STRATEGIES TO IMPROVE OVULATION SYNCHRONIZATION/TIMED ARTIFICIAL INSEMINATION PROTOCOL TO INCREASE PREGNANCY RATE IN DAIRY CATTLE

by

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B.Sc.Ag., The University of British Columbia, 2004

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

(Animal Science)

THE UNIVERSITY OF BRITISH COLUMBIA
(Vancouver)

October 2011

ABSTRACT

Physiological stressors of high milk production and intensive management systems affect fertility and pregnancy rates (PR) in lactating dairy cows. A field study was conducted on dairy farms in the Fraser Valley of British Columbia to benchmark current reproductive performance and issues that impact local dairy farms. The results indicated a substantial reduction in reproductive performance due to inaccurate estrus detection, fertilization/ovulation failure, and embryonic mortality. Ovsynch timed artificial insemination (TAI) protocol is used for the induction and synchronization of ovulation in cattle, reducing the need for estrus detection. However, PR to Ovsynch TAI are still low. Strategies to modify Ovsynch TAI protocol were investigated in this study to increase PR. In the first experiment, Ovsynch was compared with a pre-synchronization treatment, using $PGF_{2\alpha}$ before Ovsynch TAI to improve synchronization rates, and to a treatment of GnRH given 6 d after Ovsynch TAI, to reduce embryonic loss. Although no differences in PR were observed between treatments, days in milk in cows and age and weight in heifers affected PR. In the second experiment, the effects of pLH or hCG vs. GnRH in an Ovsynch TAI protocol were compared. Progesterone concentrations in pregnant cows were greater in the hCG group on days 7, 11, and 14, and greater in the pLH group on day 11 after breeding than GnRH group. There were no differences in synchronization rates or PR between treatments. High protein diets have been associated with reduced PR. Therefore, in the third experiment, cows either continued on a typical high protein diet, which was fed from calving, or switched to a lower protein diet, beginning 7 d before Ovsynch TAI and continued until pregnancy diagnosis. First and second lactation cows fed the low protein diet tended to have greater PR than cows fed the high protein diet. There were no differences in synchronization rates and progesterone concentrations between diets. Cows fed the lower protein diet had lower milk urea nitrogen and lower average daily milk production during treatment. Costs associated with treatments, labour, and producer compliance should be considered before recommending ovulation synchronization Ovsynch TAI protocol to dairy producers.

PREFACE

I conducted all the experiments presented in this dissertation (Chapters 2, 3, 4, 5). All analyses and writing presented in this dissertation are my own.

A version of Chapter 3 has been published: Gordon, M.B., Dinn, N., and Rajamahendran, R. 2010. Effects of pre-synchronization and post-insemination treatments on pregnancy rates to a timed breeding Ovsynch protocol in dairy cows and heifers. Can. J. Anim. Sci. 90: 35-44. Mr. Dinn and Dr. Rajamahendran provided support and advice throughout the study.

A version of Chapter 4 will soon be submitted for publication: Gordon, M.B., Ambrose, D.J., and Rajamahendran, R. 2011. Pregnancy rates to timed artificial insemination in lactating dairy cows treated with GnRH, pLH or hCG. Theriogenology. Dr. Ambrose and Dr. Rajamahendran provided support and advice throughout the study.

My role in the research leading to this dissertation also included the research from the following publications:

Balendran, A., Gordon, M., Pretheeban, T., Singh, R., Perera, R., and Rajamahendran, R. 2008. Decreased fertility with increasing parity in lactating dairy cows. Can. J. Anim. Sci. 88: 425-428.

Pretheeban, T., Gordon, M., Singh, R., Perera, R., and Rajamahendran, R. 2009. Differential mRNA expression in in-vitro produced pre-implantation embryos in dairy heifers and mature cows. Mol. Reprod. Devel. 76: 1165-1172.

Pretheeban, T., Balendran, A., Gordon, M.B., and Rajamahendran, R. 2010. mRNA expression of luteal genes associated with progesterone synthesis, maintenance, and apoptosis in dairy heifers and lactating dairy cows. Anim. Reprod. Sci. 121: 218-224.

Pretheeban, T., Gordon, M.B., Singh, R., and Rajamahendran, R. 2011. Comparison of expression levels of candidate genes in the endometrium of dairy heifers and lactating dairy cows. Can. J. Anim. Sci. 91: 255-264.

In the above four studies I was responsible for conducting the field portion of the experiments and assisted in data analysis and manuscript edits.

Colazo, M.G., Gordon, M.B., Rajamahendran, R., Mapletoft, R.J., and Ambrose, D.J. 2009. Pregnancy rates to timed artificial insemination in dairy cows treated with gonadotropin-releasing hormone or porcine luteinizing hormone. Theriogenology 72: 262-270. I conducted 50% of field experiment and assisted with final editing.

The Animal Care Committee of UBC approved the use of animals in the studies of this dissertation (Certificates #A06-0119 and #A06-1551).

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ABBREVIATIONS

°C	= degrees Centigrade
ADF	= acid detergent fiber
AI	= artificial insemination
BCS	= body condition score
BHBA	= beta-hydroxybutyrate
BUN	= blood urea nitrogen
CIDR-B®	= controlled internal drug release
CL	= corpus luteum
CP	= crude protein
CR	= conception rate
DF	= dominant follicle
DIM	= days in milk
DMI	= dry matter intake
E_2	= estradiol-17β
FCM	= fat corrected milk
FSH	= follicle stimulating hormone
g	= gravity
GnRH	= gonadotropin releasing hormone
GH	= growth hormone
hCG	= human chorionic gonadotropin
i.m.	= intramuscular
IU	= international units
IGF	= insulin-like growth factor
IFN-τ	= interferon-tau
JMP®	= JMP IN statistical package
LH	= luteinizing hormone
mRNA	= messenger ribonucleic acid
MUN	= milk urea nitrogen
n	= sample size or animal numbers

NDF = neutral detergent fiber

NEB = negative energy balance

NEFA = non-esterified fatty acids

pLH = porcine luteinizing hormone

POF = preovulatory follicle

P_4 = progesterone

PG = prostaglandin

$PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$

PR = pregnancy rate

PRID = progesterone releasing intravaginal device

PUN = plasma urea nitrogen

RDP = ruminally degradable protein

RUP = ruminally undegradable protein

SD = standard deviation

SEM = standard error of the mean

TAI = timed artificial insemination

TDF = total digestible fiber

TMR = total mixed ration

VWP = voluntary waiting period

ACKNOWLEDGEMENTS

First and foremost, I extend my most sincere gratitude to Dr. Rajadurai Rajamahendran for your supervision, encouragement, constructive criticism, and support during my studies. I truly appreciate everything I have learned from you. I am also very grateful to my other committee members, Dr. Kim Cheng, Dr. David Kitts, and Dr. Doug Veira for their valuable inputs during the course of my dissertation. To Dr. Nina von Keyserlingk, who was an original member of my Master's committee – thank you for your continued support, guidance, and mentorship. I must also thank Dr. Divakar Ambrose for his contributions in Chapter 4 and various other collaborations throughout my studies. I also appreciate the support received from Dr. Jim Thompson for his inputs into writing research reports, to the accomplishments he has made at the UBC Dairy Centre, and to his critical reviewing of my final dissertation. I would also like to thank Shelagh Niblock (Viterra, Chilliwack) for assistance with diet formulation (Chapter 5).

I appreciate the financial assistance received from the Viterra Fellowship, the James A. Shelford Memorial Scholarship, and Dr. Rajamahendran's research grants during the course of my projects. Various projects were partially funded by Western Canada's Genetic Centre, Natural Sciences and Engineering Research Council of Canada, Agriculture and Agri-Food Canada, and Investment Agriculture Foundation of British Columbia. I would also like to thank Bioniche, Vétoquinol, and Pfizer for in-kind contributions to some of my research.

A *huge* thank-you must go to Nelson Dinn, the manager of the UBC Dairy Education and Research Centre and his crew (Brad Duncan, Barry Thompson, Bill Kramer, Ted Toenders, Mike Duncan, Dan Peters, Cori McKay, Jason Struys, Jesse Scott, and Jeremy Hatt) for their unlimited help, assistance, and friendship. The farm would not be the same without you. During my time living at the Dairy Centre, I had many students help me and were of great friendship. There are too many of you to thank, but you know who you are! Particular thank-you goes to Camilo Andrés Díaz Pulgar, Andrés Mauricio Díaz Franco, Mark Bertens, Frank Dinnissen, Ivan Dario Ovalle Díaz, Santiago Pardo, Sina Rehle, and Ulrike Kamrad for your assistance on my research. Thanks for getting all those 5 a.m. milk samples! Thank-you to all the cows at the dairy centre for your patience – you amaze me and put a smile on my face every time I work with you; and to Ted and Amy, my favorite girls, thank-you for being crazy.

To my laboratory colleagues, Pretheeban Thavaneetharajah, Ravinder Singh, Ruwanie Perera, and Gunaretnam Iyathurai – thank you for your assistance and company rendered. To the members of the Animal Welfare program – thanks for making me a honourary! Many of you were a constant presence, giving advice, beside me in many adventures and shared moments during my studies. In particular, I am indebted to Katy Proudfoot, for her friendship, advice, and knowledge.

Most of all, thank you to my parents, family, and closest friends whom provided immense support and encouragement through all of my time in school. Finally, a special thanks to Roger – a house is not a home without a cat, and my goodness you are a special one that made living on the farm feel like home.

To my parents

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

Introduction

Conception rates (CR) in lactating dairy cows have significantly decreased from 65%, in the 1950's, to now only 30% (Figure 1.1) (Butler, 1998; Norman et al. 2009). The physiological and environmental stressors arising from high milk production within intensive management systems impair reproductive performance in dairy cows. These stressors affect the onset of post-partum ovarian activity, expression and detection of estrus, corpus luteum (CL) development and function, oocyte and embryo quality, and the uterine environment (Opsomer et al., 1998; Lucy, 2001; Thatcher et al., 2001). Good reproductive performance is therefore a key component for a profitable and sustainable dairy system.

Researchers continue to report trends for large increases in the number of days open in lactating dairy cows (Washburn et al., 2002; VanRaden et al., 2004; Hare et al., 2006a). Days open is defined as the interval of time from calving until a cow becomes pregnant. De Vries (2007) estimated that the cost per extra day open is $1.00 for first lactation cows and $1.80 for second lactation cows past 150 d after calving. These costs are similar to those reported by Meadows et al. (2005), who estimated a loss of $1.37 per cow per day for a 1-day increase in days open beyond 160 d of lactation. Increased days open leads to increased calving intervals. The recommended calving interval for dairy cows is 12 to 13 mo (Opsomer et al., 2000). Extended calving intervals reduce the percentage of cows in their peak production period, which reduce herd milk yield and therefore results in a loss of profit to the farmer (Norman et al, 2009). In order to achieve this desired calving interval, cows should conceive within 90 d after parturition, which

requires effective and timely insemination.

The common measure of reproductive performance in lactating dairy cows is pregnancy rate (PR), defined as the product of estrus detection and CR (De Vries et al., 2005). Conception rate represents the portion of cows bred within a specific period of time that become pregnant. Pregnancy rate represents the proportion of eligible cows that become pregnant within a specific period of time. Pregnancy rate is also used to determine days open after a voluntary waiting period (VWP). The VWP is defined as the period of time following calving when cows are willingly not bred; this allows time for recovery from parturition and a return of estrus. The average VWP in the US is 54.9 d (NAHMS, 2009).

According to the National Animal Health Monitoring System (NAHMS, 2009) 77.3% of dairy cows are bred at first service after a VWP by artificial insemination (AI) and the remainder by natural service from bulls. De Vries et al. (2005) observed no differences in PR between cows bred to AI or bulls. However, producers using bulls have lower herd milk production than producers using both bulls and AI and AI herds (Smith et al., 2004; De Vries et al., 2005). The greater milk production obtained in AI herds is related to superior genetics of raised replacements (Cassell et al., 2002).

Washburn et al. (2002) found that both estrus detection and CR have decreased over the span of 25 years. To overcome problems associated with estrus detection, many dairies use timed artificial insemination (TAI) programs either immediately after the VWP or after an unsuccessful AI period where cows are bred at detected estrus (NAHMS, 2009). Timed AI programs allow producers to breed cows at specific times rather than waiting for cows to show natural estrus.

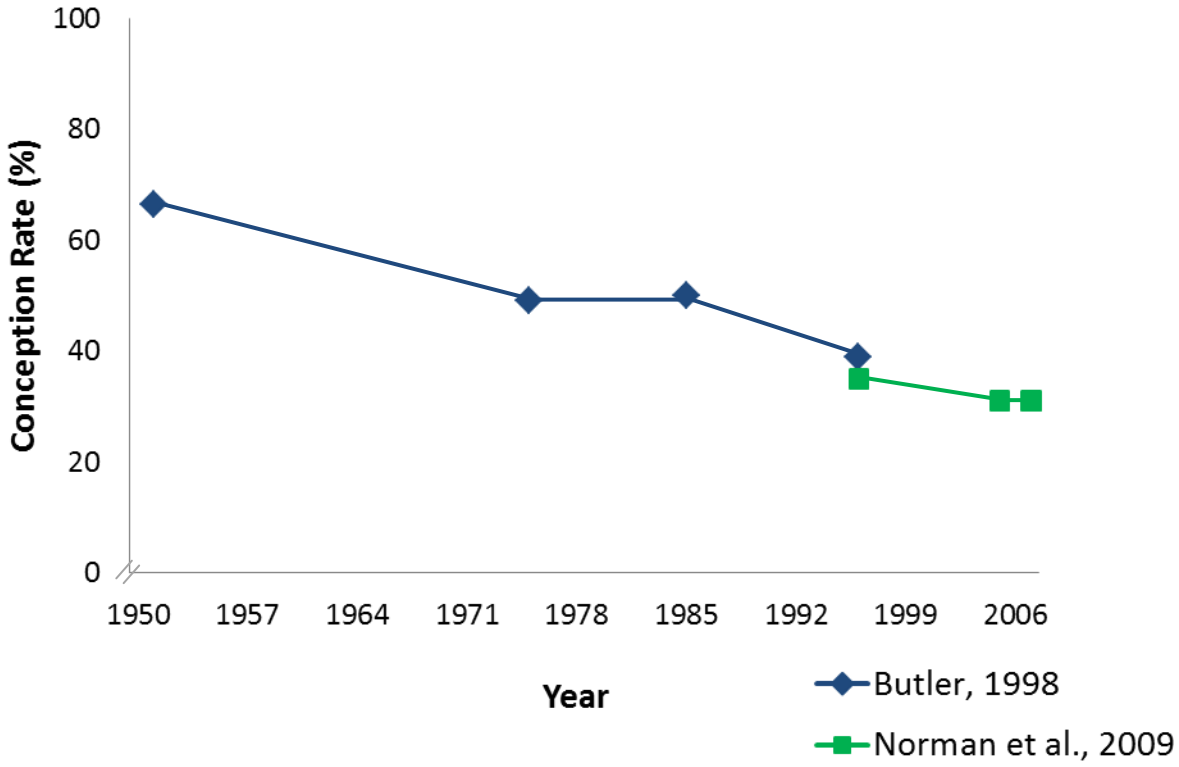

Figure 1.1 First insemination conception rates in lactating dairy cows

This graph is based on data from New York dairy herds adapted from Butler (1998) and from all US dairy herds from USDA data (Norman et al., 2009).

The Bovine Estrous Cycle

The cow is a polyestrous animal exhibiting regular estrous cycles every 21 d. The estrous cycle can be divided into four stages: estrus, metestrus, diestrus, and proestrus (Figure 1.2) and is defined as the period between two estruses. Estrus is the period characterized by sexual receptivity, where cows will also display other behavioural signs such as excitability and homosexual behaviour, allowing other cows to mount them. Estrus can last from 6 to 24 h, averaging 15 h (Senger, 2003) and is culminated by ovulation, when a dominant follicle ruptures and releases an egg. Metestrus, when the cow recovers from estrus and prepares the uterus for pregnancy, lasts for 2 to 4 d. During this period the ruptured follicle develops into a CL. This is followed by the diestrus period, which lasts between 15 to 17 d. The estrous cycle can also be divided into two distinct phases, based on the dominant structure present on the ovary. Metestrus and diestrus make up the period known as the luteal phase because of the presence of a CL, which produces the hormone progesterone (P_4). The follicular phase makes up the remainder of the days in the cycle and is the period from the regression of the CL to ovulation (proestrus and estrus). Proestrus immediately precedes estrus and begins when P_4 concentration begins to decline and terminates at the onset of estrus. During the follicular phase the primary ovarian structures are growing dominant follicles that produce the primary hormone estradiol-17β (E_2).

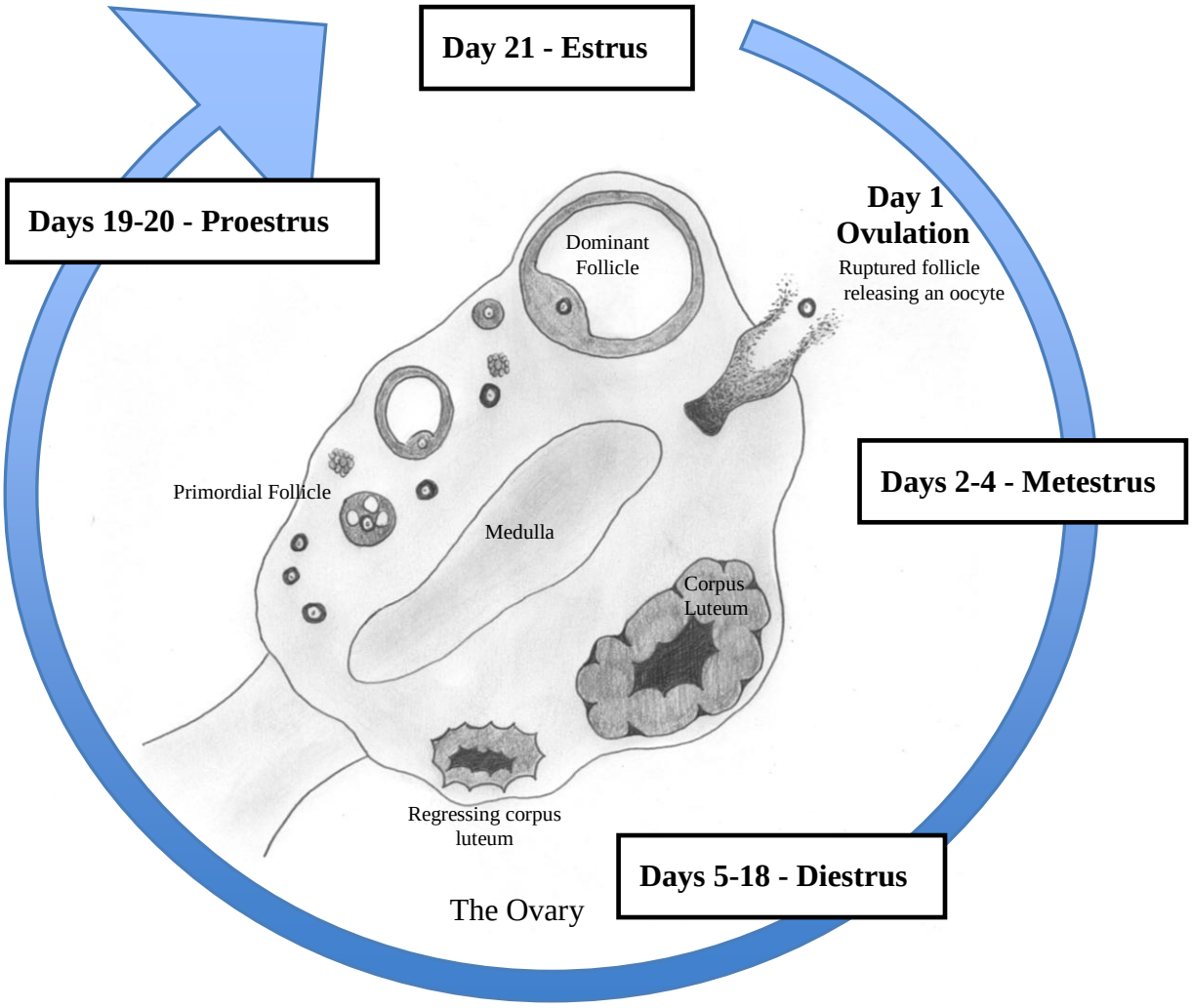

Figure 1.2 Diagram of ovarian structures present at different stages of the estrous cycle

Metetrus and diestrus make up the luteal phase of the estrous cycle based on the presence of a corpus luteum. Proestrus and estrus make up the follicular phase of the estrous cycle based on the presence of a dominant follicle and no corpus luteum.

Hormones involved in regulation of the estrous cycle

Gonadotropin releasing hormone

Gonadotropin-releasing hormone (GnRH) is a neural secreted decapeptide (Senger, 2003). The hypothalamus plays an essential role in regulating the estrous cycle because it produces GnRH, which is responsible for stimulating the biosynthesis and release of both follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior lobe of the pituitary gland (Senger, 2003). Gonadotropin-releasing hormone has a biological half-life of 4 to 5 min and during this time it must travel to the pituitary via the hypthalamo-hypophyseal portal system, bind to its receptors on the surface of gonadotroph cells and exert its action (Ramakrishnappa et al., 2005). Within the hypothalamus there are two areas where groupings of nuclei are referred to as: the tonic centre and the surge centre. Nuclei in the tonic centre are responsible for basal pulsatile release of GnRH characteristic of the luteal phase and the surge centre is responsible for the high frequency, high amplitude preovulatory release of GnRH. Regulation of GnRH release is by positive (stimulation of GnRH neurons) and negative (suppression of GnRH neurons) feedback mechanisms, which in turn control the secretion of FSH and LH (Figure 1.3). GnRH synthesis in the hypothalamus is partly modulated by short feedback loops from the pituitary and by the ovarian steroids P_4 and E_2 through a long loop feedback mechanism.

Follicle stimulating hormone and luteinizing hormone

Follicle stimulating hormone and LH are glycoproteins composed of two polypeptide chains, designated as α and β subunits. The α subunits of FSH and LH are

identical and the β subunits are different, providing each hormone a high degree of specificity and function (Senger, 2003). The gonadotropin hormones, FSH and LH, are released into the blood by the gonadotroph cells localized within the anterior lobe of the pituitary gland. The half-life of the pituitary gonadotrophs varies between 30 (LH) to 120 min (FSH) (Moor et al., 1984; Senger, 2003). Both FSH and LH are secreted in response to pulsatile release of GnRH. Higher amplitude pulses of GnRH over a short period of time result in synthesis and release of LH, whereas lower amplitude and prolonged pulses are responsible for biosynthesis and release of FSH. Follicle stimulating hormone is important for stimulating follicular growth in the ovaries and is also responsible for the production of E_2 within the ovary by binding to its receptors on the granulosa cells (Figure 1.4) (Fortune et al., 1988; Senger, 2003). Significant increases of FSH occur during various times during the estrous cycle: 1) around the time of preovulatory LH surge, 2) 12-24 h after the LH peak, which coincides with the emergence of the first follicular wave, and 3) before the initiation of the second or third follicular waves (Ireland et al., 2000). Luteinizing hormone is responsible for the events leading to ovulation of the dominant follicle and stimulating the CL to secrete P_4. Within the ovary LH targets receptors on theca interna cells and luteal cells (Senger, 2003). While LH is secreted in response to the pulsatile release of GnRH during most of the estrous cycle, just before ovulation there is a surge of LH, causing ovulation. Ovulation occurs 22 h after LH peak has been attained (Rajamahendran et al., 1989).

