 or 8.0 mg) of pLH were less effective in synchronizing ovulation (particularly during the diestrous phase). Progesterone concentrations and CL size were significantly reduced in cows given 8 versus 25 mg pLH. Therefore, there was no particular advantage of using 25 mg pLH in lieu of a standard dose (100 μg) of GnRH in a TAI protocol, and using reduced doses of pLH is definitely not recommended.

3.5 Tables and Figures

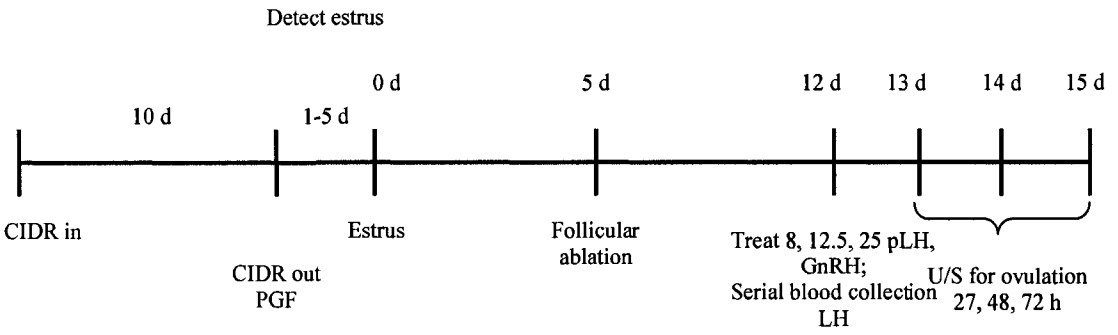


Figure 3.1. Schematic representation of the timeline of treatments for Experiment 1.

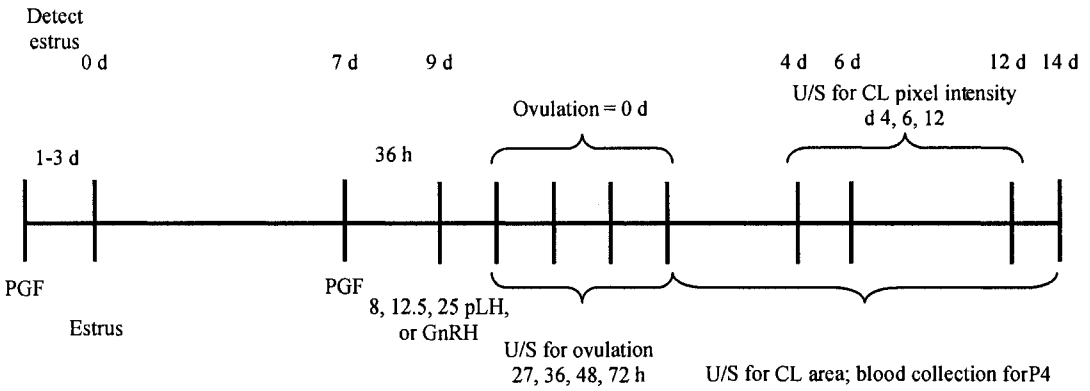


Figure 3.2. Schematic representation of the timeline of treatments for Experiment 2.