In order for E_2 to be synthesized, it requires the synergism of LH and FSH. During follicular development, LH binds to its specific receptors located on theca interna cells, activating the conversion of cholesterol to testosterone. Testosterone then diffuses

out of the theca interna cells and enters the granulosa cells. When FSH binds to its granulosal cell receptors, it causes the conversion of testosterone to E_2. Once an E_2 threshold is reached, synthesis of LH receptors occurs on the granulosa cells, so that the pre-ovulatory surge can cause ovulation of the follicle (Senger, 2003).

Inhibin

Inhibin is a glycoprotein hormone that contains an α subunit and one of two possible β-subunits (A, B). Both β subunits have the same physiologic activity. Under the influence of FSH the granulosa cells produce inhibin, which suppresses the release of FSH from the anterior pituitary (Senger, 2003). As a dominant follicle continues to grow, circulating FSH concentrations drop, inhibiting subordinate follicles from growing any larger (Lucy et al., 1992; Findlay, 1993). More recently, inhibin has also been shown to suppress E_2 secretion by granulosa cells (Jimenez-Krassel et al., 2001).

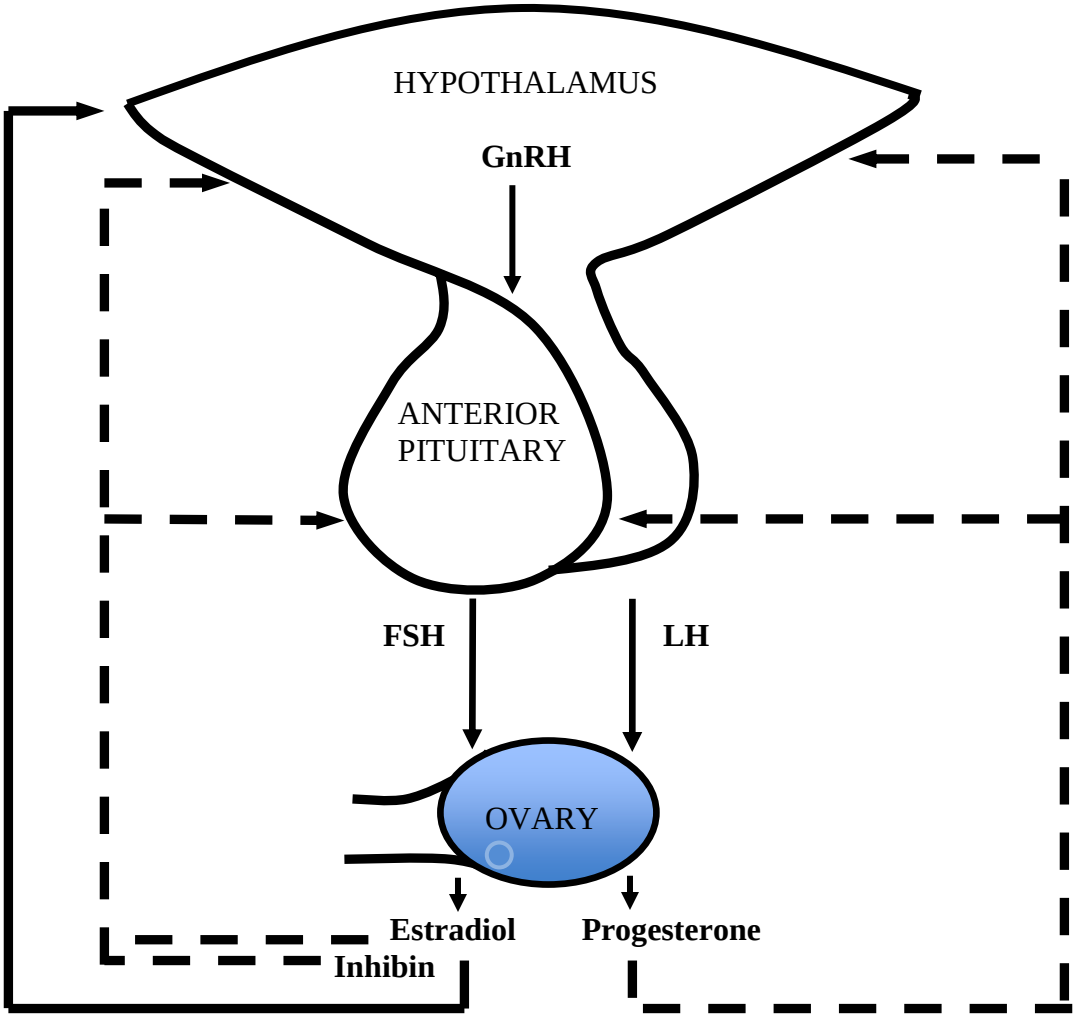

Figure 1.3 Hypothalamus-pituitary-ovarian axis

The hormones produced at each level of the hypothalamus-pituitary axis are responsible for the morphological events and behavioural changes occurring during the estrous cycle. Solid arrows indicate stimulatory effects and dashed arrows indicate inhibitory effects. Adapted from Merck Animal Health (2009).

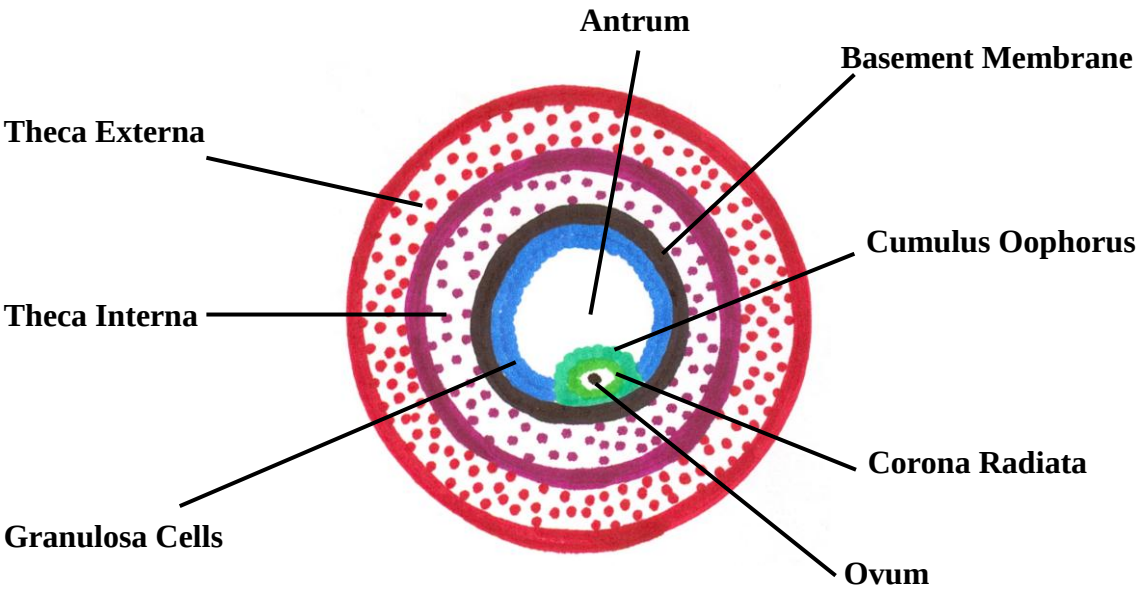

Figure 1.4 A Graaffian follicle

A dominant preovulatory follicle, also known as a Graaffian follicle, consists of three distinct layers: the theca externa, theca interna, and the granulosa cell layer. The theca externa is primarily composed of loose connective tissue. The theca externa is responsible for the production of androgens under the influence of LH. The theca externa and interna also consists of a capillary network. Separating the theca interna and the granulosa cells is the basement membrane. The granulosa cells contain FSH receptors and produce estrogen, inhibin, and follicular fluid. The cumulus oophorus and corona radiata surround the ovum during ovulation and are composed of specialized granulosa cells that provide nutritive support. The antrum is the follicular fluid filled area. Adapted from Senger (2003).

Estradiol-17β (E_2)

Estradiol-17β is a steroid hormone produced within the follicles and at basal concentrations provides negative feedback to the hypothalamus to prevent the further release of FSH and LH. As the dominant follicle grows and produces more E_2, this stimulates the nuclei in the surge centre of the hypothalamus (positive feedback) to increase both frequency and amplitude of GnRH release (Hansel and Echternkamp, 1972; Senger, 2003). This in turn results in increased frequency of LH pulses, which complete follicular maturation and result in a surge of LH. High E_2 concentrations coupled with low P_4 concentrations results in ovulation. Peaks of E_2 during mid-cycle can also be detected, corresponding with follicular development during the first and second follicular wave. Estradiol-17β is also responsible for the sexual behaviours that the cow exhibits during estrus.

Progesterone

Progesterone is a steroid hormone produced by the CL. It is secreted at metestrus and continues throughout diestrus. Progesterone concentrations begin to increase just after ovulation, as the CL develops and then stabilizes until about day 16 of the estrous cycle. Progesterone acts on the hypothalamus and pituitary exerting a negative feedback that results in the change in frequency and amplitude of GnRH and LH release, thereby decreasing LH blood concentrations. Following luteolysis of the CL, P_4 concentrations will rapidly decrease (Hansel and Echternkamp, 1972). Once P_4 decreases, a dominant follicle can grow further, thus producing more E_2 for a positive feedback to the

hypothalamus, causing the surge of LH release. A sustained high level of P_4 is important for the maintenance of the CL and for maintenance of pregnancy in the cow.

Prostaglandin$F_{2\alpha}$

Prostaglandin$F_{2\alpha}$ ($PGF_{2\alpha}$) is a 20-carbon unsaturated hydroxy-fatty acid that is derived from arachidonic acid (Senger, 2003). If the cow is not pregnant, $PGF_{2\alpha}$ is secreted by the uterine endometrium around day 16 to 18 of the estrous cycle to cause luteolysis of the CL. Corpus luteum regression ceases the production of P_4, allowing for the final growth of the dominant follicle for ovulation. Waite et al. (2005) demonstrated that $PGF_{2\alpha}$ reduces P_4 concentrations by reducing the synthesis of its precursors and increasing its metabolism. Prostaglandin$F_{2\alpha}$ also stimulates the uterine smooth muscle and promotes uterine tone and contraction.

Ovarian follicular dynamics

Typically, there are two or three waves of follicular development during the estrous cycle of a cow (Figure 1.5) (Savio et al., 1988; Sirois and Fortune 1988; Taylor and Rajamahendran, 1991), although alternatively a small proportion of animals can display just one or four waves per cycle (Savio et al., 1988; Sirois and Fortune 1988). Cows with two waves usually have a 21 d cycle, whereas cows with three waves tend to have a longer cycle of 23 d (Taylor and Rajamahendran, 1991; De Rensis and Peters, 1999). Animals with two wave cycles begin their second follicular wave on day 10 of the estrous cycle; whereas in animals that have three waves the second wave usually begins on day 9 and the third wave on day 16 (De Rensis and Peter, 1999).

Following ovulation the first wave of follicular development begins and is characterized by the recruitment and growth of a number of small follicles followed by the selection of a dominant follicle and subsequent regression of subordinate follicles. Subordinate and small follicles (4 to 6 mm in diameter) are recruited to compete for dominance by responding to a peri-ovulatory rise of FSH (Hendrickson et al., 2003). Follicles develop in response to tonic levels of FSH and LH, released by the pituitary. FSH will peak and concentrations will begin to decline once follicles reach approximately 4 mm in diameter (Ginther, 2000). While these FSH concentrations are still needed by the growing follicles, the follicles will begin to undergo deviation and the future dominant follicle(s) will continue to grow while the remaining follicles are reduced or terminated in growth to become subordinate follicles (Ginther, 2000). Dominant follicle selection is also regulated by growth factors and the interactions between follicles (Soboleva et al., 2000). The future dominant follicle contains lower insulin-like growth factor (IGF) binding proteins, which in turn results in increased availability of IGF. The responsiveness of the granulosa cells to reducing FSH concentrations depends on the bioavailability of free IGF (Mongent et al., 2002). As the selected dominant follicle proceeds towards dominance, it produces both E_2 and inhibin. Inhibin suppresses circulating FSH concentrations to below the requirements of the smaller follicles, causing their regression. An active dominant follicle is defined as having a diameter greater than 10 mm and is capable of preventing the growth of other follicles as well as the development of a new follicular wave (Lucy et al., 1992; Taylor and Rajamahendran, 1994; Ginther et al., 1996). Generally, a dominant follicle will reach a maximum diameter of 13 to 16 mm (De Rensis and Peters. 1999). In the majority of estrous cycles,

the first dominant follicle of the first follicular wave will regress, giving way to the second follicular wave growth. Follicular emergence is preceeded by a surge of FSH, which is required for the emergence of a new wave of follicular development (Driancour, 2001; Fortune et al., 2001). In two-wave cycles, the maturation of the second dominant follicle coincides with spontaneous regression of the CL and this follicle ovulates after luteolysis. Luteolysis is important because P_4 from the CL suppresses LH secretion and the growth of the dominant follicle (Adams, 1999). Alternatively, the second dominant follicle may become atretic and, if this occurs, a third follicular wave will be initiated (Lucy et al., 1992). The second and third follicular waves develop through the same series of recruitment, selection, and dominance. If no embryo is present then the uterus will secrete $PGF_{2\alpha}$. Prostaglandin$F_{2\alpha}$ causes the regression of the CL and a decrease in P_4. This in turn allows for the dominant follicle to grow further and secrete large amounts of E_2. This E_2 then causes a positive feedback to the hypothalamus, which causes an LH surge, in-turn causing ovulation of the dominant follicle.

Corpus luteum development

At ovulation, the dominant follicle present on the ovary ruptures and releases an oocyte. The remaining follicular structure, a corpus hemorrhagicum, undergoes cellular restructuring and develops into the CL, which is responsible for producing P_4. Through proliferation, reorganization, and neovascularization the theca interna and the granulosa cells of the follicle undergo luteinization and are transformed into luteal tissue. Among the changes occurring during luteinization are: 1) breakdown of the basement membrane, which separates the theca cells from the granulosa cells, 2) invasion of blood vessels into

the ruptured wall of the antral follicular space, resulting in the development of extensive vascular network, and 3) transition of a preovulatory follicular structure that secretes E_2 to a CL structure, secreting P_4 (Niswender et al., 2000). Producing progesterone is the main function of the CL and is important for the maintenance of pregnancy and prevention of luteolysis (Niswender et al., 2000).

The concentration of P_4 in the blood depends on various factors such as the amount of steriogenic tissue in the CL, blood flow to the CL, and the capacity of the steroidogenic tissue to synthesize and secret P_4 (Niswender et al., 2000). Luteinizing hormone, growth hormone (GH), and various growth factors also act as luteotropic agents on the CL. The luteolytic mechanism is a highly complex process that occurs in association with a number of hormonal, vascular, and immune mediated events within and outside the CL. Luteinizing hormone is the principle luteotropic hormone supporting the formation of the CL, and the maintenance of P_4 synthesis and secretion (Hoffman et al., 1974; Rajamahendran and Sianangama, 1992; Kawate et al., 2000). This is because LH is important for the proliferation of small luteal cells, which produce P_4 (Grazul-Bilska et al., 1995). Growth hormone can induce an increase in P_4 production by luteal cells (Wathes et al., 1995; Kobayashi et al., 2001). During early pregnancy the expression of LH and GH receptor mRNA in the CL is increased, suggesting a possible role for LH and GH in the maintenance of CL function during pregnancy (Yuan and Lucy, 1996). Insulin-like growth factors have stimulatory effects on P_4 secretion (McArdle and Holtorf, 1989). Moreover, recent findings of IGF binding proteins within the CL of cows suggest that there is a similar IGF system existing to that in ovarian follicles (Kirby et al., 1996). In ewes, Hastie and Haresign (2006) demonstrated an increase in IGF-I gene

expression, coinciding with a decrease in LH secretion, which suggests the regulatory effect of LH on the IGF system within the CL.

Angiogenesis, the formation of new blood vessels, is an important process in the formation and maintenance of a growing CL (Fraser and Wulff, 2003). There are various factors associated with the angiogenesis of the CL, including vascular endothelial growth factor, fibroblast growth factor, and endothelin-I. Vascular endothelial growth factor is an angiogenic substance important for the formation of luteal vasculature, regulating endothelial cell migration and thus P_4 secretion (Kamada et al., 2004; Yamashita et al., 2008). Fibroblast growth factor is a heparin binding growth factor, with similar functions to that of vascular endothelial growth factor (Yamashita et al., 2008). Endothelin-I is a peptide that modulates angiogenic activities in the CL and P_4 production (Girsh et al., 1996a, b).

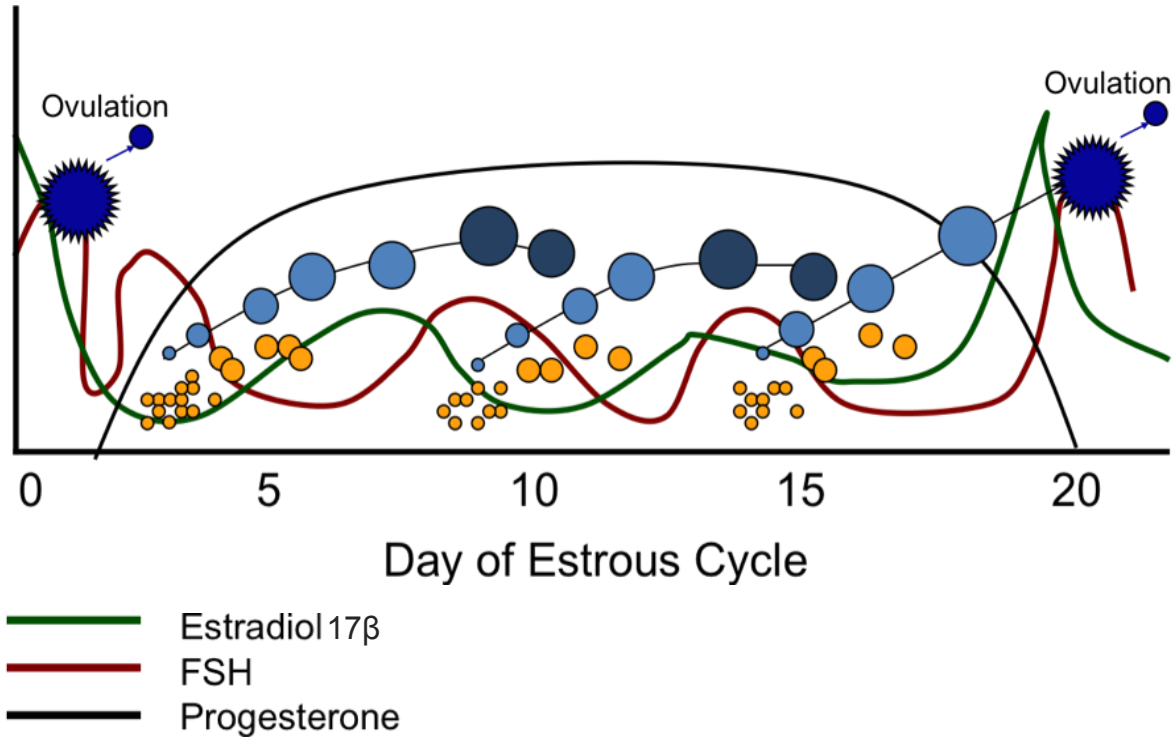

Figure 1.5 Schematic representation of follicular wave development and endocrinology of the bovine estrous cycle

Blue circles represent dominant follicles. Orange circles denote selection, recruitment, and atresia of subordinant follicles. Ovulation is followed by a rise in progesterone concentrations, produced by the newly developing corpus luteum. Follicle stimulating hormone (FSH) stimulates the growth of a cohort of follicles. Due to the presence of progesterone, the dominant follicles of the first and second wave result in atresia. The dominant follicle of the third follicular wave is able to grow in capacity because of the release of $PGF_{2\alpha}$ from the uterus around day 17, causing luteolysis of the corpus luteum. As the dominant follicle grows, estradiol-17β reaches a peak, resulting in a positive feedback mechanism, causing an LH surge and ovulation. Adapted from Merck Animal Health (2009).

Reproductive Failure in Dairy Cows

Although there has been a decline in reproductive performance in lactating dairy cows, heifers continue to show excellent reproductive fertility (Lucy et al., 1992; Balendran et al., 2008). Pregnancy rates in dairy heifers are consistently around 60-70% (Pursley et al., 1997a). Pregnancy rates among lactating dairy cattle greatly vary and the reasons for the decline in fertility are multifactorial. As examples, low PR may be a result of poor estrus expression and/or detection, increased early and late embryonic mortality, extended anovulatory periods, and reduced health and compromised immune function (Thatcher et al., 2006) or a combination of these.

Estrus detection

Estrus detection has been cited as one of the most important factors affecting reproductive success of AI programs (Everett and Bean, 1986; Lucy, 2001; Rajamahendran et al., 2001). However, most farms only have an estrus detection efficiency of about 50% (De Rensis and Peters, 1999; Rabiee et al., 2005). The duration and intensity of estrus expression in dairy cows can be variable. Estrus expression may depend on an individual cow's behaviour, social interactions between cows, floor surface, hormone production of the cow, and time of day. It is often difficult for producers to detect the onset of standing estrus since peak estrus activity often occurs at night (Lucy, 2001). Moreover, the length of time cows stand to be mounted represents less than 1% of the total duration of estrus (Rorie et al., 2002), with the average dairy cow mounted 8.5 times per estrus and many producers may only spend less than 30 min twice daily for estrus detection (Lucy, 2001). Researchers have also shown the importance of flooring

surface (concrete, rubber, dirt) and bedding substrate on the duration of estrus, the mounting activity, and the standing activity (Vailes and Brit, 1990; Fregonesi et al., 2004). Concrete is extensively used for barn flooring and has been suggested to depress cow activity 15-fold compared to dirt flooring (Vailes and Brit, 1990). Delayed post-partum cyclicity (the initation of the estrous cycle after parturition), anestrus (when cows do not show outward signs of heat), and lameness can all affect estrous behaviour of cows; coupled with increases in herd size and changes in herd management result in inefficient and inaccurate estrus detection, which leads to decreased reproductive efficiency and economic losses (Jobst et al., 2000, Lucy, 2001). Therefore, by maximizing estrus detection and the number of animals bred to AI, PR can be improved.

Embryonic mortality

Embryonic mortality refers to pregnancy loss from the time of fertilization to day 42 post-insemination. Early embryonic losses occur up to day 25, whereas late embryonic mortality occurs from day 25 to 42 post-insemination, after which the conceptus becomes the fetus. In general, losses occur during CL formation or the period of maternal recognition (Changes e Silva et al., 2002; Bilodeau-Goeseels and Kastelic, 2003). Up to 80% of the total embryo loss in cattle occurs by about day 16 after insemination (McNeill et al., 2005).

Once fertilization occurs, the embryo has to make its way to the uterus for subsequent implantation and to develop sufficiently to produce enough interferon-tau (IFN-τ) (maternal recognition) to prevent luteolysis of the CL which would occur around day 16 (Mann and Lamming, 2001). Interferon-tau prevents the initiation of a luteolytic

cascade in pregnant animals. It inhibits the expression of endometrial estrogen, oxytocin, and P_4 receptors, thereby preventing the release of $PGF_{2\alpha}$ (Spencer and Bazer, 1996). Any failure in the process could be due to a defect in the embryo itself and/or a problem with the maternal environment (Ball and Peters, 2004). After day 42 fetal losses are generally about 5 to 8% (Changes e Silva et al., 2002; Bilodeau-Goeseels and Kastelic, 2003).