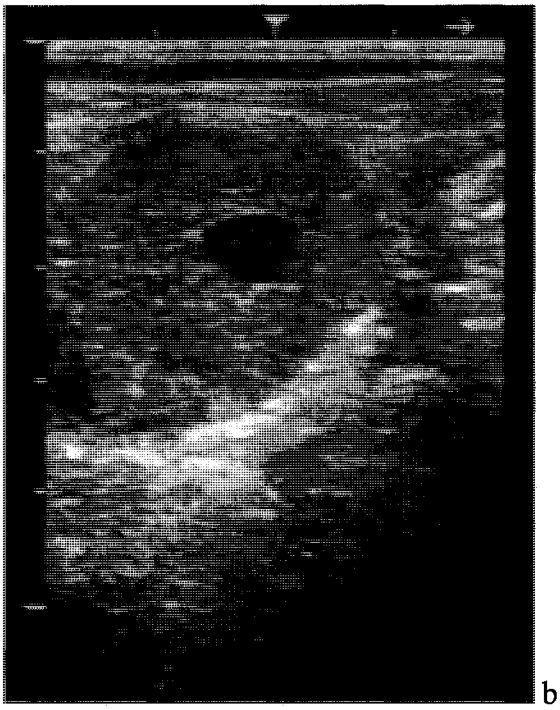
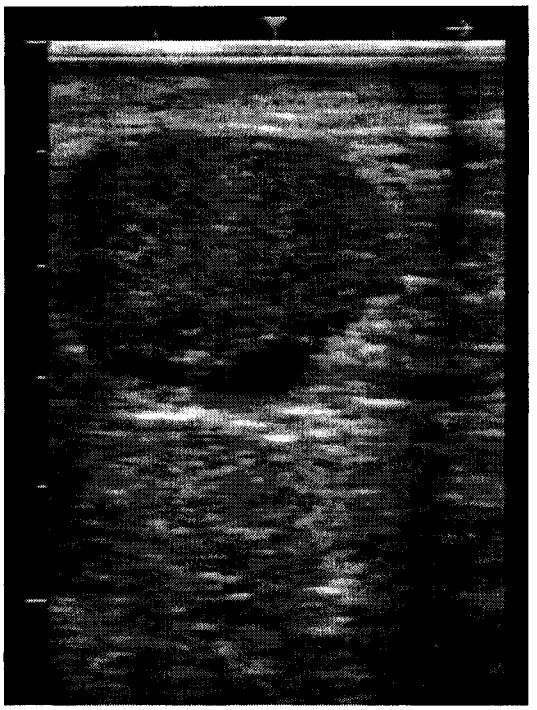
a.  b. 

Figure 3.3. Ultrasound images of a corpus luteum 12 d after estrus in cows treated with 100 μg GnRH or 25 mg pLH (panels a and b respectively) in Experiment 2.

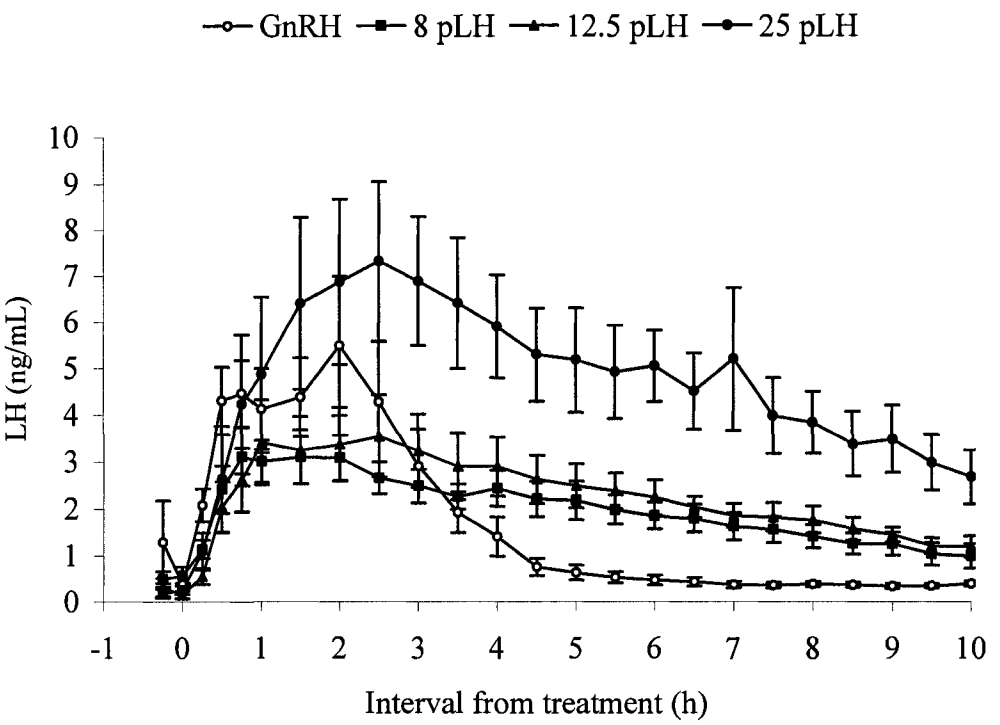


Figure 3.4. Plasma LH concentration in a subset of cows following treatment with 25, 12.5, or 8 mg pLH or 100 μg GnRH (n = 6 cows/group) in Experiment 1. There were effects of treatment, time, and a treatment by time interaction ($P < 0.01$). Treatment with 25 mg pLH resulted in a mean LH concentration that was higher ($P < 0.01$) than the all other treatments.