There are many factors associated with embryonic mortality, including genetic abnormalities, nutrition, stress, infectious causes, early fertilization, endocrine, uterine environment, and the age of the animals (Gordon, 2004). Causes of very early embryonic mortality can be associated with genetic abnormalities. Most genotypic abnormalities will cause death of the embryo within the first 2 wk of pregnancy (King, 1990). These abnormalities can arise as errors in the process of gamete formation, at fertilization, or in early development, such as with expression of lethal genes, abnormal chromosome numbers, or inappropriate gene expressions (King, 1990; Bilodeau-Goeseels and Kastelic, 2003). Expression of developmentally important genes is vital for the function and survival of embryos. In a recent study from our lab, we observed greater expression of IFN-τ and heat shock protein 70 (HSP70) in embryos from heifers than in embryos from lactating cows (Pretheeban et al., 2009). When HSP70 is expressed its synthesis enhances the ability of stressed cells to cope with increased concentrations of unfolded or denatured proteins (Nollen et al., 1999) and prevents apoptosis (Mosser et al., 1997). Our lab also compared the expression levels of some endometrial genes (interleukin 1 alpha, tumor necrosis factor, and fibroblast growth factor-2) and found greater expression in heifers compared to lactating cows (Pretheeban et al., 2011). Interleukin 1 alpha (Tanikawa et al., 2005) and tumor necrosis factor (Miyamoto et al., 2001) play an

important role in the regulation of endometrial prostaglandin production and the survival of the CL; fibroblast growth factor-2 plays an important role in regulating embryogenesis (Gospodarowicz, 1991). The differential expression of genes within embryos and within the endometrium observed between heifers and cows contributes toward embryo survival and in turn, contributes to embryonic loss observed in mature cows (Pretheeban et al., 2009; 2011).

The establishment and maintenance of pregnancy and of embryo growth are related to the ability of the CL to secrete P_4 (Schmitt et al., 1996a; Changes e Silva et al., 2002). The concentration of systemic P_4 has been shown to affect the volume of uterine secretions, the rate of embryo development, the embryo's ability to produce IFN-τ, and the development of the luteolytic signal, $PGF_{2\alpha}$ (Schmitt et al., 1996a; Changes e Silva et al., 2002; McNeill et al., 2005). Therefore, a delay in the normal rise in P_4 concentrations in the early luteal phase results in smaller embryos with sub-optimal IFN-τ secretion and reduced ability to inhibit luteolysis (Changes e Silva et al., 2002; Stronge et al., 2005). Our lab has also observed poorer embryo quality, reduced PR from transferred frozen embryos, and reduced P_4 concentrations in lactating cows compared to heifers, contributing to the lower PR observed in the field between dairy heifers and lactating cows (unpublished data).

Post-partum cyclicity

Following the regression of the CL of pregnancy, there is a variable period of anestrous before the first ovulation (De Rensis and Peters, 1999). The first 2 to 3 wk after giving birth are necessary for uterine involution, for the anterior pituitary LH stores to be

replenished, and for follicular waves to resume. Following calving, plasma E_2 concentrations will drop (Echternkamp and Hansel, 1973). This in turn terminates the inhibition of FSH, allowing plasma FSH levels to increase, which can begin to simulate follicle development as early as 7 d after parturition (Thatcher et al., 2006). A dominant follicle develops in response to both FSH and LH; however, due to inadequate amounts of plasma LH the new follicles do not always produce E_2 (Beam and Butler, 1998). Post-partum anovulation and anestrous in dairy cows is not due to a lack of follicular development, but rather the failure of a dominant follicle to ovulate (Roche et al., 2000).

The interval to first ovulation in dairy cows has typically occurred between 14 to 21 d after calving, with 5% of the herd anestrous at the start of the breeding period post-partum (typically around 50-60 DIM) (Lucy, 2001). However, Lucy (2001) compared the interval to first ovulation in US dairy herds and observed that in dairy cows in 1964 the average interval to first ovulation was 29 ± 3 d and in modern dairy cows the interval was 43 ± 5 d, with corresponding anestrous cows (> 60 d post-partum) of 0% and 38%, respectively. Not only does resumption of ovarian activity play an important role in subsequent fertility (Lucy, 2001), it also results in delayed intervals to first insemination, leading to extended calving intervals (Opsomer et al., 2000).

Post-partum metabolic disorders and nutrition

The genetic, environmental, and nutritional management of the modern day lactating dairy cow is tailored towards producing large milk yields. However, this increase in milk yield has been coupled with reduced fertility (Butler, 1998). Researchers have focused on understanding the physiological mechanisms of nutrition that influence

poor reproductive performance, with emphasis on the nutritional requirements of the dairy cow both pre-and post-partum to prevent metabolic diseases and gynecological disorders. An epidemiological study concluded that negative energy balance (NEB) as well as peri-parturient and post-partum disorders were risk factors for delayed cyclicity and prolonged luteal phases (Opsomer et al., 2000).

The onset of lactation is associated with a prolonged period of NEB during which energy intake lags behind the energy requirements of rapidly increasing milk production. This is because the cow is unable to consume dry matter (DM) as fast as the increased nutrient demands required for lactation. Negative energy balance usually begins a few days before calving and becomes visible during early lactation as a loss in body condition because the cows' body is mobilizing body reserves of fat and protein to cope with the nutrient shortage (Roche et al., 2000; Butler, 2005a). Prolonged periods of NEB are associated with a delay in the resumption of normal ovarian activity (Lucy, 2001). A cow in NEB during the early post-partum period has a reduction of post-partum LH pulse frequency and low levels of blood glucose, insulin, and IGF-I that collectively reduce E_2 production from the dominant follicle which then undergoes atresia rather than ovulation (Butler, 2003; Roche, 2006). Any dominant follicles that develop have a decreased chance of producing sufficient E_2 to induce a pre-ovulatory gonadotropin surge (Roche et al., 2000). Not only does NEB delay recovery of post-partum reproductive function, but it can also exert carryover effects that reduce fertility during the breeding period, such as reduced or sub-optimum concentrations of P_4, detrimental effects on the oocyte, altered uterine function and rate of early embryo development (Butler, 2005a).

In response to NEB, cows mobilize stored triglycerides from adipose tissue in order to maintain energy for maintenance and milk production. These triglycerides are broken down through lipolysis, producing non-esterified fatty acids (NEFA), which are metabolized in the liver to be utilized by the mammary glands where, they are converted to milk fat. However, excess NEFA are partially oxidized to the ketone bodies acetoacetate and β-hydroxybutyrate (BHBA), which in large amounts can lead to ketosis and other metabolic disorders (Drackley et al., 2007). Ketosis occurs when liver glycogen stores are depleted and the body begins to utilize fatty acids to form the ketone bodies for energy (Baird, 1982). Fourichon et al. (2000) conducted a meta-analysis on post-partum diseases and found that clinical ketosis, dystocia and retained placenta were associated with a 4 to 10% lower PR at first service, metritis was associated with 20% lower PR, and anestrous was associated with 26 more days to first service and with an 18% lower PR at first service. Retained placenta is associated with a delayed involution of the uterus and low plasma E_2 concentrations (Ball and Peters, 2004). Moreover, cows with retained placenta are more susceptible to metritis (Sandals et al., 1979), which is often associated with a persistent CL, preventing the uterus to be cleaned out (Ball and Peters, 2004). A persisitent CL prevents $PGF_{2\alpha}$ secretion, which is important because it promotes uterine tone and contraction, removing potential harmful bacteria. With high P_4 concentrations from a persistent CL, the uterine immune system becomes down-regulated, creating a uterus that is even more susceptible to infection (Sakaguchi et al., 2004).

Dairy cows are fed different diets throughout their lactation cycle to match changing energy requirements. Because cows begin to experience a state of NEB in the pre-partum period it is important to maintain dietary intake during the transition period in

order to achieve better energy status during the early weeks of lactation (Butler, 2005a). Researchers have recently shown that reduced feed intake prior to calving increased the risk of cows developing metritis (Hammon et al., 2006; Huzzey et al., 2007). Inadequate nutrient intake before calving may predispose cows to impaired immune function, such as reduced neutrophil function, subsequently increasing the risk for uterine diseases (Hammon et al., 2006). Including fat in the pre-partum diet in order to feed higher energy dense rations has been shown to exert a carryover benefit on PR during lactation (Butler, 2005a). Supplemental dietary fat may improve fertility by: 1) improving energy status of the cow leading to an earlier return to estrus during the post-partum period, 2) increasing P_4 production, and 3) increasing serum insulin concentrations, thereby stimulating the development of ovarian follicles (Ambrose and Kastelic, 2003).

Because the benefits of feeding fat may originate from specific fatty acids much work has focused on feeding high amounts of essential fatty acids to enhance reproductive performance in dairy cows (Staples et al., 1998; Staples and Thatcher, 2005; Santos et al., 2009). Omega-3 fatty acids can alter the synthesis and release of $PGF_{2\alpha}$ and are thought to improve embryonic survival (Mattos et al., 2000, 2002). Cows fed diets enriched in linoleic or linolenic fatty acids had a lesser incidence of ovarian cysts and ovulated sooner with no effect on energy balance or PR (Colazo et al., 2009a). The inclusion of flax seed, rich in omega-3 fatty acids (Ambrose et al., 2006a; Petit and Twagiramungu, 2006) or fish oil, rich in omega-6 fatty acids, improved fertility through reduced pregnancy losses in lactating dairy cows after the first post-partum AI. Thangavelu et al. (2007) demonstrated that embryonic development was enhanced in dairy cows fed unsaturated fatty acids (linoleic acids; flaxseed and sunflower) compared

to those fed saturated fatty acids. Linolenic acid supplementation affected the molecular mechanisms controlling bovine oocyte maturation *in vitro* improving embryo development (Marei et al., 2009). These molecular mechanisms included increased PGE2 concentrations and intracellular cAMP and phosphorylation of mitogen-actived protein kinases, important for the resumption of oocyte meiotic maturation.

The most common strategy used to reduce the extent of NEB and body condition score (BCS) loss in early lactation is to increase dietary energy intake by increasing the starch or fat components of the ration at the expense of forage components. Such changes in carbohydrate and fat supplies have implications for rumen function, milk composition (milk fat content), nutrient partition, and metabolic hormones. Changes in metabolic hormones interact with reproductive hormones that control ovarian function (Webb et al., 2004; Garnsworthy et al., 2008). Major metabolic hormones (insulin, IGF-I, and leptin) act at the hypothalamic, pituitary, and ovarian levels, linking changes in reproductive activity to changes in energy balance. Insulin and IGF-I stimulate steriodogenesis and proliferation of theca and granulosa cells *in vitro* (Spicer and Stewart, 1996). Circulating concentrations of insulin are affected by diet and the changes are positively correlated with changes in estradiol produced by granulosa cells from small antral follicles (Armstrong et al. 2002). Insulin serves as a metabolic signal influencing LH release by the anterior pituitary (Monget and Martin, 1997) and has been shown to play a role in regulating ovarian responsiveness to gonadotropins (Diskin et al., 2003). Leptin can stimulate the GnRH producing neurons in the hypothalamus and directly stimulate LH and FSH secretion from the pituitary (Liefers et al., 2005) and steriodogenesis within the ovary (Kendall et al., 2004; Liefers et al., 2005). Kadokawa et al. (2006) observed that

plasma leptin concentrations were positively correlated to both frequency and amplitude of LH pulses in early post-partum lactating dairy cows.

Gong et al. (2002) fed a high-starch diet to cows to increase their circulating insulin concentrations and observed an increase in the proportion of cows ovulating within 50 d of calving from 55 to 90% and a reduction in the interval to first ovulation postpartum from 48 to 34 d. A further study demonstrated that to maintain an adequate insulin-to-glucagon ratio in cows at the start of the breeding period, dietary starch concentration should be above 160 g/kg of DM and dietary fat below 44 g/kg of DM, and this should have a positive effect on ovarian function (Garnsworthy et al., 2008).

Further, it is common practice to feed lactating dairy cows a diet high in crude protein (CP) concentration (17-19%) to enhance and maintain high milk production (Canfield et al., 1990). However, excessive intake of protein, particularly ruminal degradable protein (RDP), results in high systemic concentrations of ammonia and urea (Kenny et al., 2002; Ocon and Hansen, 2003), which are associated with reduced fertility, affecting ovulation, fertilization, and development of the early embryo (Kenny et al., 2002). Early degeneration and poor development of embryos occurred in lactating dairy cows fed excess protein (Blanchard et al., 1990) and high urea concentrations decreased the fertilization rates, cleavage rates, and development in *in-vitro* bovine oocytes (De Wit et al., 2010). As well, when excess protein is fed to cows in NEB, the energetic demands of excreting this as urea may exacerbate the effects of NEB on reproduction, thereby decreasing fertility. This is supported by the fact that embryonic survival in heifers, which do not experience NEB to the same extent as lactating dairy cows, is not affected by high CP diets and associated elevated urea concentrations (Kenny et al., 2002; Ocon

and Hansen, 2003). Dietary energy and protein can directly affect the expression of mRNA encoding components of the ovarian IGF system and dietary protein concentration can also influence oocyte quality, with developmental competence being negatively correlated with plasma urea concentrations (Armstrong et al., 2001). Canfield et al. (1990) and Barton et al. (1996) observed greater PR in lactating dairy cows fed a low protein diet, however milk production was affected. With improvements in diet modeling software and consistency of predicting metabolizable protein and amino acid requirements diets can be better formulated with moderate protein concentrations that can both minimize urea nitrogen production and not harm fertility (Tylutkai and Van Amburgh, 2010).

Development of Methods to Control the Bovine Estrous Cycle

As stated previously, estrus detection is a major factor affecting reproductive success of AI programs (Everett and Bean, 1986; Lucy, 2001; Rajamahendran et al., 2001). In order for a cow to get pregnant, she must be inseminated properly and in a timely fashion. The development and advancement of AI has had a dramatic impact on the dairy cattle industry. In 1998, there was a global AI usage of over 110 million first inseminations per year (Thibier and Wagner, 2002). The use of AI as a means of breeding is used in approximately 70 to 90% of dairy farms in North America (Rajamahendran et al., 1993; Pursley et al., 1997b; NAHMS, 2009). With the implementation of AI there have been huge advancements: the elimination of venereal diseases and use of dangerous bulls, the ability to impregnate many females from a single ejaculate, improved genetics, improved record keeping on farms where AI is used, reduced feed costs, and the

reduction in transportation costs through the ability to export semen (Foote, 1996). The use of AI has also led to the development of other reproductive technologies such as cryopreservation and sexing of sperm, embryo harvesting, freezing, culture and transfer, and cloning (Patterson et al., 2003). But with the advancement of AI and the adoption of emerging reproductive technologies, the need for precise methods of controlling the estrous cycle has become essential.

Traditional methods used to synchronize the estrous cycle had been based on the manipulation of the CL either by shortening its life span through the use of estrogens or prostaglandins, or extending its lifespan with the use of P_4 or synthetic analogues to mimic the luteal phase (Rajamahendran et al., 2001). The reduced fertility and variable estrus and ovulation times made it crucial to better understand ovarian follicular dynamics. The development and use of ultrasonography in cattle was one of the major steps in understanding follicular dynamics in the bovine estrous cycle. From ultrasound imaging techniques, it was established that follicular development occurs in a wave-like pattern in normal cycling cattle (Savio et al., 1988; Sirois and Fortune 1988; Rajamahendran and Taylor, 1990; Sakaguchi et al., 2004).

Patterson et al. (2003) cite that there has been six phases in the development of methods to synchronize the estrous cycle of the cow; these include the progesterone phase, the progesterone-estrogen phase, the prostaglandin phase, the progesterone-prostaglandin phase, and more recently the GnRH-prostaglandin phase and the progesterone-GnRH-prostaglandin phase. In the 1950's it was discovered that P_4 inhibited ovulation and pre-ovulatory follicle maturation (Ulberg et al., 1951; Nellor and Cole, 1956) and efforts focused on extending the luteal phase or by establishing an

artificial luteal phase by administrating exogenous P_4 (Patterson et al., 2003). Development of long-term P_4 delivery devices such as PRID (Progesterone Releasing Intravaginal Device) (Roche, 1976), CIDR-B® (controlled internal drug release) (MacMillan and Peterson, 1993) and Synchromate B implant (Spitzer et al., 1978; Hixon et al., 1981) facilitated the use of progestogens. But lowered fertility was often experienced with progestogens, especially after treatment for more than 10 d due to a prolonged persistence of a dominant follicle and consequently the ovulation of a sub-fertile oocyte (Xu et al., 1997). The progesterone-estrogen phase used P_4 to lengthen, and estrogens to shorten the lifespan of the CL; however, short-term progestogen treatments, with the use of estrogens, were not completely effective in synchronizing estrus in cattle (Wiltbank et al., 1975). This led to the development of estrus synchronization regimens that involved a short-term progestogen treatment combined with $PGF_{2\alpha}$ shortly before or at the termination of the progestogen treatment, in order to fully luteinize any CL present at the time of progestogen removal (Xu et al., 1997). Some researchers reported satisfactory fertility (Folman et al., 1990; Kastelic et al., 1996; Pancarci et al., 2002; Cavalieri et al., 2004), while others reported a reduction in fertility (Folman et al., 1984; Beal et al., 1988).

In the 1970's, $PGF_{2\alpha}$ was discovered to be the natural luteolytic agent in cattle (Lauderdale et al., 1974; Rajamahendran et al., 1977) and as a result, $PGF_{2\alpha}$ and analogues became the preferred treatment for estrus synchronization in cattle (MacMillan and Day, 1982, Odde, 1990; Xu et al., 1997). Some studies have shown that fertility of cows inseminated after induced estrus from a single injection of $PGF_{2\alpha}$ or double injections 12 to 14 d apart are similar or superior to that of cows inseminated after natural

estrus (Kastelic, 1990; Xu et al., 1997). However, since the interval from $PGF_{2\alpha}$ to ovulation is often variable, $PGF_{2\alpha}$ followed by a fixed TAI is associated with reduced fertility (Dailey et al., 1983; Stephens and Rajamahendran, 1998). Researchers, who used $PGF_{2\alpha}$ programs on cows, reported that the stage of the estrous cycle at the time of the second $PGF_{2\alpha}$ treatment affected both the percentage of cows showing estrus in response to the treatment and the PR to inseminations performed at the synchronized estrus. Cows in late luteal phase had a greater estrus response and PR than cows in early or mid- luteal phases (Xu et al., 1997). The effect of stage of the estrous cycle at the second $PGF_{2\alpha}$ treatment on PR may be partially explained by lower P_4 concentrations in early or mid luteal phases. Xu et al. (1997) examined estrus synchronization using two treatments of $PGF_{2\alpha}$ 13 d apart with and without CIDR for 5 d before the second $PGF_{2\alpha}$ injection and observed improvements in estrus synchronization, PR to synchronized insemination, and an increase in the percent of cows responding to the second $PGF_{2\alpha}$ treatment; but only with cows in the early and middle stages of the luteal phase at the time of the second $PGF_{2\alpha}$ treatment (Xu et al., 1997).

While synchronization of estrus using P_4 and/or luteolytic agents is based on controlling the lifespan of the CL, estrus culminates from the growth of a dominant follicle in coordination with the regression of a CL (Thatcher et al., 2001). It is now evident that variations in efficacy of synchronization protocols based on P_4 and/or $PGF_{2\alpha}$ are related to the stage of follicular development when the synchronization treatment is initiated. Thus, synchronization of follicular growth and CL regression has the potential to increase precision of estrus synchronization, optimize follicle quality and maximize PR (Thatcher et al., 2001).

Ovulation Synchronization

Through the development of past hormonal applications, and with improved understanding of follicular growth and CL dynamics, new methods of synchronizing ovulation have been used in an attempt to eliminate the dominant follicle and initiate a new follicular wave. Based on this, Thatcher et al. (1989) proposed a method that synchronized both follicular development and CL regression – an injection of GnRH agonist followed 7 d later with $PGF_{2\alpha}$. This resulted in a better estrus synchronization rate (defined as the proportion of cows in estrus at a specified time) and PR compared to those treated with two injections of $PGF_{2\alpha}$ 12 d apart (Twagiramungu et al. 1995, Pursley et al., 1997b; Momcilovic et al., 1998). However, another study found that there were no differences in PR when it was compared to cows treated with one $PGF_{2\alpha}$ injection (Thatcher et al., 2001). While estrus synchronization programs attempt to synchronize animals so that they come into heat at similar times, many still resulted in variability of when the animal would come into heat and these programs still required the need for estrus detection. Pursley et al. (1995) demonstrated that an additional injection of GnRH given 48 h after the $PGF_{2\alpha}$, as reported by Thatcher et al. (1989), would induce a timed ovulation at approximately 30 h later. This allowed for the control of both the CL and ovarian follicular growth, without need for estrus detection. This protocol, Ovsynch (Pursley et al., 1995; 1997a; 1997b), focuses on synchronizing ovulation, rather than estrus and so allows for TAI. Several researchers have compared the efficacy of Ovsynch to $PGF_{2\alpha}$ programs. Researchers compared cows treated with Ovsynch TAI to cows treated with two treatments of $PGF_{2\alpha}$ 12 or 14 d apart and bred at natural estrus or with

TAI and found that Ovsynch cows had either higher PR (Pursley et al., 1997a; Hirad et al., 1999; Small et al., 2001) or similar PR to those bred to natural estrus (Lucy et al., 2001; Rabiee et al., 2005). A study comparing Ovsynch to a CIDR-based ovulation synchronization TAI protocol found no differences in PR between the two treatments (Aali et al., 2008).

Altering timing of treatments

Pursley et al., (1998) evaluated the effect of the interval between the last GnRH treatment and time of AI and found that pregnancy per AI decreased in cows inseminated 32 h after the GnRH treatment, and maximum percentage of cows pregnant was obtained when TAI was performed 16 h after the final treatment of Ovsynch. Another protocol Cosynch, involves TAI at the same time as the last GnRH treatment in Ovsynch (Geary and Whittier, 1998). Although Cosynch reduces cow handling, it does not maximize the CR to TAI due to the timing of ovulation from the treatment with GnRH (Pursley et al., 1998; Dalton et al., 2001). However, Small et al. (2001) and Rabiee et al. (2005) observed no differences in PR if the last GnRH treatment and TAI are concurrent. Recently, Brusveen et al (2008) found no advantage of Cosynch at 72 h vs. 48 h after the $PGF_{2\alpha}$ treatment. If the second GnRH injection is delayed, then more cows are detected in heat prior to GnRH injection, cows become asynchronized and timing of insemination is off. Richardson et al. (2002) also used a Select Synch protocol where animals that come into heat after the $PGF_{2\alpha}$ treatment, but before the designated TAI, are inseminated, and those animals that are not detected in heat are then subsequently given the second GnRH treatment and then inseminated at a fixed time. Also, if the interval is less than 7 d

between the GnRH and $PGF_{2\alpha}$ injection, the ability to effectively regress a newly developed CL is reduced.

A meta-analysis by Rabiee et al. (2005) on the efficacy of Ovsynch found that PR of cows on Ovsynch programs did not significantly differ from those with natural breeding programs and identified that the PR obtained with the $PGF_{2\alpha}$ induced estrus, Select Synch, and modified Ovsynch (including Cosynch) programs were comparable with the Ovsynch program. The economic benefit of Ovsynch is based on the reduction of intervals to first AI, reduced number of days open, and reduced culling for infertility (Tenhagen et al., 2004a; Rabiee et al., 2005). Ovsynch can greatly improve reproduction in herds with poor estrus detection rates (Pursley et al., 1997a, b; Schmitt et al., 1996b).

Progesterone supplementation with CIDR/PRID during Ovsynch

El-Zarkouny et al. (2004) compared Ovsynch alone to Ovsynch with a CIDR insert for 7 d starting at first GnRH injection and found a higher PR at days 29 and 57 for CIDR supplementation. It was suggested that the positive effects might be attributed to the fact that under P_4 dominance, the dominant follicle either grows, degenerates, or ovulates and a new follicular wave emerges (Kastelic et al., 1996; El-Zarkouny et al., 2004; Cavalieri et al., 2004). Meanwhile Richardson et al. (2002) compared a modified SelectSynch to that with additional CIDR supplementation found that dairy heifers, but not beef heifers, treated with the P_4 had higher PR.