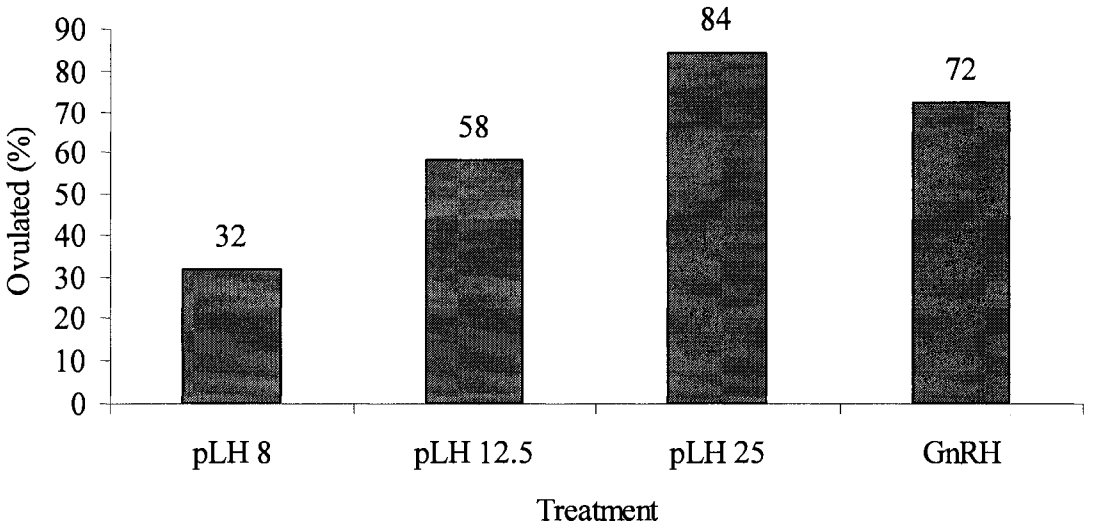


Figure 3.5. Ovulation rate in diestrus cows given 25, 12.5 or 8 mg pLH or 100 μg GnRH (n = 18 or 19/group) in Experiment 1. The ovulation rate was higher ($P < 0.05$) in cows given 25 mg pLH or GnRH than those given 8 mg pLH, and tended to be higher in those given 25 vs 12.5 mg pLH.