Pre-synchronization

Initiation of Ovsynch at certain stages of the estrous cycle can influence its effectiveness. Administration of GnRH during the late luteal phase (day 15 to 17) may not result in ovulation and formation of a CL (El-Zarkouny et al., 2004). At this stage, cows that have two wave cycles commonly have a small potentially dominant follicle that is not yet responsive to the GnRH treatment. Consequently, a new CL does not develop, and at the time of $PGF_{2\alpha}$ injection given 7 d later, the CL has already regressed and the cow may even be in heat. A second problematic stage occurs early in the estrous cycle (day 2 to 3) when spontaneous ovulation has already occurred and the new potentially dominant follicles are too small to ovulate in response to GnRH treatment. Consequently, the dominant follicle will be aged at the time of the second GnRH treatment, producing less fertile oocytes (Vasconcelos et al., 1999; Thatcher et al., 2001; El-Zarkouny et al., 2004; Navanukraw et al., 2004).

One way to target the initiation of Ovsynch during a favourable stage in the estrous cycle is to presynchronize the estrous cycle before the first treatment of Ovsynch (Moreira et al., 2001; El-Zarkouny et al.; 2004; Navanukraw et al., 2004). The Presynch protocol originally involved two treatments of $PGF_{2\alpha}$ 14 d apart with a 12 d interval before the start of Ovsynch (Cartmill et al., 2001; Moreira et al., 2001; Navanukraw et al., 2004) and resulted in increased PR to TAI compared with Ovsynch alone, some even higher by 10 to 12%. Several studies incorporated an extended interval of 14 d between Presynch and Ovsynch into the design of their field studies and still found beneficial results (Pancarci et al., 2002; Fricke et al., 2003; Navanukraw et al., 2004). This extended interval allows the injection of $PGF_{2\alpha}$ to fall on the same day during successive weeks,

which is important for the compliance of producers to follow through on giving the treatments to the cows (Navanukraw et al., 2004). Navanukraw et al. (2004) reported that the proportion of cows ovulating after the first GnRH injection did not differ between treatments, but that the proportion of cows ovulating after the second GnRH injection was numerically greater for Presynch cows. The stage of the cycle at administration of the first GnRH injection of Ovsynch on days 5 to 10 may provide a more favourable P_4 environment during development of the ovulatory follicle, which may affect PR (Navanukraw et al., 2004). Also, when subjected to a Presynch protocol using two injections of $PGF_{2\alpha}$ administered 12 to 14 d apart, cyclic cows should express estrus once or twice before TAI (Navanukraw et al., 2004). With increases in the number of estruses occurring during the post-partum period, PR to AI has increased (Thatcher and Wilcox, 1972). A positive effect of a $PGF_{2\alpha}$ treatment on PR to AI has been reported in cattle because it permits uterine clearance (Roche, 1976; MacMillan and Day, 1982).

Variable methods of presynchronizing cattle before the initiation of Ovsynch have been examined, but none found the improvements as with Presynch.; Researchers (Cordoba and Fricke, 2001; LeBlanc and Leslie, 2003) have shown that presynchronizing with a single injection of $PGF_{2\alpha}$ at 10 or 12 d before Ovsynch did not improve PR compared with Ovsynch alone; whereas Cartmill et al. (2001) did observe an increase in PR. Peters and Pursley (2002) reported that there were higher proportions of cows in the luteal phase at the initiation of Ovsynch for cows presynchronized with $PGF_{2\alpha}$ 10 d and GnRH 7 d before the initiation of Ovsynch; however, PR were not affected.

Portaluppi and Stevenson (2005) examined the timing of AI after a Presynch + Ovsynch treatment and found that inseminating at 48 or 72 h after $PGF_{2\alpha}$, when GnRH

was administered at 48 h after $PGF_{2\alpha}$, produced lower PR than inseminating and injecting GnRH at 72 h after $PGF_{2\alpha}$. However, other researchers observed either no difference or decrease in PR when cows received TAI at 72 h compared with 48 h after induced luteolysis (Sterry et al., 2007; Brusveen et al., 2008; Hillegass et al., 2008). Brusveen et al. (2008) observed greater PR in cows that were administered GnRH 56 h after $PGF_{2\alpha}$ and TAI at 72 h due to a more optimal interval between the LH surge and AI. El-Zarkouny et al. (2004) compared Presynch/Ovsynch with or without the CIDR and found that the P_4 supplementation had no effect on pregnancy.

Improving Pregnancy Rates Using Post-insemination Treatments

Other strategies that try to improve PR focus on minimizing embryonic mortality. Ovulation and fertilization occur in about 76% (Santos et al., 2004) to 90% of treated animals (Diskin and Sreenan, 1980); however, PR is drastically low 35 d later. Low P_4 concentrations early post-insemination are associated with delayed CL formation and reduced pregnancy in cattle due to asynchrony between the uterus and the embryo (Schmitt et al. 1996a; Changes e Silva et al. 2002). Since most pregnancy loss occurs by about day 16, approaches have focused on this time; these include P_4 supplementation, use of GnRH and human chorionic gonadotropin (hCG).

Progesterone administered early in the estrous cycle advances the maturation of the uterine endometrium and stimulates changes in the uterine environment resulting in a more accelerated growth of the embryo (Barnes, 2000). However, administration of exogenous P_4 post-insemination as a method for increasing PR has had limited success (Robinson et al., 1989; MacMillan and Peterson, 1993; Van Cleeff et al., 1996; Ambrose

et al., 1998; Villarroel et al., 2003). Best results have been found when P_4 is administered over the first 4 d of the cycle (Barnes, 2000; McNeill et al., 2005). A study using CIDR insert 7 d after insemination tended to increase PR (Stevenson et al., 2007).

Studies have demonstrated that giving GnRH (Kastelic et al., 1990) or hCG (Sianangama and Rajamahendran, 1992) between day 5 to 7 or 11 to 14 (Franco et al., 2006) of the estrous cycle can induce the formation of an accessory CL, thereby increasing P_4 concentrations and possibly reducing embryonic loss. Treatment with GnRH or hCG given between day 5 to 7 post-AI, focuses on enhancing CL function, ovulating the first-wave dominant follicle, and increasing P_4 secretion. Treatment with GnRH or hCG given on day 11 to 14 coincides approximately with maternal recognition of pregnancy and with the rise or peak of the second follicular wave in cows with three wave cycles (Peters et al., 2000; Franco et al, 2006). Ovulation of a dominant follicle at this time also results in a decrease in E_2 production, which in turn results in an inhibition of up-regulation of oxytocin receptors and consequent inhibition of $PGF_{2\alpha}$ secretion (Peters et al., 2000). Up-regulation of oxytocin stimulates the synthesis of P_4 (Miyamoto and Schams 1991).

There have been several experiments where hCG and GnRH were administered anywhere from the day of insemination up to day 14 post AI (Lewis et al., 1990; Sianangama and Rajamahendran, 1992; Ryan et al., 1994; Schmitt et al., 1996a; Ambrose et al., 1998; Peters et al., 2000; Tefera et al., 2001; Howard et al., 2006; Stevenson et al., 2007). Both the methods and the timing of the treatments have produced variable results. Differences in experimental design and physiological condition of cattle among studies are large, and these differences may explain some of the variation in the effects of GnRH

and hCG on PR (Lewis et al., 1990). The stage at which GnRH or hCG is given is significant, and its effects depend on the follicular status at the time of treatment (Lewis et al., 1990).

In a study by Sianangama and Rajamahendran (1992) comparing hCG treatment on day 0, 7, or 14, to no treatment, significant increases in P_4 concentrations from day 18 to 42 after breeding and in PR were observed in cows treated on day 7 or day 14. Tefera et al. (2001) found no effects on the rate of embryonic mortality with the administration of hCG on day 4 or GnRH on day 12 post-AI. In an experiment by Schmitt et al. (1996a) the induction of an accessory CL with hCG on day 5 or 6 after insemination increased P_4 concentrations, but did not increase PR in fertile heifers or lactating dairy cows during summer heat stress. A meta-analysis of studies on the effect of GnRH administered 11 to 14 d after insemination on PR also found a significant improvement in PR in treated animals (Peters et al., 2000). The addition of GnRH or hCG treatment after a TAI protocol may further help to overcome the low PR still observed with Ovsynch.

Human Chorionic Gonadotropin and Porcine Luteinizing Hormone

Unlike exogenous treatment with GnRH, hCG targets gonadal cells directly and has a longer half-life (3 to 4 d vs. hours) than an exogenous GnRH-induced LH surge, exerting a longer stimulatory and luteotropic influence on the developing CL (Schmitt et al., 1996a). Moreover, Stevenson et al. (2007) have shown that hCG is more effective at inducing ovulation than GnRH in dairy cattle and that the resulting CL has greater P_4 concentrations compared with a CL induced by GnRH (Rajamahendran and Sianangama, 1992; Schmitt et al., 1996a; Sianangama and Rajamahendran, 1996).

Exogenous treatment with porcine luteinizing hormone (pLH) also acts directly on the ovary and has a similar duration in the blood to that of a naturally occurring LH surge (10 h) (Ree et al., 2009). It has also been demonstrated in cattle and buffalo that when plasma P_4 concentrations are higher, pLH is more effective than GnRH at inducing ovulation (de Araujo Berber et al., 2002). Moreover, one study has reported that dairy heifers induced to ovulate with pLH had higher P_4 concentrations 9 d after pLH treatment than those treated with GnRH (Ambrose et al., 2005).

While exogenous treatment with hCG or pLH may be more effective treatments than treatment with GnRH, costs must also be considered. Gonadotropin releasing hormone is only a deca-peptide, and so is easily made; whereas hCG and pLH are glycoproteins consisting of long polypeptide chains, which are much more complex (Senger, 2003). Therefore both hCG and pLH are considerably more expensive per treatment ($9.95 and $14.00, respectively) compared to GnRH ($2.80). As such, there have also been fewer studies conducted using these hormones.

Rationale and Objectives of the Dissertation

Synchronization protocols were developed as management tools for producers to reduce the problems faced with estrus detection and to improve PR. Through the development of past hormonal protocols, and with the better understanding of follicular growth and CL dynamics, current methods of synchronization are based on eliminating the dominant follicle and initiating a new follicular wave. The Ovsynch TAI protocol is proven to be a useful management tool to minimize the need for estrus detection, but it does not necessarily improve PR. However, what are some further hormonal and

management strategies to this protocol that could be developed to increase PR? It is also important to determine what the current reproductive issues that impact local dairy farms are, in order to best develop and apply new reproductive technologies for dairy cattle.

In this dissertation I first set out to conduct a benchmark field study on the reproductive performance of lactating dairy cows on local dairy farms in British Columbia. A similar field study was conducted on dairy farms in the Fraser Valley of British Columbia area over 15 years ago (Rajamahendran et al., 1993) and the information is outdated. This study is important in order to determine how best to apply new management strategies to overcome poor fertility in lactating dairy cows. While Ovsynch TAI is a management tool commonly used by dairy producers because it reduces the need for estrus detection, it does not necessarily increase PR. As described in the literature review above, there are some potential strategies that may improve PR to Ovsynch TAI. A pre-synchronization treatment before Ovsynch TAI can increase PR by increasing synchronization rates to TAI and a post-insemination treatment after Ovsynch TAI could increase PR by reducing embryonic mortality. As well, pLH or hCG may be more effective than GnRH in an Ovsynch TAI protocol. Finally, nutrition plays a large role in fertility in lactating dairy cows and feeding a lower protein diet during Ovsynch TAI and early embryonic development may also increase PR.

Therefore, the objectives of this dissertation were: 1) to provide a benchmark study utilizing surveys and P_4 profiles taken from local dairy farms to assess reproductive parameters (Chapter 2) and 2) increase PR in cows bred to an Ovsynch TAI protocol. Focus on the second objective was achieved in three separate experiments: 1) to determine if ovulation synchronization rates, PR and P_4 profiles improve in cows and

heifers following a Presynch + Ovsynch or an Ovsynch + Post-TAI GnRH (Chapter 3), 2) to determine if ovulation synchronization rates, PR and P_4 profiles improve following a modified Ovsynch protocol using pLH or hCG in lieu of GnRH, (Chapter 4), and 3) to determine if ovulation synchronization rates, PR and P_4 profiles improve following cows fed a low protein diet during Ovsynch TAI (Chapter 5).

CHAPTER 2: A FIELD STUDY ON THE CURRENT REPRODUCTIVE PERFORMANCE OF DAIRY COWS IN THE UPPER FRASER VALLEY AREA OF BRITISH COLUMBIA

Introduction

Poor reproductive performance remains one of the primary reasons for culling lactating dairy cows (Norman et al., 2007). Lower fertility leads to increased insemination costs and increased involuntary culling (USDA, 2007). Numerous factors can influence reproductive performance, such as the onset of ovarian activity, efficiency of estrus detection, the incidence of early embryonic mortality, and maintenance of pregnancy (Starbuck et al., 2004). Moreover, synchronization programs are becoming standard components in current breeding management of cows in most dairy industries (MacMillan, 2010). Therefore, it is very important to benchmark current reproductive performance and issues (e.g. estrus, detection, embryonic loss) that impact dairy farms in order to apply new reproductive technologies to enhance fertility in dairy cows. The last field study conducted on dairy farms in the Fraser Valley of British Columbia area was performed over 15 years ago (Rajamahendran et al., 1993) and the information is outdated.

The concentration of P_4 in plasma or milk is closely related to the estrous cycle of dairy cows (Laing and Heap, 1971; Rajamahendran et al., 1976). Progesterone concentrations are used to confirm estrus and diagnose non-pregnancy, and monitor ovarian activity, early embryonic loss, and ovarian disorders (Ginther et al., 1974; Rajamahendran et al., 1993; Opsomer et al., 1998; Opsomer et al., 2000). The objective of this field study was to obtain information to benchmark the current management of

reproduction on commercial dairy herds in British Columbia with the aid of surveys and milk P_4 data.

Materials and Methods

Milk sampling and data collection

Nine farms in the Upper Fraser Valley area of British Columbia (Agassiz-Chilliwack area) volunteered for this study. Producers were provided with plastic sampling vials containing a milk fat preservative (BroTab10, Systems Plus Ltd., Ontario, CA) and were asked to collect foremilk samples from cows 30 and 37 d post-partum, on the day of breeding (0), and on days 7, 14, 21, and 28 post-breeding. Samples were refrigerated until collected at weekly intervals, where upon they were frozen at -20°C until subsequent analysis. For cows that had milk samples collected, pregnancy after first insemination was confirmed by rectal palpation from a herd veterinarian was collected.

A questionnaire (Appendix A) on reproductive farm management was mailed out to all farms (n = 97) within the Upper Fraser Valley region of BC, which also included the 9 farms that participated in milk sample collection. The surveys gathered basic farm management information, with a focus on reproductive management. Prepaid postage was included on return envelopes with the surveys.

Radioimmunoassay of progesterone in milk

Milk P_4 concentrations (ng/mL) were determined using a commercially available solid-phase radioimmunoassay kit (Coat-A-Count Progesterone, Diagnostic Products, Los Angeles, CA). This method was previously validated in our laboratory for the

measurement of P_4 in milk (Rajamahendran et al., 1993). Milk samples and reference standards (0.1 mL) were added to tubes coated with a P_4 specific antibody. Reference standards contained between 1 ng/mL and 40 ng/mL P_4. Buffered I^{125}-labelled P_4 (1.0 mL) was added to all tubes, which were shaken on a vortex, incubated for 3 h at room temperature and then decanted. The tubes were counted for radioactivity on a gamma counter for 1 min. The coefficients of variation within (intra) and between (inter) assays were 7 and 9%, respectively. The sensitivity of the assay was 0.03 ng/mL.

Analysis of data

Data collected from the questionnaires were summarized using descriptive analysis. Progesterone concentrations on days 30 and 37 post-partum and on the day of breeding (day 0) were classified into two groups: < 1 ng/mL and ≥ 1 ng/mL. Cows were considered cycling if at least one P_4 sample (day 30 or 37) was ≥ 1 ng/mL. Cows were defined as being in estrus if the milk P_4 concentrations on the day of breeding were < 1 ng/mL. Ovulation was assumed in those animals that were in estrus on the day of breeding and then had P_4 concentrations ≥ 1 ng/mL 7 d later. Animals were assumed pregnant on day 28 if P_4 concentrations were < 1 ng/mL on day 0, followed by P_4 concentrations ≥ 1 ng/mL on days 7, 14, 21, and 28 (presumptive PR). Non-pregnancy was determined if P_4 concentrations dropped below 1 ng/mL on any of days 7, 14, and 21 or if day 0 concentrations were > 1 ng/mL.

Progesterone data were analyzed using Least Squares ANOVA for repeated measures using JMP®. Progesterone data on day 7 were also categorized into three categories: low (< 1 ng/mL), intermediate (≥ 1 ng/mL, but < 5 ng/mL), and high (≥ 5

ng/mL). A concentration of ≤ 1 ng/mL was assumed to indicate non-pregnancy. The accuracy of non-pregnancy and pregnancy determinations by milk P_4 analysis was determined by comparing results of the radioimmunoassay with diagnoses of pregnancy by a veterinarian around 40 d post-insemination via rectal palpation. To determine the association among data classifications, P_4 concentrations, and farm pregnancy data, Chi-square was used.

Results

Survey data

Of the 97 surveys mailed out to dairy producers in the Upper Fraser Valley area, 23 were returned (23.7% compliance). Three farms had sold their dairy herd over the past year. Among the farms that participated in the survey, herd size ranged from 19 to 450 milking cows, with an average of 157 ± 30 (SEM). All herds consisted of purebred Holsteins with the exception of one farm (Holstein and Jersey). Artificial insemination was employed on all but one farm. Inseminations were performed by the farmer in 50% of the farms, 25% relied on an AI technician for all inseminations and the remainder relied a combination of farmer, AI technician and/or a bull.

Free-stall housing was used on 90% of the farms; the remainder used loafing areas. Cows were milked using herringbone (50%), parallel (45%), or inline (5%) systems. All farms had concrete flooring. Although the two farms with loafing areas had shavings on top of concrete, and one farm had 50% rubber flooring. Seventy-five percent of the farms milked twice daily, while the remainder milked three times daily.

All farms were closed farms and raised their own stock. Seventy-five percent of farms were members of the Dairy Herd Improvement program of Canada. The average VWP before inseminating cows post-partum was 68 ± 3 days in milk (DIM), with the range between 50 and 90 DIM. Only 33% of the producers used heat detection aids, such as pedometers and activity measurements. All producers had a routine suited to their management system for observing estrus and relied on standing estrus to determine breeding. Many farms also used other visual signs of the cow to help determine if a cow was in estrus (slime, curiosity, alertness, mounting, and bawling).

Producers were asked to list their top three reasons for culling in the past year and their top three health concerns. Fertility/reproductive problems were the top reason for culling, followed by mammary health (mastitis, high somatic cell count), and lameness (hoof and leg health). Other reasons for culling were voluntary culling, and low milk production. Top health concerns were hoof health (lameness and fungus), mastitis and mammary health, and reproductive disorders. Post-partum and metabolic diseases were also important concerns.

Fifty-eight percent of the farms used a form of estrus synchronization for breeding their animals; the majority of those farms (71%) used the Ovsynch protocol and the remainder used $PGF_{2\alpha}$ treatment. The average number of days open was 120 ± 5 DIM, with a range of 85 to 150.

The nine farms that had milk samples collected were similar to the average farms from the questionnaire survey. All had purebred Holsteins housed in free-stalls. The herd size ranged from 80 to 250 milking cows and AI was employed on all farms. A summary

of data collected from the current study and from the 1993 survey are compared in Table 2.1.

Post-partum cyclicity

Three hundred and sixty-eight day 30 and day 37 post-partum milk samples were provided by participating farms. Seventy-two percent of all animals were cycling by 37 DIM. There was only 2% (6 cows) that were anestrus, where P_4 concentrations were less than 1 ng/mL throughout the entire sampling period (all 7 samples). First service PR tended (P = 0.10) to be higher for cows that were cycling by day 37 (41.9%) vs. those that were not cycling (31.8%).

Progesterone concentrations at breeding

The nine participating farms provided a total of 241 milk samples taken on the day of breeding. All samples were for first services only. Of the 241 observations of milk P_4 samples at breeding, 86.4% contained P_4 concentrations that were < 1 ng/mL. The proportion of breeding-day milk P_4 concentrations that were < 1 ng/mL varied greatly among participating farms (Figure 2.1) (72 to 100% range; P = 0.04), but was not associated with herd size. Table 2.1 compares milk P_4 data from the 1993 study and the current study.

Progesterone concentrations post-breeding

Based on milk P_4 observations on days 0 and 7, 81.7% cows ovulated and had a functional CL on day 7. Of the day 7 milk P_4 concentrations > 1 ng/mL, 72% were

between 1 and 5 ng/mL, the remainder were ≥ 5 ng/mL. Two hundred and forty-one sets of milk P_4 observations for each of days 0, 7, 14, 21, and 28 were obtained from participating producers. Based on milk P_4 concentrations, by day 28, 51.2% of the cows were considered pregnant. Of the 241 milk P_4 breeding sets, 39.4% were diagnosed pregnant by a veterinarian. Presumptive pregnancy loss between days 21 to 28 was 8.8% and between days 28 to 40 was 11.8%. Sixty-six percent and 77.2% of the cows diagnosed pregnant by the farm were assumed pregnant, based on P_4 concentrations, on days 21 and 28, respectively. There were no differences in P_4 concentrations on days 7 and 14 between pregnant and non-pregnant cows. There were differences in P_4 concentrations between pregnant and non-pregnant cows on days 21 (9.09 ± 0.76 vs. 4.04 ± 0.56 ng/mL; $P < 0.0001$) and 28 (9.03 ± 0.80 vs. 4.59 ± 0.74 ng/mL; $P < 0.0001$) (Figure 2.2).

Discussion

In 1993, our lab conducted a similar survey on 27 dairy farms of the Fraser Valley area of British Columbia, where the average lactating herd size was 65 and similar management information was obtained (Rajamahendran et al., 1993). Compared to the 1993 study, the average herd size in the current study more that doubled, to 158 cows. In 2009, British Columbia had 545 dairy farms, with the average herd size of 135. Moreover, milk production is largely concentrated (75%) within the Fraser Valley region of BC (BCMPA, 2011). Depending on management conditions, improper breeding, when cows are not in estrus and P_4 concentrations are greater than 1 ng/mL, is reported to occur in 5 to 30% of all inseminations (Rajamahendran et al., 1993; Sturman et al., 2000). In

our current study, the proportion of cows bred when milk P_4 concentrations were ≥ 1 ng/mL on the day of breeding was 13.6%. The 1993 study reported a low rate of error in estrus detection of 4.8%. This was partially attributed to smaller herd sizes (Rajamahendran et al., 1993). Smaller herds require less time and labour and it is easier to watch for cows in estrus within the herd. Although herd size among the farms participating did not have an affect on the rate of accurate estrus detection, the average herd size was much larger in the current study. The range of estrus detection errors among the participating farms could be attributed to management practices on the farm, as well as to the use of synchronization protocols (specifics of which were not known), or when the milk samples were collected relative to breeding (compliance of farms collecting milk samples at the correct time). Inefficient and inaccurate estrus detection leads to decreased reproductive efficiency and increased economic losses (Jobst et al., 2000, Lucy, 2001).

The interval to first ovulation in US dairy herds has typically reported to be 43 ± 5 d, with corresponding anestrous cows (> 60 d post-partum) of 38% (Lucy, 2001). Gautam et al. (2010) determined that first post-partum ovulation occurring beyond 35 DIM was associated with reduced PR (Gautam et al., 2010). These authors observed that 34.9% of cows were not cycling by 35 DIM and that these cows were more likely not to conceive on first AI and more likely not to become pregnant within 100 d post-partum compared with cows with normal resumption of ovarian activity. While in the current study it cannot be determined when cows began cycling from the two post-partum milk samples collected, 28% were not cycling by 37 DIM and there was a tendency for those cows not cycling by 37 DIM to have lower first service PR compared with cows that had resumed

ovarian activity. While only 2% (six cows) of the cows followed through first service insemination were anestrus, this does not correspond with the total number of anestrous cows on the farms, only that those six cows were diagnosed in estrus inaccurately.