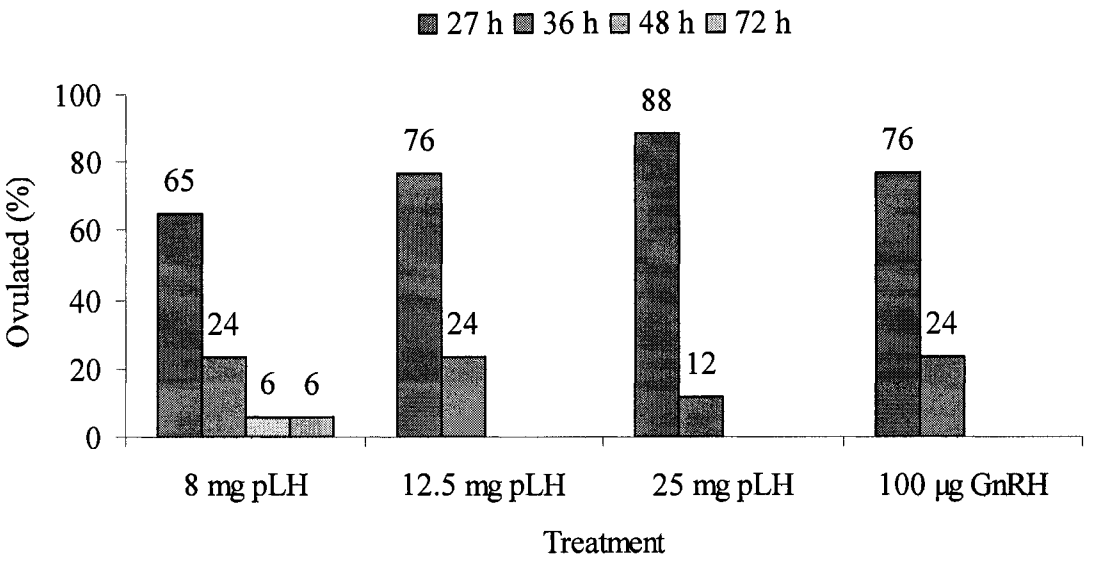


Figure 3.6. Interval from treatment to ovulation in proestrus cows given 25, 12.5, or 8 mg pLH or 100 µg GnRH (n = 17/group) in Experiment 2. All cows ovulated (100%); the mean interval from treatment to ovulation did not differ among groups, but was most variable ($P < 0.01$) in cows given 8 mg pLH.

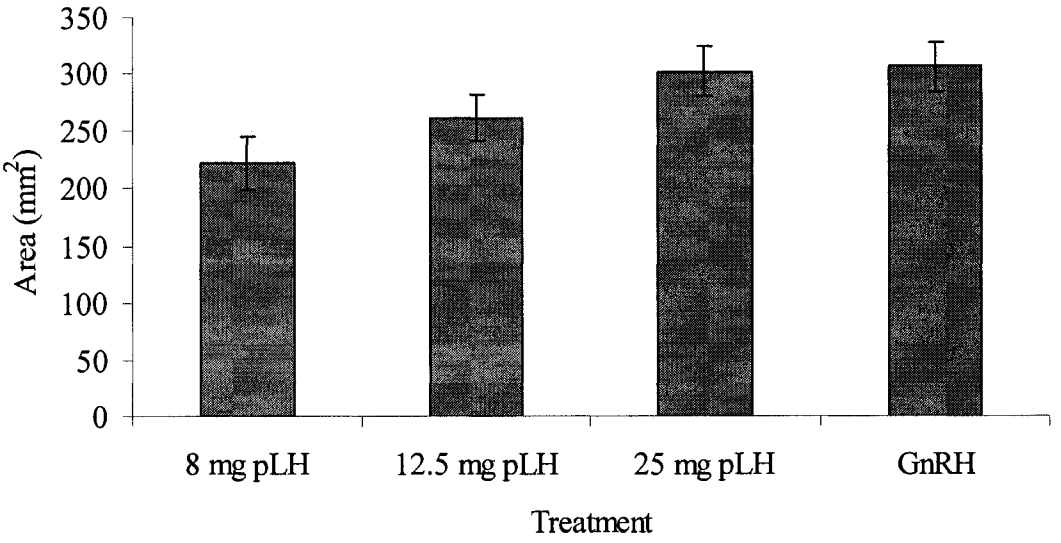


Figure 3.7. Mean area of CL in 17 cows given 25, 12.5, or 8 mg pLH or 100 μg GnRH (Experiment 2). The area was larger ($P < 0.02$) in cows given 25 mg pLH or GnRH than cows given 8 mg pLH, but did not differ from 12.5 mg pLH.

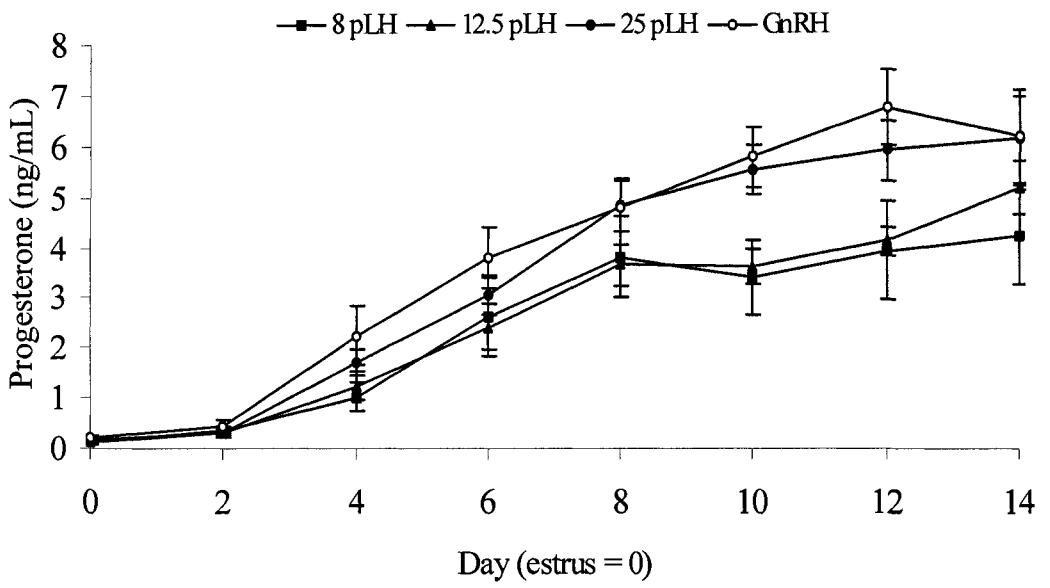


Figure 3.8. Plasma progesterone concentration in 17 dairy cows given 25, 12.5, or 8 mg pLH or 100 μg GnRH (n = 4 or 5/group) to induce ovulation in Experiment 2. Mean plasma progesterone was higher ($P < 0.04$) in cows given 25 mg pLH or GnRH than those given 8 mg pLH. Progesterone concentration was lower in cows treated with 12.5 mg pLH than in cows treated with GnRH ($P < 0.02$), and tended ($P < 0.07$) to be lower than the 25 mg pLH treatment group.

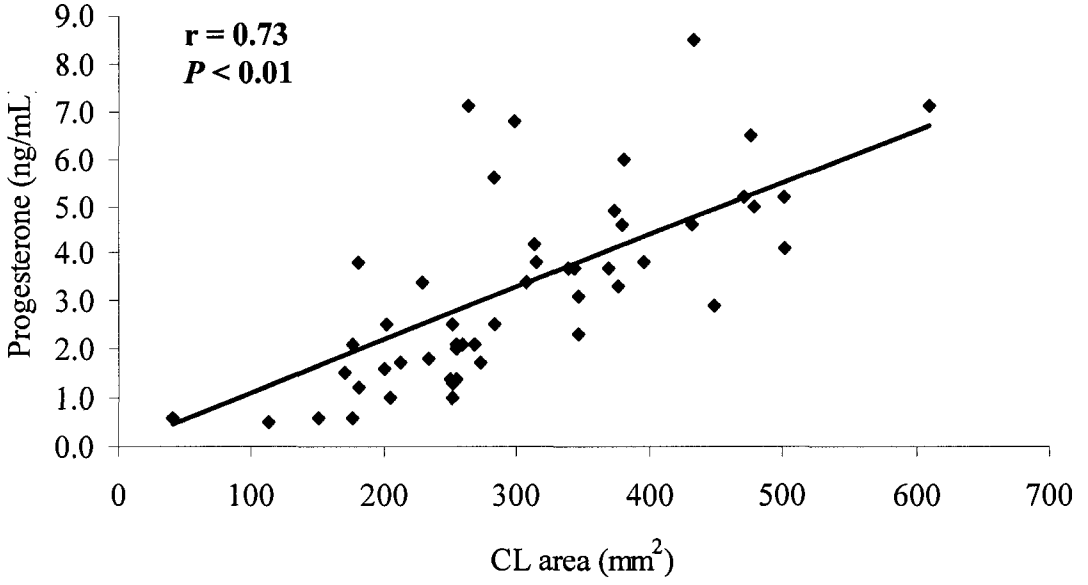


Figure 3.9. Relationship between CL area and progesterone concentration.