While P_4 concentrations can be used for determining non-pregnancy, they cannot be accurately used to determine pregnancy because P_4 is not a pregnancy-specific hormone. Other researchers have observed a 72% (Rajamahendran et al., 1993), 73% (Cavestany and Foote, 1985), and 77% (Zaied et al., 1976; Pennington et al., 1985) accuracy of 21-day milk P_4 status as a predictor of pregnancy. In this study I observed 66 and 77.2% accuracy for 21 and 28-d milk P_4 as pregnant. These differences can be attributed to non-pregnant cows experiencing extended cycles longer than 21 days, and/or embryonic loss but not CL regression (Rajamahendran et al., 1993). Although 51.2% of cows had high P_4 concentrations at day 28, only 39.4% were diagnosed pregnant at day 40, a difference of 11.8% percentage units. Researchers have estimated embryonic loss in cows with a delayed return to estrus to be between 15-20% (Wood, 1973; Wijeratne, 1973). More recent literature has reported estimates of pregnancy loss between day 28 and 42 at 7.1 to 11% (Starbuck et al., 2004), attributing the higher values from earlier studies to greater embryonic loss prior to day 28.

Progesterone production by the CL is important for the establishment and maintenance of pregnancy, and embryo growth (Schmitt et al., 1996; Changes e Silva et al., 2002). Any delay in the normal rise of P_4 concentrations in the early luteal phase results in smaller embryos with sub-optimal IFN-τ secretion and reduced ability to inhibit luteolysis (Changes e Silva et al., 2002; McNeill et al., 2005). Of the animals that were in estrus on day 0, 18.3% did not have milk P_4 concentrations ≥ 1 ng/mL on day 7. Of those

animals that did have P_4 concentrations ≥ 1 ng/mL, 72% were between 1 and 5 ng/mL, the remainder were ≥ 5 ng/mL. Several researchers have reported that a delay in the normal rise in P_4 concentration between days 4 and 5 post-insemination and cows with low P_4 values on day 5 to 7 have been associated with negative effects on embryo survival (Ambrose et al., 1998; Changes e Silva et al., 2002; Starbuck et al., 2004; McNeill et al., 2005; Stronge et al., 2005). Delayed CL formation can result in asynchrony between the uterus and the embryo and is associated with a marked and progressive reduction in PR in cattle (Schmitt et al., 1996; Changes e Silva et al., 2002). Inseminated cows subsequently observed to be non-pregnant have been shown to have significantly lower milk P_4 concentrations between days 10 and 16 after insemination than those that were subsequently diagnosed pregnant (Lamming et al., 1989). In general, most embryonic losses occur during CL formation and the maternal recognition period around days 14 to 16 (Changes e Silva et al., 2002; Bilodeau-Goeseels and Kastelic, 2003). In this study there were no differences in P_4 concentrations between pregnant and non-pregnant cows on days 7 or 14, but P_4 concentrations were greater for pregnant cows on days 21 and 28. Cavestany and Foote (1985) reported that as milk P_4 concentrations increased above 5 ng/mL, the proportion of cows diagnosed pregnant at palpation increased considerably, indicating a highly functional CL.

Implications

The results of this study show potential losses of pregnancies from inaccurate estrus detection and improper breedings, fertilization and ovulation failure, and/or embryonic mortality as the major reproductive problems encountered by the dairy

producers in the Fraser Valley. Moreover, post-partum cyclicity had a negative impact on subsequent first service PR.

Post-partum cyclicity is a huge management concern to dairy producers because it can extend intervals to first services, and can affect estrus detection and PR. Moreover, embryonic mortality reduces PR. As observed in this study, many producers are more frequently using estrus and ovulation synchronization (Ovsynch) methods as a reproductive management tool. Using a pre-synchronization treatment may help to improve synchronization of animals to Ovsynch TAI, as well as benefit those animals with post-partum cyclicity disorders As well, combining Ovsynch with a post-insemination treatment to combat embryonic mortality may be a simple way for producers to improve PR using a timed insemination protocol.

Table 2.1 Survey and progesterone data

Information collected	2006	1993
No. of surveys mailed out	97	-
No. of farms participating	23	27
Compliance	23.7%	-
Average herd size	157	65
Range	19 – 450	15 - 175
Pure Holstein herd	96%	93%
AI usage	96%	96%
AI employed only by farmer	50%	50%
Free-stall housing	90%	85%
Concrete flooring	100%	-
Closed farm	100%	-
DHI members	75%	-
Twice daily milking	75%	-
Type of milking parlour		
Herringbone	50%	63%
Parallel	45%	0%
In-line	5%	18%
Stanchion	0%	15%
Polygon	0%	4%
Voluntary waiting period	68 d	-
Range	50 - 90 d	-
Use of heat detection aids	33%	20%
Use of embryo transfer	0%	66%
Use of estrus/ovulation synchronization protocols	58%	-
Ovsynch use	41%	-
Average days open	120	-
Range	85 - 150 d	-
Top 3 reasons for culling	1. fertility/reproduction	-
	2. mammary health	-
	3. lameness	-
Top 3 health concerns	1. hoof health	-
	2. mammary health	-
	3. reproductive disorders	-
Milk progesterone (P_4)		
cycling by 37 DIM	72%	-
day 0 P_4 >1 ng/mL	13.6%	4.8%
day 7 P_4 < 1 ng/mL	18.3%	-
day 21 P_4 > 1 ng/mL	66%	71%
day 28 P_4 > 1 ng/mL	77%	-
1st service pregnancy rate	39.4%	53%

Data in 2006 column is from the current field study while data in 1993 column is adapted from Rajamahendran et al., 1993.

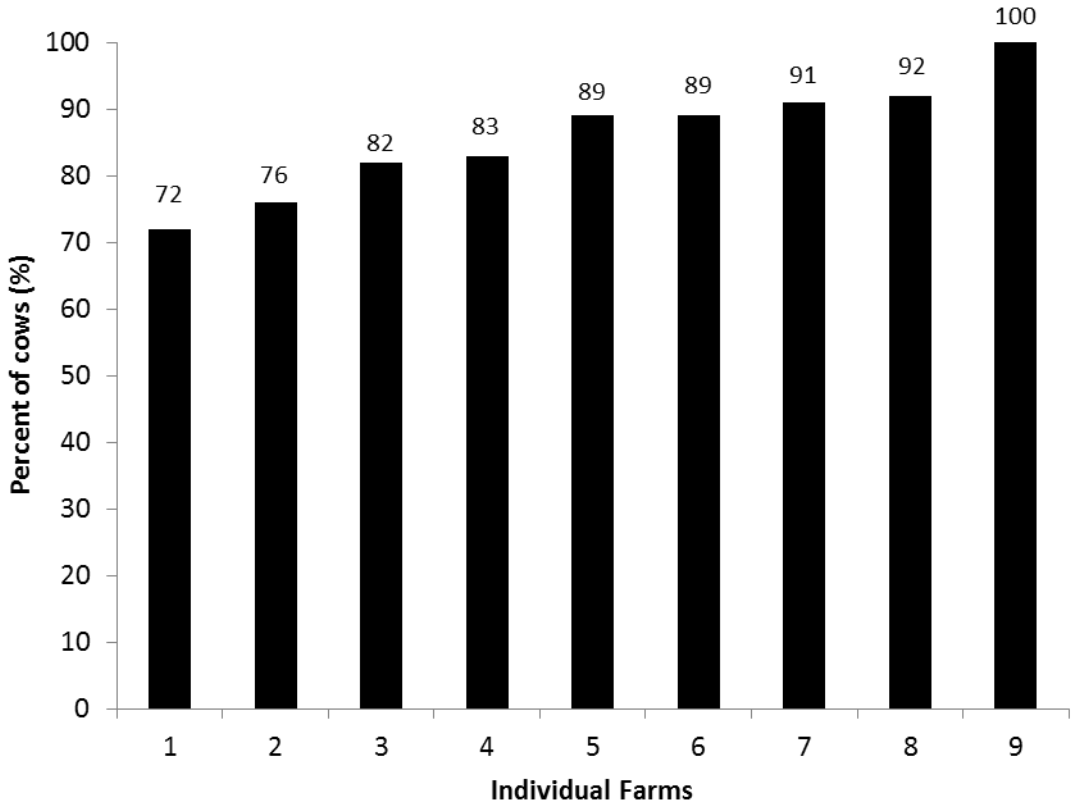

Figure 2.1 Percent of cows bred that were in estrus

Milk samples were collected from lactating dairy cows from nine farms within the Upper Fraser Valley of British Columbia on the day the animals were first bred. Cows were assumed to be in estrus if milk progesterone concentrations were < 1 ng/mL. Statistical difference between farms $P = 0.04$; n = 22-39 cows per farm.

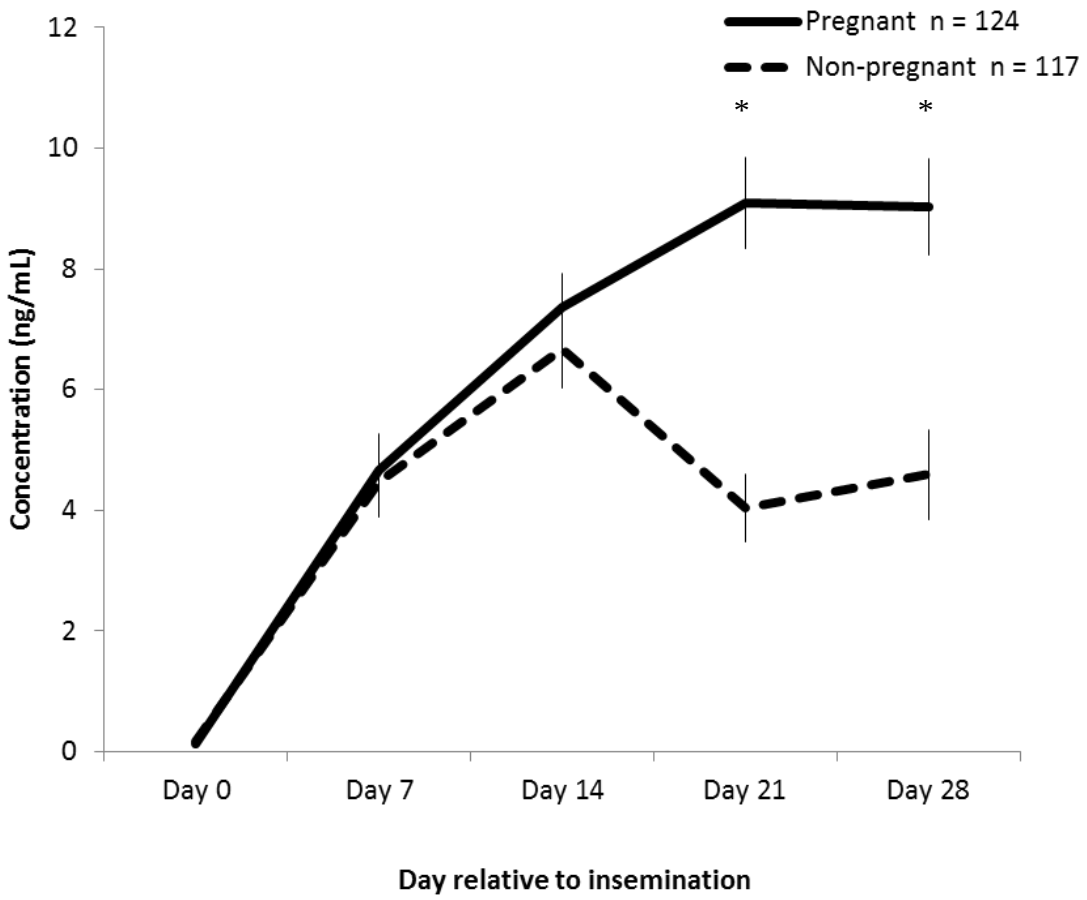

Figure 2.2 Milk progesterone concentrations of pregnant and non-pregnant cows

Milk samples were collected from lactating dairy cows from farms within the Upper Fraser Valley of British Columbia on day 0 (day of insemination), 7, 14, 21, 28. * indicates significantly different $P < 0.0001$. Vertical lines represent SEM bars.

CHAPTER 3: EFFECTS OF PRE-SYNCHRONIZATION AND POST-INSEMINATION TREATMENTS ON PREGNANCY RATES TO A TIMED BREEDING OVSYNCH PROTOCOL IN DAIRY COWS

Introduction

In response to challenges faced with estrus detection and poor fertility, the development of the Ovsynch protocol has allowed for the control of follicular development and CL regression thereby allowing for a TAI (Pursley et al., 1997a). Unfortunately, despite PR following Ovsynch having similar results to AI at natural estrus (Pursley et al., 1997a, 1997b), these rates are still far below the PR achieved over 50 years ago in lactating dairy cows (Starbuck et al., 2004). Initiation of Ovsynch during the late luteal phase (day 15 to 17) may not result in ovulation and formation of a CL (El-Zarkouny et al., 2004). As well, a second problematic stage occurs early during the first 2 to 3 d of the estrous cycle, when the new potentially dominant follicles are too small to ovulate in response to GnRH treatment, resulting in aged follicles at the time of the second GnRH treatment, producing less fertile oocytes (El-Zarkouny et al., 2004; Navanukraw et al, 2004). Such challenges have resulted in the pre-synchronization of estrus and ovulation in order to initiate the Ovsynch protocol at the optimum time. One pre-synchronization method, Presynch, involves two treatments of $PGF_{2\alpha}$, 14 d apart, with a 12 or 14 d interval before the start of the Ovsynch protocol (Moreira et al., 2001; Fricke et al., 2003). Presynch increases the probability that cows are in the early luteal phase at the initiation of Ovsynch, increasing the probability of ovulating the dominant follicle of the first follicular wave; thereby, improving synchrony of ovulation following the second GnRH treatment (Cartmill et al., 2001).

Embryonic mortality is a major cause for pregnancy loss and further reduces PR in dairy cows (Fricke et al., 2003). Various causal factors have been cited but the contribution of an inadequate CL to reduced P_4 production has received the most attention. The effect to increase endogenous P_4 production to enhance PR using luteotropic hormones has been inconsistent. The timing of GnRH injection is significant, and its effects depend on the follicular status at the time of treatment (Lewis et al., 1990). The administration of GnRH on day 5 or 7 has been shown to induce the formation of an additional CL, arising from the ovulation of the first-wave dominant follicle present at the time of treatment (Schmitt et al., 1996a; Howard et al., 2006). In a recent study Howard et al. (2006) observed increased serum P_4 but no differences in CR when GnRH was administered 5 d after AI. Moreover, practical implementation of hormonal protocols becomes important for farmer compliance, especially those producers who assign groups of cows to initiate protocols on a weekly basis (Fricke et al., 2003). As well as corresponding with the first-wave dominant follicle, a treatment of GnRH 6 d following Ovsynch TAI falls on the same day of the week as the final GnRH treatment in Ovsynch.

Although fertility in dairy heifers remains high, management and time spent on estrus detection is less when compared to lactating dairy cows (Tenhagen et al., 2005). Any delay of first service insemination will increase age at first calving as well as increase associated costs (Heinrichs, 1993). The use of Ovsynch protocol may help to overcome these issues. Previous work using Ovsynch protocol on heifers found unsatisfactory PR compared to using natural estrus (Pursley et al., 1997a). These results have been attributed to a high proportion of heifers expressing estrus before TAI (Pursley et al., 1997a; Rivera et al., 2005), resulting from an inconsistent response to the first

GnRH treatment of the Ovsynch protocol and differences in their follicular wave pattern compared to lactating dairy cows (Rivera et al., 2004). Pre-synchronization before the initiation of Ovsynch may help to overcome these premature ovulations in heifers. Moreover, heifers are also susceptible to embryonic loss and any improvement of PR, especially after Ovsynch treatment, will have economic benefits with reduced timed to first calving.

Clearly, it is important to determine which strategies are better at enhancing PR in lactating dairy cows and heifers before or following Ovsynch protocol. The main objective of this study was to determine the effect of pre-synchronization with two injections of $PGF_{2\alpha}$ or a post-insemination treatment with GnRH on PR to a timed breeding Ovsynch protocol in dairy cows and heifers. The other objectives were to determine: a) treatment effects on cycle status, ovulation synchronization response, presumptive PR, and embryonic losses based on P_4 concentrations, b) relationships between DIM and body condition of cows at breeding on PR among different treatments, and c) relationships between age and weight at breeding of heifers on PR among different treatments.

Materials and Methods

Animals

This study was conducted at the UBC Dairy Education and Research Centre, Agassiz, BC, from July 2006 to July 2007 and involved 225 multiparous and primiparous lactating Holstein cows, and 87 nulliparous heifers. Cows and heifers were housed in free stalls and provided total mixed rations twice daily to meet or exceed NRC requirements.

Cows were milked at 0500 and 1500 h. Cows were weighed and scored for body condition at 35, 65, and 95 d post-partum. Heifers were weighed before the experiment at day -38, at the time of breeding (day 0) and then 28 d post-TAI. All animals were weighed on 2 consecutive days at the same time each day and then an average was taken to determine body weight. Body condition score (BCS) was determined using a quarter point scale from one to five (where 1 = emaciated, 5 = fat [Ferguson et al., 1994]). All animals were handled in accordance with the guidelines of the Canadian Council on Animal Care (1993).

Treatments

Cows and heifers were randomly assigned to one of three treatments: Ovsynch protocol, Presynch + Ovsynch, or Ovsynch + Post-AI GnRH. Treatments were balanced for parity and post-partum diseases (retained placenta, metritis, ketosis, milk fever), similar DIM for cows and similar age for heifers. Cows that were enrolled in the experiment were between 28 and 68 DIM and heifers were between 13 and 15 mo of age. Breeding groups consisted of 6 to 12 animals per group. Ovsynch treatment consisted of two injections of GnRH (Fertiline®, Vetoquinol NA Inc., Lavaltrie, QC, Canada; 100 μg, i.m.) 9 d apart with an injection of $PGF_{2\alpha}$ (Lutalyse®, Pharmacia Animal Health, Orangville, ON, Canada; 25 mg i.m) 48 h before the second GnRH treatment. Timed AI was performed 16 to 18 h after the second GnRH treatment. Presynch treatment involved two injections of $PGF_{2\alpha}$ (Lutalyse®, 25 mg i.m.) 14 d apart followed by Ovsynch protocol 14 d after the second treatment of $PGF_{2\alpha}$. Post-AI treatment involved Ovsynch followed by a GnRH treatment (Fertiline®, 100 ug, i.m.) given 6 d after TAI. All animals

were subjected to ultrasound examination for pregnancy determination using a transrectal 7.5 MHz linear-array transducer (Aloka SD500, Japan) between 35 to 40 d after TAI for pregnancy determination. A detailed schedule of the treatment protocols is shown in Figure 3.1.

Blood and milk sampling

Progesterone concentrations in blood plasma from heifers and whole milk from cows were used to assess ovarian function among animals. Ten samples were collected from each animal on days -38, -31, -24, -10, -3, 0 (day of TAI), 7, 14, 21 and 28. Milk samples were collected directly from the teat by hand stripping before morning milking into a plastic vial containing a dissolvable milk fat preservative (BroTab10, Systems Plus Ltd., Ontario, CA). The blood was withdrawn via coccygeal vein into Vacutainer tubes (Becton, Dickinson and Company) containing sodium heparin. Plasma samples were immediately separated by centrifugation (100x *g*) for 20 min at room temperature. All samples (milk and plasma) were stored at about -20 ˚C until subsequent P_4, analysis by radioimmunoassay. Studies have demonstrated that when milk is obtained immediately prior to milking, milk P_4 concentrations are not different from corresponding blood plasma concentrations (Ginther et al., 1976; Hoagland and Barnes, 1984).

Concentrations of P_4 (ng/mL) in plasma and milk were determined using a commercially available solid-phase radioimmunoassay kit (Coat-A-Count Progesterone, Diagnostic Products, Los Angeles, CA). This method was previously validated in our laboratory for the measurement of P_4 in milk and blood (Rajamahendran et al., 2001). Briefly, samples or reference standards (0.1 mL) were added to individual tubes coated

with a P_4 specific antibody. Reference standards contained between 1 and 40 ng/mL P_4. Buffered I^{125}-labelled P_4 (1.0 mL) was added to all tubes, which were mixed on a vortex and incubated for 3 h at room temperature and then decanted. The tubes were counted for radioactivity in a gamma counter for 1 min. Coefficients of variation within (intra) and between (inter) assays were 7 and 9%, respectively. The sensitivity of the assay was 0.03 ng/mL.

Animals were considered anestrus at the start of Ovsynch if P_4 concentrations were ≤ 1 ng/mL on day -38, -31, -24, and -10. The proportion of animals cycling at the beginning of the experiment, on day -38 and -31 was based on elevated P_4 concentrations (> 1 ng/mL) in milk or blood (indicative of a functional CL). If the herd manager detected animals in heat between day -3 and 0, animals were bred. Those animals on Post-AI treatment still received GnRH 6 d after AI. Synchronization response to treatment in animals was based on animals with P_4 concentrations ≤ 1 ng/mL on the scheduled timed breeding date. Animals that were detected in estrus before the scheduled timed insemination were considered not synchronized. Presumptive PR for animals on day 21 and 28 was based on synchronized animals with P_4 concentrations ≤ 1 ng/mL on day 0, followed by an increase to > 1 ng/mL on days 7, 14, 21, and 28.

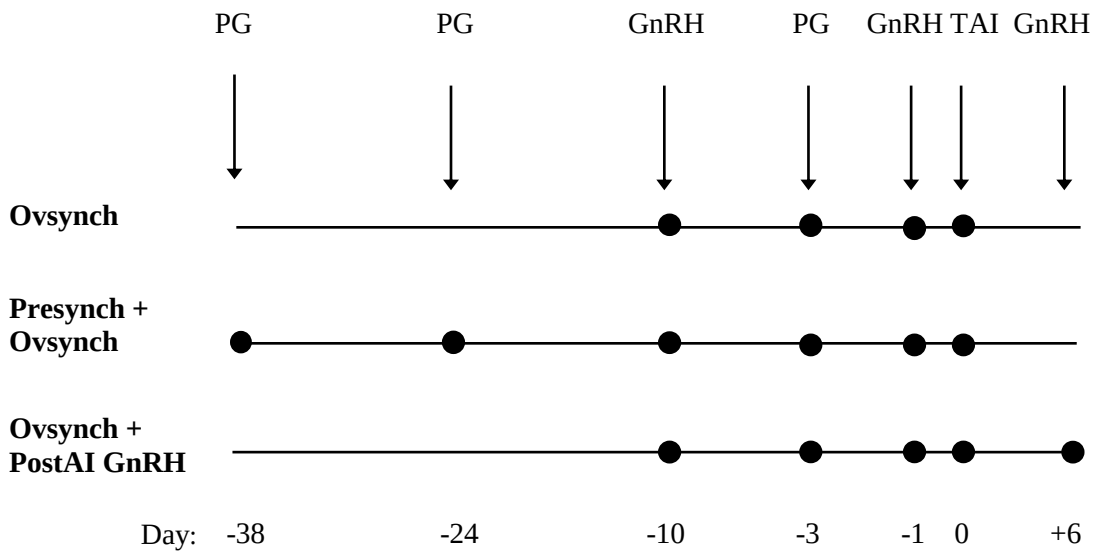

Figure 3.1 Schematic diagram of treatment schedules

Ovsynch treatment was initiated in all animals on day -10. Presynch + Ovsynch treated animals received their first $PGF_{2\alpha}$ treatment on day -38 and their second treatment on day -24. Timed artificial insemination (TAI) was performed on day 0. Ovsynch + Post-AI GnRH treated animals received GnRH 6 d after TAI. Cows were bred between 62 and 106 DIM. Heifers were bred between 13 to 16 mo of age.