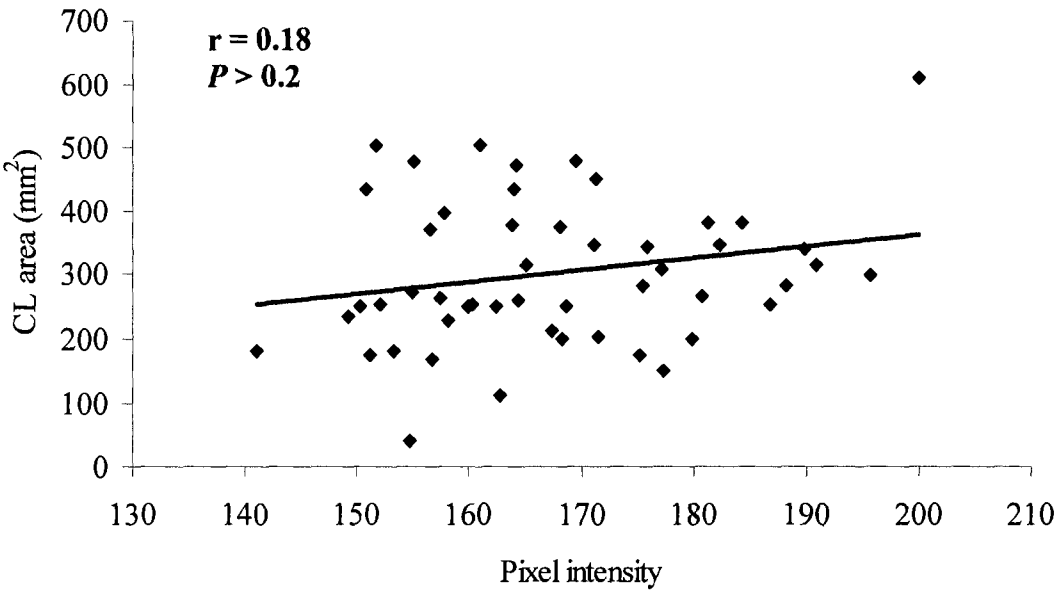


Figure 3.10. Relationship between pixel intensity and CL area.

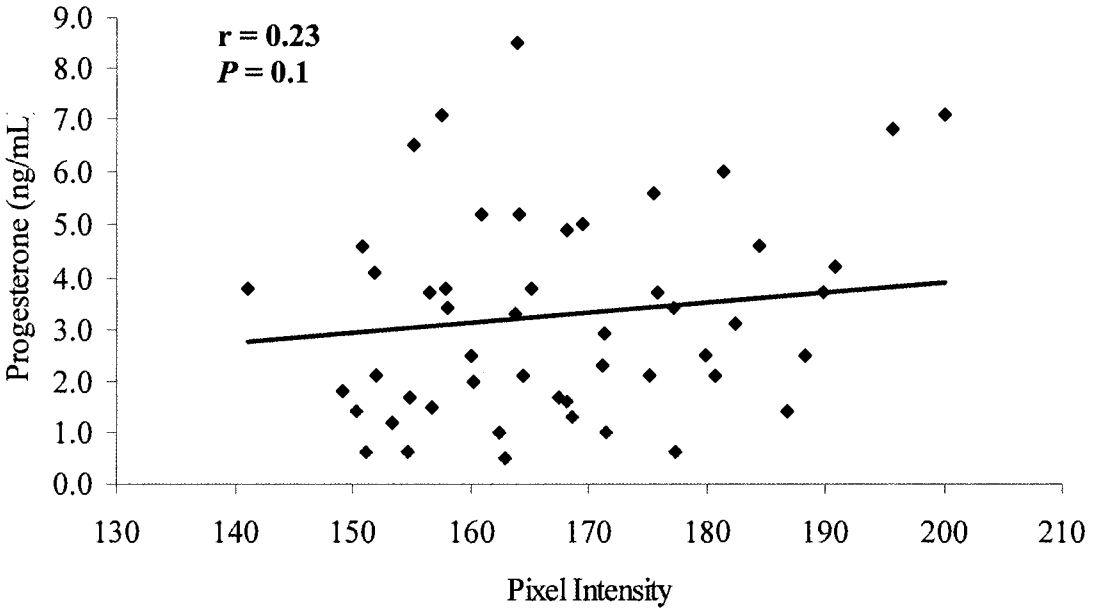


Figure 3.11. Relationship between pixel intensity and progesterone concentration.