Analysis of data

Pregnancy rate data were analyzed by Chi-square using an ordinal logistic procedure using JMP®. Covariates considered in the analysis of PR were treatment, parity, DIM and BCS at TAI. In heifers, body weight and age at TAI were also considered. In the model BCS, DIM, body weight, and parity were considered continuous variables. Pregnancy rates comparing parity and treatment were also analyzed by dividing cows into four groups (heifers, first lactation, second lactation, and ≥ third lactation). After it was determined that there were significant differences in PR of cows at different DIM and heifers at different ages and weight, a Chi-square analysis was also done with these variables as a categorical variables in response to PR. The percentage of cows with plasma P_4 concentrations ≤ 1 ng/mL or > 1 ng/mL was analyzed as categorical data (high/low) and were analyzed by Chi-square; this type of analysis was used for synchronization rates and determining the stage of estrous cycle. Mean plasma concentrations of P_4 at the various days collected were analyzed using a MIXED Model for repeated measures to compare treatments, synchronized, and pregnant to non-pregnant animals. Significant α level was set at $P \leq 0.05$, and $0.05 < P \leq 0.10$ were considered a tendency towards a difference.

Results

Pregnancy and synchronization rates

Overall PR for Ovsynch, Presynch + Ovsynch, and Ovsynch + Post-AI GnRH treatments were 49.0%, 51.0%, and 48.1%, respectively. There were no significant differences in PR among treatments in cows (42.5%, 48.0%, and 44.9%) or heifers

(65.5%, 58.6%, 58.6%) for Ovsynch, Presynch + Ovsynch, and Ovsynch + PostAI GnRH, respectively. There were also no differences in synchronization rates in cows (86.3%, 83.8%, 82.9%) or heifers (79.3%, 72.4%, 69.0%) for Ovsynch, Presynch + Ovsynch, and Ovsynch + PostAI GnRH treatments, respectively. As well, PR among treatments was not different in synchronized cows (47.6%, 56.5%, 51.6%) or heifers (73.9%, 66.7%, 60.0%) for Ovsynch, Presynch + Ovsynch, and Ovsynch + PostAI GnRH, respectively.

A difference in PR was found between heifers and cows (P = 0.008). Figure 3.2 presents synchronization rates and PR in cows and heifers, regardless of treatment. Synchronized heifers also had greater PR to TAI compared with synchronized cows (P = 0.03). Overall, cows had a greater synchronization response than heifers (P = 0.02). This was due to a higher percentage of heifers that expressed estrus before scheduled timed inseminations than cows (19.5% vs. 6.2%; P = 0.0004). There were 17 heifers and 13 cows that were detected in heat and bred before scheduled TAI. Pregnancy rate, excluding only these animals, was 61.4% and 46.0% for heifers and cows, respectively. There were no differences in PR following AI observed between primiparous and multiparous animals among treatments. Pregnancy rates according to treatment and parity are shown in Table 3.1.

For those animals that were synchronized on day 0, 25.4% did not have a P_4 value ≥1 ng/mL by day 7. For these animals, there were no differences on PR between cows (42.8%) and heifers (44.3%). However, animals that had P_4 concentrations ≥ 1 ng/mL on day 7 had a greater PR than those animals with < 1 ng/mL (56.7% vs. 43.9%; P = 0.04).

There was a larger proportion (P = 0.04) of heifers (83.7%) with P_4 concentrations ≥ 1 ng/mL on day 7 than cows (73.4%).

There were no differences in embryonic loss between treatments. On day 21 post-AI, there were no differences observed in presumptive PR between cows and heifers (P = 0.21); however, by day 28 heifers had greater PR than cows (P = 0.01; Table 3.2). This corresponds to an embryonic loss of 9.9% for cows and only 1.2% for heifers between day 21 and 28. Cows that were in their third parity or greater tended to have a greater embryonic loss than first or second parity cows between days 21 and 28 (15.4% vs. 5.1% and 7.6%, respectively; P = 0.06). Overall, embryonic loss increased as parity increased (P = 0.01) between days 21 to 28, but not between days 28 to 40 (P = 0.96). Embryonic loss from days 28 to 40 was 6.0% and 9.0% for heifers and cows, respectively.

Cycle status and progesterone concentrations

Table 3.2 presents cycling status based on P_4 concentrations in animals. There were no differences between treatments in the number of animals that were cycling at the start of the experiment (85%, 78.6%, and 82.5% for Ovsynch, Presynch + Ovsynch, and Ovsynch + Post-AI, respectively). There was a difference observed in the number of cows (77.3%) vs. heifers (94.1%) cycling at the start of the experiment (P < 0.001). There was a larger proportion of animals (P = 0.05) with P_4 concentrations below 1 ng/mL on day -31 in the Presynch + Ovsynch group (53.4%) compared with the Ovsynch (37%) or the Ovsynch + PostAI (41.9%) groups. Presynch animals had lower mean (± SEM) P_4 concentrations on day -31 than animals in the Ovsynch group (1.84 ± 0.27 vs. 2.98 ± 0.27 ng/mL; P = 0.003). Heifers in the Presynch + Ovsynch group had greater mean P_4

concentrations than the Ovsynch group on day -24 (4.45 ± 0.50 vs. 2.85 ± 0.50 ng/mL; *P* = 0.03), however no differences were observed for P_4 in cows on that day. On day -10, 4.1% of cows were considered to be anestrous. Induction of ovulation in the anestrous cows in response to the first GnRH treatment on day -10 in Ovsynch and Presynch + Ovsynch treatments was 33.3%, with no differences among treatments. In contrast, all heifers were cycling by day -24 (*P* = 0.05).

There was a greater percentage of animals observed in the luteal phase (P_4 concentrations >1 ng/mL, high) on day -10 in the Presynch + Ovsynch group (74.5%) compared with the Ovsynch (59.4%) and the Ovsynch + PostAI group (60.9%; *P* = 0.05) (Table 3.2), while no differences were observed in P_4 concentrations among treatments on day -3. Animals had lower mean P_4 concentrations in the Ovsynch group on day -10 (2.79 ± 0.33 vs. 3.77 ± 0.37 ng/mL; *P* = 0.04) and day -3 (3.28 ± 0.42 vs. 4.47 ± 0.40 ng/mL; *P* = 0.04) compared to animals in the Presynch + Ovsynch group. There was a greater percentage of heifers than cows with high P_4 concentrations for Ovsynch and Presynch + Ovsynch (*P* = 0.04) on day -10, but no differences observed on day -3. Overall, animals had greater PR when concentrations of P_4 were high on day -10 or day -3 (Table 3.3). Heifers had greater PR when P_4 concentrations were high on day -3 (*P* = 0.004). Cows had greater PR based on P_4 concentrations being high on day -10 (*P* = 0.01), but no differences were observed on day -3.

There were no differences observed between treatments for mean P_4 concentrations on days 0, 7, 14, 21, and 28 for synchronized animals. In pregnant animals, mean P_4 concentrations were greater in the Ovsynch + PostAI group compared to the Ovsynch group on day 21 for heifers (7.41 ± 0.95 vs. 4.60 ± 0.77 ng/mL; *P* = 0.03)

and day 28 for cows (9.92 ± 0.78 vs. 6.54 ± 0.76 ng/mL; $P = 0.02$). Pregnant animals had greater mean P_4 concentrations than non-pregnant animals on day 7 ($P = 0.004$), 21 ($P < 0.0001$), and 28 ($P < 0.0001$). Synchronized heifers had lower P_4 concentrations than synchronized cows on day 0 (0.10 ± 0.03 vs. 0.19 ± 0.02 ng/mL; $P = 0.003$) and on day 14 (4.56 ± 0.55 vs. 5.77 ± 0.32 ng/mL; $P = 0.05$). In pregnant animals, heifers had lower P_4 concentrations than cows on day 0 (0.06 ± 0.03 vs. 0.20 ± 0.02 ng/mL; $P = 0.0002$) and 28 (6.05 ± 1.10 vs. 8.96 ± 0.80 ng/mL; $P = 0.03$) and a tendency for lower P_4 concentrations than cows on day 14 (4.91 ± 0.55 vs. 6.11 ± 0.39 ng/mL; $P = 0.08$) and 21 (5.58 ± 0.66 vs. 6.94 ± 0.45 ng/mL; $P = 0.09$).

Relationship between stage of lactation and body condition score on pregnancy rate in lactating cows

There was a significant ($P = 0.01$) effect of the stage of lactation (DIM) at TAI on PR. The average DIM at the time of insemination was 76.7 ± 0.4, with similar distribution between treatment groups. Lactating cows that were > 76 d post-partum at TAI had greater PR than cows that were bred between 62 to 76 d post-partum (52.6% vs. 39.1%, respectively; $P = 0.03$). The stage of lactation at AI had the greatest effect ($P = 0.04$) on PR in the Ovsynch treated cows (Figure 3.3).

Overall, there was no significant affect of BCS on PR. However, cows in the Ovsynch group with poorer BCS at 95 d post-partum had lower PR ($P = 0.03$). Whereas there were no differences observed in the Presynch + Ovsynch and Ovsynch + PostAI ($P > 0.10$) groups.

Relationship between age and body weight and pregnancy rate in heifers

The average weight of heifers at the initiation of the experiment was 381 ± 2.8 kg. The average age of heifers at breeding was 445 ± 1.8 d. Age and weight had an influence on pregnancy outcome, regardless of treatment (P = 0.003). Heifers that had a body weight of < 380 kg at start of the experiment had poorer PR than those weighing more (47.4% vs. 70.8%, respectively; P = 0.02). Heifers that were bred when < 445 d of age (14.6 mo) were less likely to become pregnant than those that were older (48.9% vs. 75%, respectively, P = 0.01) (Figure 3.4).

Discussion

In this study two different strategies to improve ovulation and PR before and following an Ovsynch TAI program for dairy cattle were examined. There were no differences observed in PR among Ovsynch, Presynch + Ovsynch, and Ovsynch + PostAI GnRH treatments among lactating cows or heifers. Most studies have reported PR by TAI using Ovsynch to be between 30 to 40% in lactating cows, which is in the range (42.5%) of what was observed in this study (Pursley et al., 1997a,b; Lucy, 2001).

Presynch protocol has resulted in increased PR, some as high as 10 to 12%, following TAI compared with Ovsynch alone (Cartmill et al., 2001; Fricke et al., 2003; Navanukraw et al., 2004). The incorporation of an extended interval of 14 d from 12 d, between Presynch and Ovsynch still has beneficial results and allows the injection of $PGF_{2\alpha}$ to fall on the same day during alternate weeks, which is important for the compliance of dairy producers (Fricke et al., 2003; Navanukraw et al., 2004). Our study observed similar PR in our Presynch + Ovsynch group to other studies; El-Zarkouny et

al. (2004) observed increased PR with Presynch (12 d interval) from 37.5% to 46.8% and Navanukraw et al. (2004) reported increased PR with Presynch (14 d interval) from 37.3% to 49.6%.

Presynch animals had significantly lower mean P_4 concentrations on day -31 and a greater proportion of animals with P_4 values below 1 ng/mL than animals in the Ovsynch group, indicating a response to the first $PGF_{2\alpha}$ treatment (Navanukraw et al., 2004). As previous studies have indicated, more cows should be expected to be between day 5 to 12 of the estrous cycle at the initiation of the Ovsynch protocol (El-Zarkouny et al., 2004) in response to pre-synchronization treatments with $PGF_{2\alpha}$. Although there was a greater proportion of animals in the Presynch + Ovsynch group that were observed in the luteal phase at the initiation of Ovsynch and had higher mean P_4 concentrations on day -10 and day -3 compared with animals in the Ovsynch group, this did not translate into increased synchronization rates (El-Zarkouny et al., 2004). Other work examining the use of Presynch has found increased synchronization rates (Navanukraw et al., 2004). Moreover, greater concentrations of serum P_4 during the period before $PGF_{2\alpha}$ treatment has been shown to improve fertility of lactating dairy cows that were subsequently inseminated (Table 3.3) (Navanukraw et al., 2004). Although, this study and others (Navanukraw et al., 2004) demonstrate that high P_4 concentrations before insemination can have beneficial effects on PR by providing a more favourable environment for the development of the ovulatory follicle, high P_4 concentrations may also be detrimental for animals treated with Ovsynch TAI. When P_4 is elevated, it acts centrally to inhibit both the tonic and surge modes of GnRH release and subsequent ovulation (Robinson et al., 2000). However, the preovulatory LH surge induced by a GnRH treatment lasts only for

4 to 6 h, which is substantially shorter than a naturally occurring LH surge (Rahe et al., 1980). This may have an affect on the activation of genes controlling P_4 production, CL function, and embryo development (Stocco et al., 2007).

A review of studies on the treatment of cows and heifers with GnRH at times ranging from 6 h before to 6 d after AI, found that overall, first service PR in GnRH treated cattle were about 5% points greater than those in control cattle (Lewis et al., 1990); This is similar to what was observed in this study. Researchers reported increased PR in dairy cows following GnRH treatment 7 d (Ambrose et al., 2000) and 5 or 11 d (Willard et al., 2003) after Ovsynch TAI, while more recently Howard et al. (2006) observed no increase in pregnancy. Schmitt et al. (1996a) examined treatments of GnRH agonist or hCG given 5 d after AI found 91% of the cows and heifers treated with hCG and 93% of the cows and heifers treated with GnRH agonist formed an accessory CL, after induced estrus. Another study indicated that administration of a synthetic GnRH on day 6 of the estrous cycle could induce formation of an accessory CL in 75% of heifers (Rusbridge et al., 1992). In pregnant animals, mean P_4 concentrations were significantly greater in the Ovsynch + Post-AI group compared to the Ovsynch group on day 21 for heifers and day 28 for cows, indicating a response from the result of an accessory CL. Other authors have observed increases in P_4 from post-AI treatments of GnRH (Schmitt et al., 1996a; Willard et al., 2003; Howard et al., 2006).

Even though cows had greater synchronization rates, heifers still had higher PR, demonstrating greater fertility in heifers. Rivera et al. (2004, 2005) reported a high proportion (19 to 24%) of heifers that come into estrus before the day of TAI in an Ovsynch protocol. Although our study found a similar proportion of heifers that came

into estrus before scheduled timed breeding as in other studies, our PR were far superior compared to these studies and others (Pursley et al., 1997a) and are similar to those achieved with breeding at natural estrus (Pursley et al., 1997a). Both Pursley et al. (1997a) and Rivera et al. (2004) observed PR that were 20 to 40% less in heifers bred to Ovsynch TAI than heifers that received AI to a natural estrus (Pursley et al., 1997a; Rivera et al., 2004). The ability of the CL to secrete P_4 is important for the establishment and maintenance of pregnancy, and embryo growth (Schmitt et al., 1996a). Delayed CL formation is associated with a marked and progressive reduction in PR in cattle, perhaps due to asynchrony between the uterus and the embryo (Schmitt et al., 1996; Changes e Silva et al., 2002). In this study, 25.4% of animals that were synchronized for TAI did not have a P_4 concentration ≥ 1 ng/mL by day 7 and as a result, had lower PR. Moreover, heifers had a larger proportion with P_4 concentrations ≥ 1 ng/mL than cows on day 7, which may contribute to better reproductive performance. Interestingly, synchronized heifers had lower P_4 concentrations than synchronized cows on day 14 and pregnant heifers had lower P_4 concentrations than cows, on day 14, 21, and 28. Lactating cows have a higher rate of metabolism and as a result have a faster clearance rate of P_4 (Starbuck et al., 2004). It was expected that cows would have lower P_4 concentrations due to faster clearance rate associated with lactation. It is possible that local (uterine tissue and lumen) concentrations of P_4 are more relevant for embryo survival than peripheral concentrations (Balendran et al., 2008).

There were no differences in PR to TAI between primiparous and multiparous cows in this study. Some studies have reported that first lactation cows have greater PR than second or greater lactation cows after synchronization following Ovsynch

(Tenhagen et al., 2004b; Stevenson and Phatak, 2005). Others have reported greater PR in multiparous cows (Jobst et al., 2000). Cartmill et al. (2001) also reported greater fertility due to pre-synchronization in multiparous but not primiparous cows, however this was not observed in the present study. Multiparous cows have reduced fertility compared to primiparous cows; studies have shown that as parity increases, PR decreases (Stevenson and Phatak, 2005; Balendran et al., 2008). Possible reasons for better fertility in primiparous cows include a reduced risk of metabolic disorders in early lactation (Gröhn and Rajala-Schultz, 2000) and fewer reproductive problems (Huszenicza et al., 1987). Meanwhile, it has also been reported that primiparous cows have a later onset of cyclicity (Huszenicza et al., 1987). The reason for the variation between primiparous cows and older cows observed across studies remains speculative and could be associated with differences in management of primiparous cows compared to multiparous cows and differences between farms (Tenhagen et al., 2004). In this study, pregnancy loss significantly increased between days 21 to 28 as parity increased, but was unaffected between days 28 to 40. Presumptive PR at day 28 was greater in heifers than cows, corresponding to a greater pregnancy loss in cows (9.9% vs. 1.2%). In general, most losses occur before day 19 post-insemination, during fertilization, CL formation, or maternal recognition (Changes e Silva et al., 2002). However, it is difficult to distinguish between fertilization failure and early embryonic loss. The pregnancy loss between days 21 and 28 could be associated with embryonic loss and/or an extended estrous cycle. Recent literature reported estimates of pregnancy loss between weeks 5 to 7 at 7.1% (Starbuck et al., 2004), and 11% (Navanukraw et al., 2004). The larger proportion of heifers with a functional CL on day 7 after inseminations and the lower embryonic loss

both contribute to better reproductive performance and may be reasons why heifers had greater PR than cows, even though they had lower synchronization rates. Recently, our lab also observed higher P_4 concentrations on days 4 and 7 post-insemination in heifers compared with lactating dairy cows, indicating better functional CL; these heifers also produced a higher yield of better quality embryos than 2nd and 3rd parity lactating cows (unpublished data).

This study and others have shown that cows receiving TAI early post-partum (< 75 or 76 DIM) had lower PR than cows receiving TAI later during lactation (Pursley et al., 1997a; Stevenson and Phatak et al., 2005). Interestingly, the only significance in pregnancy outcome in relation to DIM was with the Ovsynch group. As well, there was a trend for cows with poorer BCS in the Ovsynch group to also have poorer PR. Cows that are earlier in lactation are likely to be closer to peak lactation period, at a time when they are in NEB. Negative energy balance during the early post-partum period results in a reduction of post-partum LH pulses and a delay in resumption of ovarian activity (Lucy, 2001; Butler, 2003). This supports that the Presynch + Ovsynch or Ovsynch + PostAI GnRH treatments may benefit those cows that are in greater NEB or peak lactation as there were no significant differences observed between cows bred > 75 DIM or < 75 DIM in this treatment group. In lactating dairy cows, resumption of ovarian activity plays an important role in subsequent fertility (Lucy, 2001). Negative energy balance is associated with changes in LH and IGF, which in turn cause inhibitory effects on ovarian follicular growth and development, resulting in the failure of a dominant follicle to ovulate (Lucy, 2001). A dominant follicle in cows in NEB requires more time and a larger size to establish blood estradiol concentrations capable of triggering ovulation (Butler, 2003).

Energy balance within the cows was not specifically examined in this study, but this aspect would be interesting to research further.

Although it is thought to be important to reduce the age to first calving in order to have cows begin producing milk earlier in life, results from this study demonstrate poorer reproductive performance in heifers bred less than 14.6 mo of age or weighing less than 380 kg. Body weight and age are important factors in the reproductive maturity of heifers and can result in increased calving difficulties, decreased milk production, and increased days open in first lactation (Heinrichs, 1993). Moreover, the poor PR observed in the other studies (Pursley et al., 1997a) using Ovsynch in heifers might have been a result of the age or weight of the animals that were being used in those experiments.

With considerations of costs between treatments, the Ovsynch treatment alone would cost just over $10.00 each; whereas the Presynch + Ovsynch treatment and the Ovsynch + Post-AI treatment costs approximately $20.00 and $13.00 each, respectively. While the two injections required for the Presynch treatment and the one post-insemination treatment have been scheduled so that they occur on the same day of the week as the Ovsynch injections, the additional treatments still require additional handling of cows, increasing labour and possibly reducing farmer compliance. However, the significant increase in PR in those cows bred when in earlier DIM can easily offset these costs. Stevenson (2001) estimated that the value of a new pregnancy was between $253 and $274 in programmed AI breeding protocols.

In summary, PR between the three treatments were not significantly different in cows or heifers. While synchronization rates were greater in lactating cows, heifers had higher PR, likely due to better functional CLs at day 7 post-insemination and lower

embryonic losses. Presynch or PostAI GnRH treatment with Ovsynch have beneficial results on those cows that are bred earlier in lactation (≤ 76 DIM). Moreover, this study demonstrates that Ovsynch can be an advantageous reproductive management tool that can be used on dairy heifers that have reached optimal breeding age. Although Ovsynch is a useful management tool, there is still room for improvement with perhaps a more effective hormone than GnRH or with nutritional changes for cows during the use of Ovsynch treatment.

Table 3.1 Effect of treatment on pregnancy rate in heifers and lactating dairy cows

Treatments				
Parity	Ovsynch	Presynch + Ovsynch	Ovsynch + PostAI GnRH	*P value*
Heifers	62.5 (29)	58.6 (29)	58.6 (29)	0.82
1	44.8 (29)	41.7 (24)	42.3 (26)	0.97
2	33.3 (15)	45.5 (22)	47.1 (7)	0.69
≥ 3	44.8 (29)	55.0 (29)	44.1 (34)	0.63
P value	0.16	0.57	0.60	

Values represent the percent of animals pregnant. The values in parenthesis represent n.

Table 3.2 Cycle status of lactating dairy cows and heifers based on P_4 concentrations

Parameter	Treatment					
	Ovsynch		Presynch + Ovsynch		Ovsynch + PostAI GnRH	
	Cows	Heifers	Cows	Heifers	Cows	Heifers
	n = 71	n = 29	n = 74	n = 29	n = 76	n = 29
Percent cycling at the start of the experiment [+]	81.7% [a]	93.1% [b]	73.0% [a]	93.1% [b]	77.6% [a]	96.3% [b]
Percent in luteal phase at initiation of Ovsynch treatment [§]	55.6% [a]	69% [b]	69.9% [b]	86.2% [c]	61.8% [a]	58.6% [a]
Percent synchronized at TAI following Ovsynch treatment [#]	94% [a]	92% [a]	89.9% [a]	91.3% [a]	87.3% [a]	91.4% [a]
	n = 67	n = 24	n = 69	n = 22	n = 71	n = 21
Percent presumed pregnant at 21 d post TAI [†]	65.7% [a]	75% [a]	66.7% [a]	72.4% [a]	63.4% [a]	71.4% [a]
Percent presumed pregnant at 28 d post TAI [†]	56.7% [a]	75% [b]	59.4% [a]	71.4% [b]	52.1% [a]	66.7% [b]
Percent confirmed pregnant on 40 d post TAI [*]	47.6% [a]	73.9% [b]	56.5% [a]	66.7% [b]	51.6% [a]	60% [b]

[+] Animals were considered cycling if P_4 concentrations were > 1 ng/mL on days -38 and -31.

[§] Animals were considered in luteal phase if P_4 concentrations were > 1 ng/mL.

[#] Animals were considered synchronized at TAI if P_4 concentrations were ≤ 1 ng/mL.

[†] Animals were considered pregnant on days 21 day 28 if P_4 concentrations were ≤ 1 ng/mL day 0 (TAI) and were > 1 ng/mL on days 7, 14, 21, and 28.