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CHAPTER 4
GENERAL DISCUSSION AND CONCLUSIONS

4.1 General discussion

High rates of reproductive efficiency are important to dairy producers to ensure reasonable economic returns. In order to achieve this, cows should become pregnant within 100 d post-calving. Dairy producers use AI to breed cows because it enables rapid genetic improvement and eliminates the need for breeding males. One of the primary limitations to achieving adequate reproductive rates in dairy herds using AI, is estrus detection. According to LeBlanc (2005), current average insemination rates (cows inseminated / cows eligible for breeding x 100), conception rates (cows pregnant / cows inseminated x 100), and pregnancy rates (cows pregnant / cows eligible for breeding x 100) in Canada are 35, 38, and 13%, respectively, which are very similar to the recent findings of Ambrose and Colazo (2007) in a study involving 23 dairy herds in Alberta. A TAI protocol eliminates the need for estrus detection by allowing producers to inseminate cows on a fixed schedule. Each cow that remains non-pregnant past 100 d in milk costs $4.70/d (Plaizier et al., 1997). At the current average calving interval of 14.2 months, the typical cow is open for 40 days longer than recommended. At a cost of nearly $5.00/d, this totals $200 per cow per lactation, or approximately $20,000 lost revenue for the average dairy farmer. By implementing a TAI program, it is reasonable to expect a pregnancy rate of approximately 25%. However, because the risk of pregnancy loss seems to be higher with GnRH-based TAI programs, it is important to find cost-effective ways of reducing the risk. One approach is to enhance CL function as an indirect means of reducing pregnancy losses.

The purpose of this research, therefore, was to determine if using pLH *versus* GnRH to synchronize ovulation in a TAI program, would increase ovulatory response and improve CL function in cattle. We also investigated if reduced doses of pLH would effectively synchronize ovulation and improve CL function, thereby improving the effectiveness of the TAI protocol, yet keeping the costs economical. However pLH at the full dose of 25 mg was only as effective as GnRH at stimulating an ovulatory response, whereas the low dose of 8 mg pLH did not produce an adequate ovulatory response. The size (area) of the CL, and plasma progesterone concentrations were also not different between the 25 mg pLH dose and GnRH. Again, the 8 mg pLH treatment induced a smaller CL and lower plasma progesterone concentrations, whereas 12.5 mg pLH yielded intermediate results.

This research demonstrated that a dose below the recommended 25 mg of pLH would not adequately replace GnRH in an Ovsynch/TAI protocol. It also indicated that mean LH concentrations during diestrus were higher and more sustained with pLH treatments compared to treatment with 100 μg GnRH.

4.2. Conclusions

Based on the findings of the present study, although pLH treatment sustained higher LH concentrations over a longer interval, replacing the standard dose (100 μg) of GnRH with pLH for synchronization of ovulation in mature cattle offerred no real advantage. Furthermore, reducing the dose of pLH from 25 mg to 8 mg was not effective in synchronizing ovulation; it induced a smaller CL and lower progesterone concentrations, and therefore an 8 mg dose of pLH is not recommended for use in mature cows. Ovulation rates during diestrus were 72 and 84% for GnRH and 25 mg pLH, respectively. Although these differences were not significant in the present study,

differences may become significant if animal numbers are increased. Furthermore, in recent research by Colazo et al. (2008) involving three dairy herds, replacing the second GnRH treatment with 25 mg pLH in an Ovsynch/TAI protocol increased pregnancy rates. Therefore, there may be a practical on-farm application of this strategy. Although the recommended full dose pLH treatment is approximately 5 times more expensive than a dose of GnRH, a producer may have to determine the value of using pLH treatment within his own herd.

4.3 Future research

Although the CL was not larger and plasma progesterone concentrations were not higher in the 25 mg and 12.5 mg pLH treatment groups compared to GnRH-treatment, the effect of the extended LH exposure on the developing CL is not known. Extended LH exposure may have a positive effect on oocyte maturation or affect the proportion of small *versus* large luteal cells in the CL; both factors may influence early embryo survival. Further research is required to investigate the effects of different doses of pLH on the proportional composition of luteal cells in induced CL, and compare the CL of untreated cows to those of cows treated with different doses of pLH. The in vitro progesterone production potential of CL tissue obtained from the above treatments also remains to be investigated. Although no differences in CL size or function were apparent among cows treated with 100 μg GnRH or 25 mg pLH, determining embryo and fetal survival rates following these treatments within a TAI protocol would also be important to enhance our understanding of how LH profiles during the periovulatory period affect oocyte competence and embryo development.

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