[*] Diagnosed pregnant via ultrasonography.

Superscripts with different letters (a, b, or c) indicate significant differences between columns ($P < 0.05$).

Table 3.3 Pregnancy rate according to progesterone concentrations during Ovsynch treatment

		First GnRH treatment (day -10)			$PGF_{2\alpha}$ treatment (day -3)		
		>1 ng/mL of P_4	≤1 ng/mL of P_4	*P value*	>1 ng/mL of P_4	≤1 ng/mL of P_4	*P value*
Cows	Proportion of cows	62.8% (91/145)	37.2% (54/145)		70.3% (97/138)	29.7% (41/138)	
	Pregnancy rate	52.6% (48/91)	31.5% (17/54)	0.01	46.4% (45/97)	43.9% (18/41)	0.79
Heifers	Proportion of heifers	77.6% (45/58)	22.4% (13/58)		79.2% (42/53)	20.8% (11/53)	
	Pregnancy rate	55.6% (25/45)	84.6% (11/13)	0.05	73.8% (31/42)	27.3% (3/11)	0.004

Data presented is based on Ovsynch and Presynch + Ovsynch groups only. Ovsynch consisted of administration of GnRH on days -10 and -1 and $PGF_{2\alpha}$ on day -3. Animals were diagnosed pregnant via ultrasonography.Values in brackets are n.

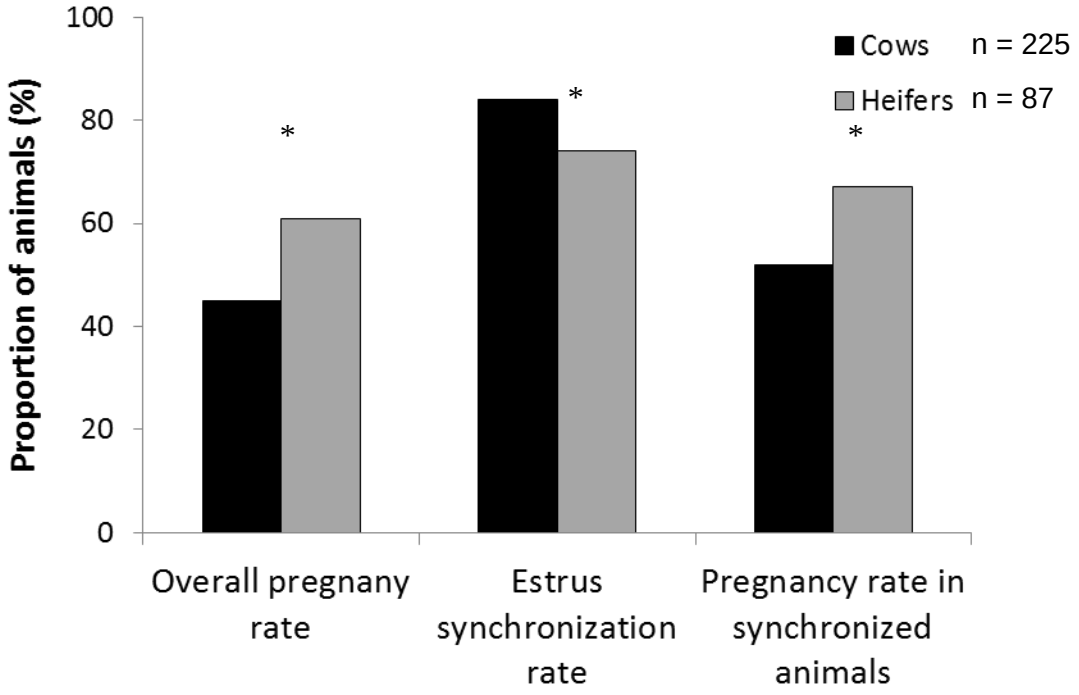

Figure 3.2 Synchronization and pregnancy rates

Overall pregnancy rate, estrus synchronization rate, and pregnancy rate in synchronized heifers and lactating dairy cows subjected to timed AI. The synchronization rate to treatment was based on progesterone concentrations of < 1 ng/mL on the day of TAI and the percentage of animals pregnant based on pregnancy diagnosis via ultrasonography on day 40 post-TAI. * Significance of $P < 0.03$.

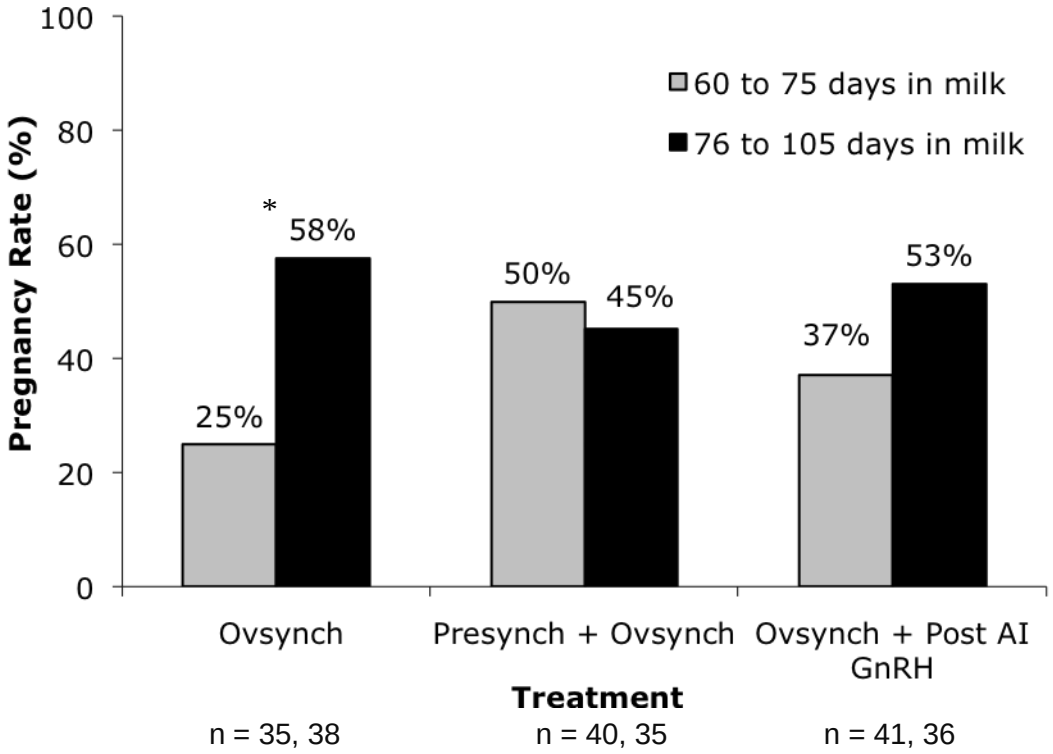

Figure 3.3 Effect of days in milk on pregnancy rates in lactating dairy cows

Lactating dairy cows bred to Ovsynch TAI when less than 76 days in milk had lower pregnancy rates than those cows that were bred when greater than 76 days in milk. There was no difference in pregnancy rate in lactating dairy cows bred during Presynch + Ovsynch or Ovsynch + Post AI GnRH protocols. * Significance of $P = 0.0007$.

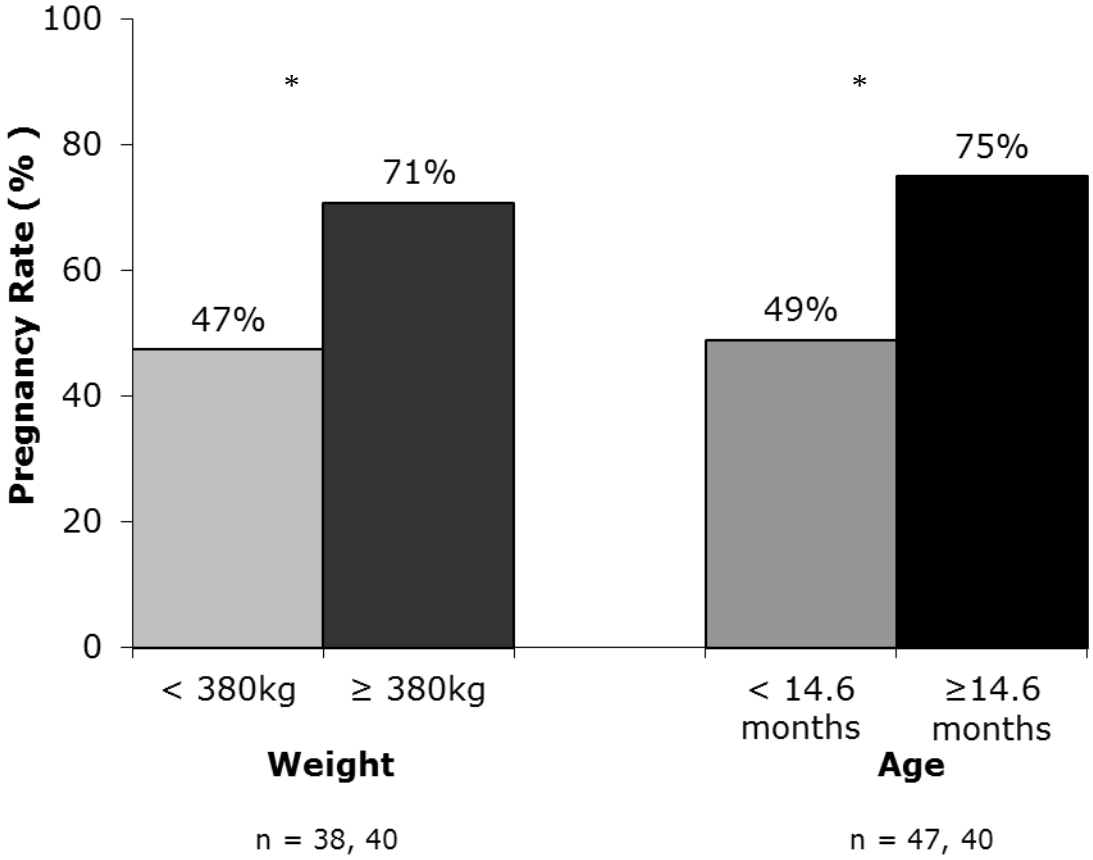

Figure 3.4 Effect of age and weight on pregnancy rates in dairy heifers

Regardless of treatment, heifers that were bred at timed AI when weighing less than 380 kg or when less than 14.6 mo of age had lower pregnancy rate than those heifers that were bred when weighing 380 kg or more or that were 14.6 mo of age or older. * Significance of $P < 0.02$.

CHAPTER 4: OVULATORY RESPONSE, SYNCHRONIZATION RATES, PREGNANCY RATES, AND P_4 CONCENTRATIONS TO TIMED ARTIFICIAL INSEMINATION IN LACTATING DAIRY COWS TREATED WITH GnRH, pLH, OR hCG

Introduction

Ovulation synchronization and TAI programs have been developed in response to challenges faced with estrus detection and poor fertility in lactating dairy cows. One such program, Ovsynch, which consists of two treatments of GnRH given 7 d before and 48 h after treatment with $PGF_{2\alpha}$, allows for the control of ovarian follicular and CL development and TAI, eliminating the need for estrus detection (Pursley et al., 1997a). Although cows bred to Ovsynch TAI have similar PR to cows bred at natural estrus (Pursley et al., 1997a, 1997b; Rabiee et al., 2005), PR are still far from satisfactory (Starbuck et al., 2004).

Initiating Ovsynch treatment at particular stages of the estrous cycle has been associated with reduced PR (El-Zarkouny et al., 2004). Initiating Ovsynch during the late luteal phase (days 15 to 17 of the estrous cycle) may result in premature estrus due to spontaneous CL regression, leading to asynchrony; while initiating treatment during the first 2 to 3 d of the cycle, when the new potential dominant follicles are too small to ovulate in response to GnRH, results in aged, less fertile oocytes being ovulated at the second GnRH treatment (El-Zarkouny et al., 2004; Navanukraw et al., 2004). Researchers have aimed to increase PR to Ovsynch using pre-synchronization treatments with $PGF_{2\alpha}$ in order to increase the probability that cows are in the early diestrus at the initiation of Ovsynch and thereby improve synchrony of ovulation following the second GnRH treatment (Cartmill et al., 2001; El-Zarkouny et al., 2004; Navanukraw et al.,

2004; Gordon et al., 2010). Moreover, it has been reported that pregnancy losses occur at a higher rate after TAI using a GnRH-based Ovsynch protocol, than after insemination at detected estrus (Vasconcelos et al., 1999; Lucy, 2001; Ambrose et al., 2006b). Another strategy used to increase PR uses human chorionic gonadotropin (hCG) (Lewis et al., 1990; Rajamahendran and Sianangama, 1992; Schmitt et al., 1996a) or GnRH (Lewis et al., 1990; Schmitt et al., 1996a; Howard et al., 2006; Gordon et al., 2010) post-insemination to induce the formation of an accessory CL, in order to increase endogenous P_4 and reduce early embryonic loss. However, both pre-synchronization and post-insemination treatments have had varied or minimal success on increasing PR (Lewis et al., 1990; Rabiee et al., 2005; Howard et al., 2006; Gordon et al., 2010).

As demonstrated by Navanukraw et al. (2004) and Gordon et al. (2010), animals in a luteal phase during the period before $PGF_{2\alpha}$ treatment in an Ovsynch TAI program show improved fertility. However, treatment with exogenous GnRH may be less effective to induce ovulations when there are high P_4 concentrations. When P_4 is elevated, it acts centrally to inhibit both the tonic and surge modes of GnRH release, thus suppressing LH release, expression of estrus and subsequent ovulation (Robinson et al., 2000). Colazo et al. (2008) observed that elevated plasma P_4 concentrations are associated with reduced pituitary release of LH and reduced ovulatory response in GnRH-treated cattle. Treatment with exogenous LH is not affected by endogenous P_4 concentrations, as it acts directly on the ovary (Martinez et al., 1999). It has been demonstrated in cattle and buffalo that on day 9 of the estrous cycle, when plasma P_4 concentrations are higher, pLH is more effective than GnRH at inducing ovulation (de Araujo Berber et al., 2002). Moreover, the preovulatory LH surge induced by an exogenous GnRH treatment lasts for 4 to 6 h,

which is substantially shorter than a spontaneously-occurring LH surge, which can last up to 10 h (Chenault et al., 1975; Rahe et al., 1980; Colazo et al., 2008). Ree et al. (2009) observed treatment with pLH had a similar duration of blood elevation to that of a naturally occurring LH surge. Ambrose et al. (2005) reported that dairy heifers induced to ovulate with pLH had higher P_4 concentrations 9 d after pLH treatment than those treated with GnRH. Therefore, using pLH in lieu of GnRH, may likely increase ovulation response to the first treatment, synchronization to treatment, and post-insemination P_4 concentrations, thereby increasing PR.

Human chorionic gonadotropin (hCG) also targets gonadal cells directly, has a longer half-life than an exogenous GnRH-induced LH surge, and has a luteotropic effect resulting in increased P_4 concentrations in the luteal phase (Schmitt et al., 1996a; De Rensis, 1999). The biological half-life of hCG is 3 to 4 days vs. hours for endogenous LH, thereby exerting a longer stimulatory and luteotropic influence on the developing CL (Schmitt et al., 1996a). Studies have shown that the ovulation of the first-wave dominant follicle can be induced with an injection of hCG and that the resulting CL had better capability to secrete P_4 compared with the CL induced by GnRH (Rajamahendran and Sianangama, 1992; Schmitt et al., 1996a; Sianangama and Rajamahendran, 1996). Human chorionic gonadotropin has also been shown in buffalo to increase plasma P_4 concentrations (Carvalho et al., 2007). As well, hCG has been shown to be more effective at inducing ovulation than GnRH in dairy cattle (Stevenson et al., 2007). Thus, we hypothesized that hCG would induce more ovulations than GnRH or pLH, thereby increasing synchronization response and P_4 concentrations post-insemination, contributing to improved PR.

Therefore, the objectives of this study were to compare the effects of 100 μg GnRH vs. 1,000 IU hCG, or 25 mg pLH on ovulatory response, synchronization response, P_4 concentrations, PR to TAI, and preovulatory follicle (POF) size on PR and pregnancy losses in lactating dairy cows.

Materials and Methods

Animals

This study was conducted at the UBC Dairy Education and Research Centre, Agassiz, BC, from July 2007 to July 2008. Lactating Holstein dairy cows (n = 167) were assigned randomly to one of three treatments blocked by parity, lactation stage, and number of services (only first, second, and third services were used). Cows that were enrolled in the experiment for first service breeding were between 55 to 75 DIM. Cows were housed in free-stalls and provided a TMR twice daily and milked at 0500 and 1530 h. Rations were formulated for lactating dairy cows according to NRC (2001) guidelines for lactating dairy cows. Main ingredients were corn and grass silage, grain (barley or corn), hay (alfalfa or grass) and mineral and vitamin supplements. Cows were weighed and scored for body condition at the start of treatments. Animals were weighed on 2 consecutive days at the same time each day and then an average was taken to determine body weight. Body condition was determined using a quarter point scale from 1 to 5 (where 1 = emaciated, 5 = fat [Ferguson et al. 1994]) by the same observer. All animals were handled in accordance with Canadian Council on Animal Care (1993).

Treatments

The control group (Ovsynch; n = 57) received 100 μg GnRH (day -10; Fertiline®, Vetoquinol NA Inc., Lavaltrie, QC, Canada; i.m.) followed by $PGF_{2\alpha}$ (day -3; Estrumate®, Schering-Plough Animal Health, Pointe-Claire, QC, Canada; i.m.) and the second 100 μg GnRH treatment followed 48 h later. The other two groups received either 25 mg pLH (Lutropin®-V, Bioniche Animal Health, Bellville, ON, Canada; n = 57; i.m.) or 1,000 IU hCG (Chorulon®; Intervet Canada Ltd., Whitby, ON, Canada; n = 53; i.m.) in lieu of the GnRH treatments (Figure 4.1). All cows were inseminated 14 to 16 h following second GnRH, pLH or hCG treatment.

Milk sampling and hormone assays

Milk samples were collected from 132 cows, 35 cows were missed or did not have full sets of milk samples. Nine milk samples were collected from each cow on days -10, -3, 0 (TAI), 7, 11, 14, 21, 25 and 28. Milk samples were collected directly from the teat by hand stripping before morning milking into a plastic vial containing a dissolvable milk fat preservative (BroTab10, Systems Plus Ltd., Ontario, CA). All samples were then stored at about -20 ˚C until subsequent P_4 analysis using a commercially available solid-phase radioimmunoassay kit (Coat-A-Count Progesterone, Diagnostic Products, Los Angeles, CA). This method was previously validated in our laboratory for the measurement of P_4 in milk (Rajamahendran et al., 2001). Briefly, sample or reference standard was added to tubes coated with a P_4 specific antibody. Buffered I^{125}-labelled P_4 was added to all tubes, which were shaken on a vortex, incubated for 3 h at room temperature and then decanted. The tubes were counted for radioactivity in a gamma

counter for 1 min each. Inter-assay and intra-assay coefficients of variation were 9 and 7%, respectively, and sensitivity of the assay was 0.03 ng/mL.

Uterine and ovarian examination

Ultrasonograpghy was only conducted on the first 112 cows that entered the experiment. Animals (n = 38, 37, 37 for GnRH, hCG, and pLH, respectively) were subjected to transrectal ultrasound examination using a scanner equipped with a 7.5-MHz linear-array transducer (Aloka-SD500, Aloka Co., Tokyo, Japan) for the presence of follicular or luteal tissues on the ovaries on days -10, -3, and -1. Ultrasonography on day -10 determined the proportion of cows with CL or follicles, and on day -3 determined the ovulatory response to first pLH, hCG, or GnRH treatment. Ultrasonography on day -1 was also used to determine response to $PGF_{2\alpha}$ treatment and the diameter of the POF. Synchronization response to treatment was based on animals with P_4 concentrations ≤ 1 ng/mL on the scheduled TAI date. Pregnancy was determined by ultrasonography at 40 d post-TAI. Presumptive pregnancy on day 28 was based on synchronized animals with P_4 concentrations ≤ 1 ng/mL on day of TAI proceeding with an increase to > 1 ng/mL which remained consistently elevated on days 7, 11, 14, 21, 25, and 28.

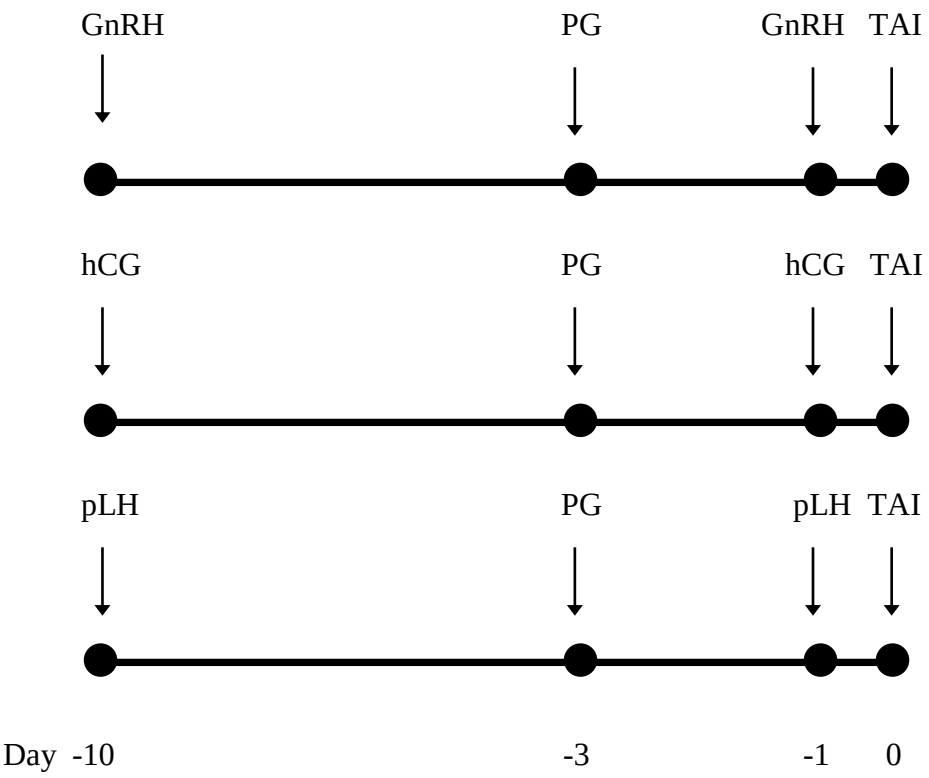

Figure 4.1 Schematic diagram of treatment schedules

For treatment groups GnRH (100 μg), hCG (1,000 IU), and pLH (25 mg) timed artificial insemination (TAI) was performed 14 to16 h after the second GnRH, hCG, or pLH injection.

Analysis of data

Pregnancy data were analyzed by Chi-square using an ordinal logistic procedure using JMP®. Covariates considered in the analysis were treatment, parity, DIM, and BCS at TAI, and the number of services. Body condition score, DIM, and parity were considered continuous variables. Pregnancy rates per TAI comparing parity and treatment were also analyzed by dividing cows into two groups (first lactation and second or greater lactation). The percentage of cows with ≤ 1 ng/mL of plasma P_4 or > 1 ng/mL plasma P_4 or < 4 ng/mL and ≥ 4ng/mL (on day -10) were treated as categorical data and were analyzed by Chi-square test; this was used for synchronization response to treatment and determining the stage of estrous cycle for ovulatory response. Mean plasma concentrations of P_4 were analyzed using a Least Squares ANOVA for repeated measures to compare treatments, synchronized, and pregnant to non-pregnant animals. Significant α level was set at $P \leq 0.05$, and P values > 0.05 but ≤ 0.10 were considered a tendency towards difference.

Results

Ovulation and synchronization response

No differences were observed among treatments in ovulation response to the first hormonal treatment given on day -10 (Figure 4.2; $P = 0.56$). As well, ovulatory response to first treatment did not affect PR to TAI (25.4% and 18.9%, for cows that ovulated vs. those that did not ovulate, respectively; $P = 0.40$). The distribution of cows that had high P_4 concentrations (≥ 1 ng/mL) on day -10 did not differ between treatments (Table 4.1). Ovulatory response to first treatment on day -10, tended to be lower in cows with P_4

concentrations ≥ 1 ng/mL, vs. animals with P_4 concentrations < 1 ng/mL (46.9% and 62.5%, respectively; *P* = 0.08). However, cows that had P_4 concentrations ≥ 4 ng/mL in the GnRH group had lower ovulatory response on day -10 compared with cows with P_4 concentrations < 4 ng/mL; whereas the ovulatory response on day -10 based on P_4 concentrations ≥ 4 ng/mL did not differ in the hCG and pLH groups (Table 4.1).

There were no differences in synchronization response among treatments (Figure 4.2; *P* = 0.42). Synchronization response did not increase due to ovulatory response from treatment on day -10 (84% and 77.1%, for animals that did not ovulate vs. those that did ovulate, respectively; *P* = 0.38).

Mean diameter of POF on day -1 was not affected by either treatment administered on day -10 (17.3 ± 1.1 mm, 18.0 ± 1.1 mm, and 16.7 ± 1.2 mm for GnRH, hCG, and pLH treatments, respectively) nor ovulatory response to the treatment on day -10 (17.2 ± 0.87 mm vs. 17.6 ± 0.97 mm for cows that ovulated and did not ovulate, respectively).

Pregnancy results

Means (± SEM) for parity, DIM, and BCS were 2.53 ± 0.12, 96.3 ± 3.72, and 2.98 ± 0.03, respectively. Days in milk and BCS did not affect pregnancy. Overall PR did not differ (*P* = 0.74) among treatment groups (Figure 4.2), except that there was a trend for primiparous cows to have greater PR than multiparous cows (37.8% vs. 26.1%, respectively; *P* = 0.10).

Progesterone concentrations and pregnancy losses

There were no differences in P_4 concentrations on days -10 (P = 0.31), -3 (P = 0.53), 0 (P = 0.23), 7 (P = 0.25), 11 (P = 0.28), or 14 (P = 0.89) for the GnRH, hCG, and pLH groups, respectively (Figure 4.3).

In pregnant cows, there was a tendancy towards a difference in P_4 concentrations between groups on day 7 (2.48 ± 1.20, 7.29 ± 1.51, 4.54 ± 1.63 ng/mL; P = 0.06), and differences on days 11 (2.91 ± 0.59, 6.37 ± 0.74, 5.94 ± 0.80 ng/mL; P = 0.002), and 14 (4.26 ± 0.86, 8.06 ± 1.08, 5.45 ± 1.16 ng/mL; P = 0.04) for the GnRH, hCG, and pLH groups, respectively (Figure 4.4). Based on P_4 concentrations, presumptive embryonic losses between day 28 and 40 post-TAI were 16.7%, 8.3%, and 9.3% for cows treated with GnRH, hCG, and pLH, respectively (P = 0.41).

Discussion

When starting an Ovsynch protocol, the first administration of GnRH is given at a random stage of the estrous cycle, generally causing either luteinization or ovulation of the largest follicle in approximately 85% of cows (Thatcher et al., 1989; Pursley et al., 1995, Pursley et al., 1997a). Our hypothesis that replacing GnRH in an Ovsynch program with hCG or pLH would increase the proportion of cows ovulating in response to the first treatment of the synchronization protocol was not supported. We observed similar ovulatory response to GnRH as other studies (Pursley et al., 1995; Ambrose et al., 2005). Ree et al. (2009) also found no differences in ovulatory response in non-lactating cows given pLH in lieu of GnRH in an Ovsynch program, whereas another study in lactating cows observed an 18% increase in ovulatory response of animals given pLH (Colazo et

al., 2009b). Other researchers have reported high ovulatory response of nearly 80% in heifers given pLH (Martinez et al., 1999) and 78% (Schmitt et al, 1996a) to 93% (Stevenson et al., 2007) in cows given hCG, however, these treatments were given at precise stages of the first follicular wave (days 5 to 7), unlike in the present study, in which randomly cycling cows were used.

Since pLH and hCG act directly on the ovary and are not affected by endogenous P_4 concentrations we hypothesized that cows treated with pLH or hCG would have a higher ovulatory response even if P_4 concentrations were high. Although the overall ovulatory response was not different among treatments, pLH and hCG were more effective than GnRH at inducing ovulation when P_4 concentrations were high (≥ 4 ng/mL). de Araujo Berber et al. (2002) also demonstrated in cattle and buffalo that when plasma P_4 concentrations were greater, pLH was more effective than GnRH. Bello et al. (2006) demonstrated a positive relationship between ovulatory response to first GnRH and response to the following $PGF_{2\alpha}$ treatment. Other researchers have demonstrated a positive relationship between ovulatory response to first GnRH and synchronization response and PR in both lactating dairy (Vasconcelos et al., 1999; Moreira et al., 2000; Bello et al., 2006) and beef cows (Colazo et al., 2004); however, this was not observed in the present study. We had hypothesized that an enhanced ovulatory response to first treatment would result in an increased proportion of synchronized cows to TAI and increased PR but since no changes in ovulatory response occurred, no increases in synchronization or PR occurred. The synchronization responses to TAI observed in this study were similar to those observed in others using a standard Ovsynch protocol (El-Zarkouny et al., 2004; Gordon et al., 2010). While Colazo et al. (2009b) reported

enhanced ovulatory response to the first treatment with pLH, this also did not result in increased PR. Colazo et al. (2009b) suggested that the administration of pLH on day -10 sustained the growth of an existing dominant follicle and suppressed the emergence of a new follicular wave in those cows leading to follicles with prolonged growth, which have been shown to result in lower PR (Revah and Butler, 1996; El-Zarkourny et al., 2004; Navanukraw et al., 2004). Colazo et al. (2009b) also did not find any significant association between POF size and PR, but found an interaction between ovulatory response to first treatment and POF diameter, with the latter being greater in cows that did not ovulate after the first pLH treatment. Ovulatory response to the second treatment of GnRH, pLH, and hCG was not measured in this study. In our study the mean diameter of the POF was not affected by first treatment administered or ovulatory response to first treatment.

As found in both the present study and by others (Stevenson and Phatak, 2005; Balendran et al., 2008), PR decreased as parity increased. Researchers have reported PR to Ovsynch TAI between 30 and 40% in lactating dairy cows (Pursley et al., 1997a, b; Lucy, 2001), which is slightly higher than the 28.1% achieved in this study. Other studies have also reported no differences in PR when replacing both GnRH treatments in an Ovsynch TAI program with hCG in cows (De Rensis et al., 2002) or only the second GnRH treatment with hCG in heifers (Schmitt et al., 1996b). However, in a parallel study to this, we found PR was greater when only the second GnRH treatment was replaced by pLH (Colazo et al., 2009b). The first treatment of GnRH may synchronize a new follicular wave (through FSH release), which does not occur when pLH or hCG

treatments replace GnRH. The emergence of a new wave of follicular development requires a surge of FSH release (Driancour, 2001; Fortune et al., 2001).

Another potential advantage with using hCG or pLH is the ability to enhance the function of the CL post-insemination, through increased P_4 concentrations. Progesterone concentrations have been shown to increase conception and embryo quality (Changes e Silva et al., 2002). Rajamahendran and Sianangama (1992) and Schmitt et al. (1996a) have shown increased post-ovulatory P_4 concentrations using hCG when treatment was given between days 5 to 7 after estrus, to induce an accessory CL from the the first-wave dominant follicle. In these studies, the increased P_4 concentrations could be due to the combined effect on the original CL and the induced CL. Meanwhile, Ambrose et al. (2005) reported an increase in P_4 concentrations on day 9 post-ovulation in dairy heifers subjected to a pLH synchronized treatment reported, and Ree et al. (2009) reported no differences in non-lactating cows treated with pLH (Ree et al., 2009). The researchers that used hCG (De Rensis et al., 2002; Schmitt et al., 1996b) in an Ovsynch TAI protocol did not measure P_4 concentrations post-insemination. In our study we found no differences in mean P_4 concentrations between treatment groups after TAI. However, P_4 concentrations among pregnant animals were greater for the hCG group on day 7 than the GnRH group, greater for the hCG and pLH groups on day 11 than for the GnRH group, and greater for the hCG group on day 14, than pLH and GnRH group (Figure 4.4). Interestingly, cows in the GnRH group had numerically higher presumptive embryonic losses between days 28 to 35 (16.7% vs. 8.3%, and 9.3% for, hCG and pLH treatments). Recent estimates of pregnancy losses vary from 7.1% (Starbuck et al., 2004) to 11% (Navanukraw et al., 2004) from days 28 to 49 of gestation. In an observational study at

the University of Alberta herd (Ambrose et al., 2006b) involving 1545 breeding records, PR was lower (29.6 vs. 38.6%) and embryonic loss higher (5.1 vs. 0%) in dairy cows subjected to an Ovsynch program (using GnRH) than those inseminated after estrus detection. Treatment with pLH (Ambrose et al., 2005; Ree et al., 2009) and hCG (Schmitt et al., 1996a) results in a prolonged duration of elevated LH. The extended exposure to the LH-like action of hCG and pLH may improve oocyte maturation and competence, enhancing fertilization (Colazo et al., 2009b), as well as inducing increased luteinization of post-ovulatory follicles (Schmitt et al., 1996b) compared to GnRH, thereby reducing embryonic mortality. In the study where only the second treatment of GnRH was replaced by hCG (Schmitt et al., 1996b) administration of hCG eliminated the occurrence of short estrous cycles, and the authors suggest administering hCG at 36 h vs. 48 h after $PGF_{2\alpha}$. Further investigation is warranted to determine the influence of elevated LH concentrations during the peri-ovulatory period on oocyte maturation and post-fertilization development. Response of the follicle to respond to LH is dependent on the LH receptor population located on theca interna cells within the follicle. Luteinizing hormone is responsible for the events leading to ovulation of the dominant follicle and stimulating the CL to secrete P_4. Differences between treatments with GnRH, hCG, and pLH may be due to the response of the follicle and/or the levels and duration of LH achieved. The formation, function, and maintenance of the CL are regulated by various luteotropic factors before and after ovulation, as well as by the inhibition of several luteolytic factors during the period of active CL function (Stocco et al., 2007). We recently reported that genes regulating angiogenic, steroidogenic, and luteotropic factors are highly expressed in heifers compared to lactating dairy cows, and that apoptosis

seemed to be more evident in the CL of lactating cows, suggesting that CL of lactating dairy cows have reduced luteotropic and steroidogenic capacities, playing a critical role in the reduced PR observed in lactating dairy cows (Pretheeban et al., 2010). Since pLH and hCG exert a longer stimulatory and luteotropic influence on the developing CL (Schmitt et al., 1996a), they may have an effect on the activation of genes controlling P_4 production, CL function, and embryo development.

Timing of insemination with respect to differing ovulatory responses to the three hormonal treatments, GnRH, pLH, and hCG, was not considered in this study. Previous studies have determined that AI around 16 h after the last GnRH treatment in an Ovsynch program is optimal (Pursley et al., 1998; Brusveen et al., 2008). Ovulation in lactating dairy cows occurs normally about 24 to 30 h after the LH surge (Rajamahendran et al., 1989). Ree et al. (2009) observed no differences between the mean time interval from treatment to ovulation (28.1 ± 0.7 h, 29.1 ± 1.0 h, 33.5 ± 2.8 h, and 29.1 ± 1.0 h) when treatments of 25, 12.5, or 8 mg pLH and 100 μg GnRH were administered. However, numerically, more cows treated with 25 mg pLH ovulated during the initial 27 h after treatment than cows in the GnRH group. Ree et al. (2009) used non-lactating animals; therefore, conducting a similar study in lactating dairy cows is warranted. Moreover, to our knowledge, no researcher has yet examined the timing of ovulation after a treatment with hCG. While LH release after GnRH treatment is almost instantaneous, pLH and hCG both act directly on the ovary, and ovulation after their treatment may occur sooner/differently after treatment than a GnRH treatment. For example, a Co-synch program, where insemination and treatment for synchronizing ovulation occur concurrently, 48 h after $PGF_{2\alpha}$, may be better suited for the use of pLH or hCG in a

modified Ovsynch program. Investigations regarding the influence of elevated LH concentrations during the peri-ovulatory period on oocyte maturation and post-fertilization development may provide better understanding on timing of ovulation and insemination.

In the present study we chose to use 25 mg pLH and 1,000 IU hCG based on previous studies examining the efficacy of various doses to induce ovulation. Ree et al. (2009) examined the efficacy of different doses of pLH and concluded that reduced doses of pLH (8.0 or 12.5 mg) were not as effective as either 25 mg pLH or 100 μg GnRH in synchronizing ovulations, particularly during diestrus. Efficacy of hCG to induce ovulation was not different between 100 μg GnRH or 500, 1,000, 2,000, or 3,000 IU of hCG (Burns et al., 2008), indicating that the ovulatory capacity of even a low dosage (500 IU) of hCG is equivalent to that of 100 μg GnRH. Dose requirements are important to consider for their associated costs, since both pLH and hCG are more costly per treatment than GnRH.

Gonadotropin releasing hormone is considerably cheaper per treatment than hCG and pLH ($10.00 vs. $25.00 and $33.00, respectively). Moreover, repeated use of hCG can sometimes lead to cows becoming immunogenic against hCG (Schmitt et al., 1996b). Although our study found no differences in PR when using hCG or pLH in lieu of GnRH in an Ovsynch program, because of increased costs, we do not recommend their use. However, replacing the second GnRH treatment with hCG or pLH has the potential to increase PR as this has already been shown with pLH (Colazo et al., 2009b).

In summary, hCG and pLH treatments induced greater ovulatory responses only when P_4 concentrations were greater than 4 ng/mL on day -10. However this did not

translate to increased synchronization response or PR. Greater P_4 concentrations post-insemination were only observed in pregnant animals in the hCG (days 7, 11, and 14) and pLH (day 11) groups compared to those in the GnRH group. In addition, mean POF diameter did not affect PR. The optimal time of insemination when using pLH or hCG in a TAI program deserves further investigation. But cost of treatment needs to be considered when examining the potential benefits of using pLH or hCG in lieu of GnRH.

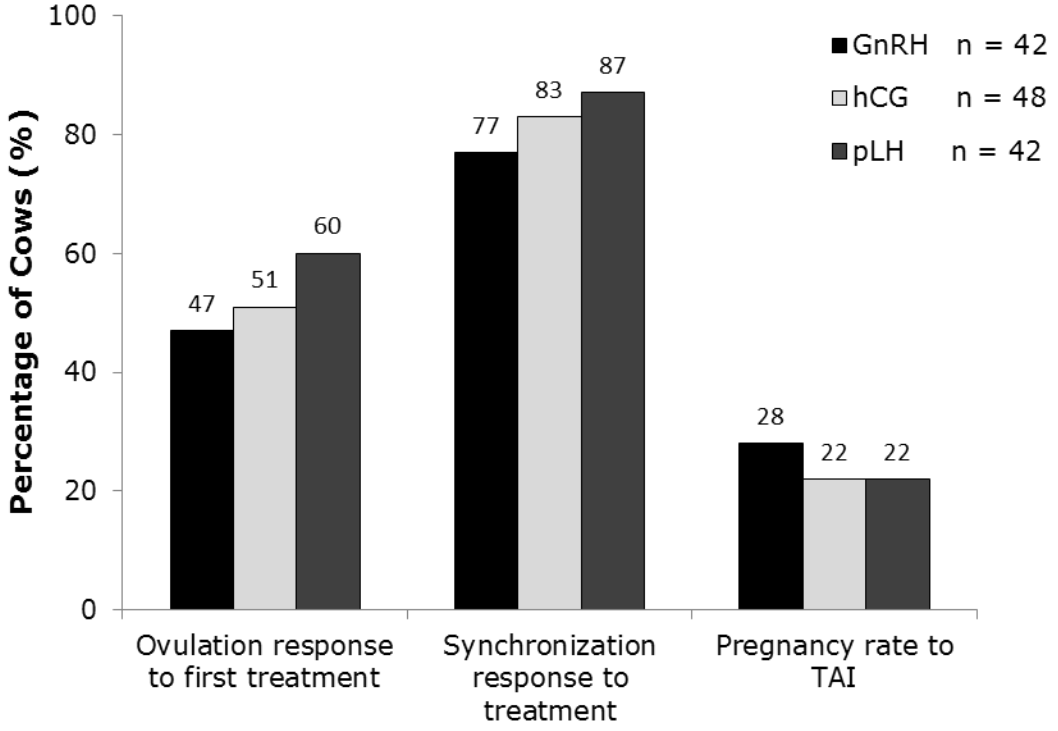

Figure 4.2 Ovulation, synchronization, and pregnancy rates among treatments

The percentage of cows that ovulated to first hormonal treatment (GnRH, hCG, or pLH) on day -10, the synchronization response to treatment based on progesterone concentrations of < 1 ng/mL on day 0, and percentage of animals pregnant based on pregnancy diagnosis on day 40 post-TAI (n = 42, 48, 42 for GnRH, hCG, and pLH respectively).

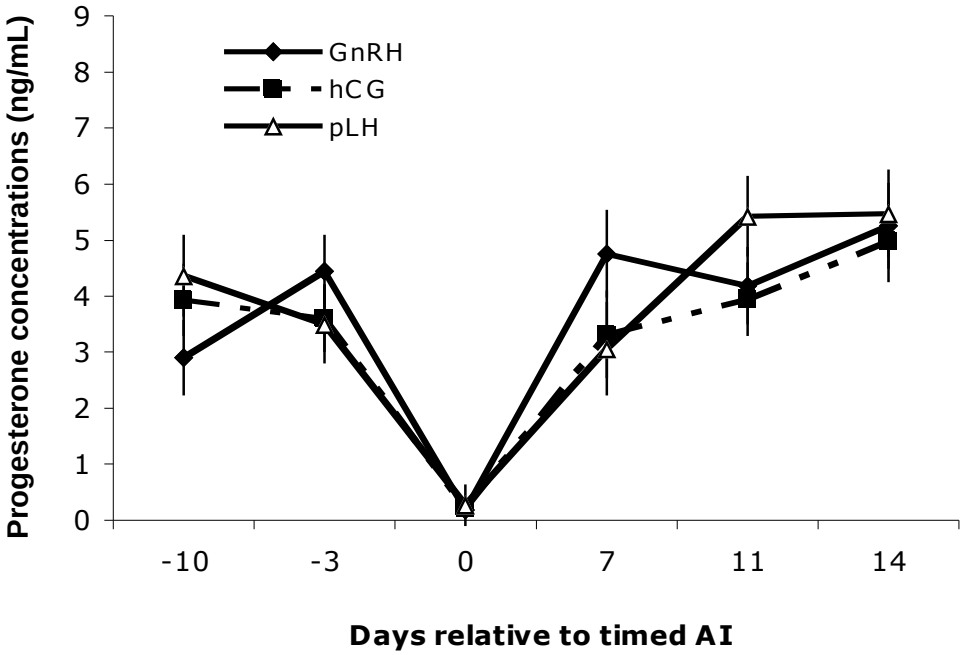

Figure 4.3 Milk progesterone concentrations among treatments

Milk samples were collected on days -10, -3, 0, 7, 11, and 14 after timed artificial insemination (TAI). Lactating cows received either 100 µg GnRH, 1,000 IU hCG, or 25 mg pLH during an Ovsynch TAI program (n = 42, 48, 42 for GnRH, hCG, and pLH respectively).Vertical bars represent SEM.

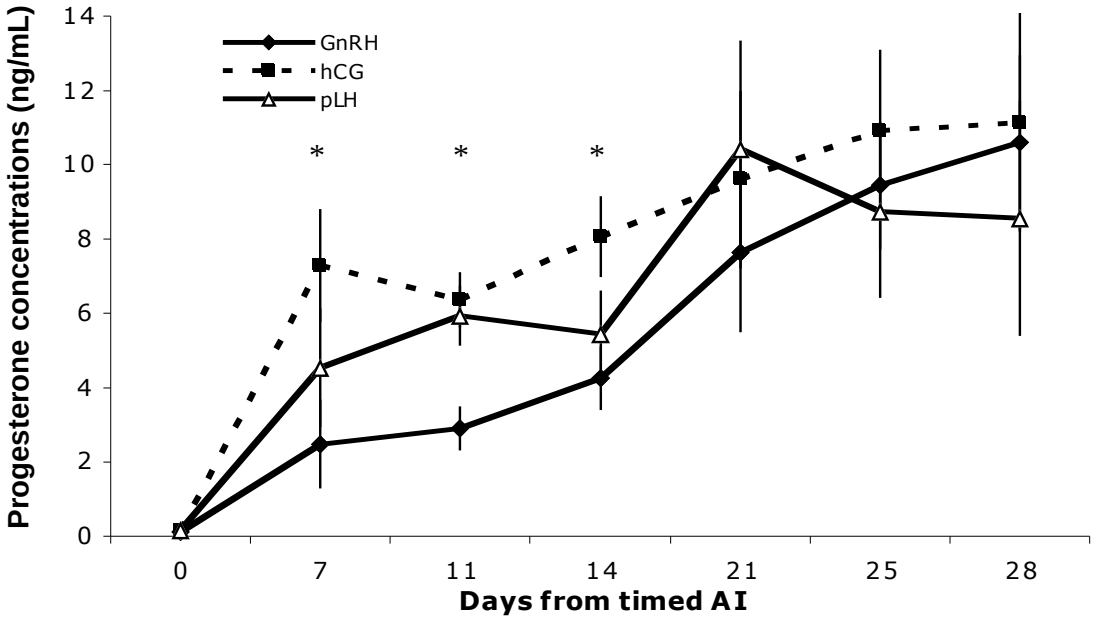

Figure 4.4 Milk progesterone concentrations among treatments in pregnant cows

A treatment of 100 µg GnRH, 1,000 IU hCG, or 25 mg pLH was given to synchronize ovulation in an Ovsynch TAI program (n = 42, 48, 42 for GnRH, hCG, and pLH respectively). The hCG group has greater progesterone concentrations than the GnRH group on days 7, 11, and 14. The pLH group had greater progesterone concentrations than the GnRH group on day 11. * P = 0.06, P = 0.002, and P = 0.04, respectively. Vertical bars represent SEM.

Table 4.1 Ovulation responses to first treatment

	Treatment			
	GnRH	**hCG**	**pLH**	***P-value***
	n = 42	n = 48	n = 42	
Cows with $P_4 \geq 1$ ng/mL	61.2 %	67.3 %	60.0 %	0.72
Cows with $P_4 \geq 4$ ng/mL	32.7 %	36.4 %	33.3 %	0.91
% of cows ovulating to first treatment:				
$P_4 \geq 1$ ng/mL	39.1 %	50.0 %	52.6 %	0.64
$P_4 < 1$ ng/mL	61.5 %	50.0 %	73.3 %	0.46
P-value	0.19	1.0	0.21	
$P_4 \geq 4$ ng/mL	20.0 %	58.3 %	58.3 %	0.12
$P_4 < 4$ ng/mL	57.7 %	45.8 %	63.3 %	0.46
P-value	0.04	0.48	0.76	

Ovulation responses to first treatment in lactating cows subjected to a GnRH-based, hCG-based, or pLH-based Ovsynch protocol based on progesterone (P_4) concentrations. Values represent the proportion of animals.

CHAPTER 5: EFFECT OF FEEDING A LOW PROTEIN DIET DURING OVSYNCH TIMED ARTIFICIAL INSEMINATION AND POST-BREEDING ON OVULATION SYNCHRONIZATION, PREGNANCY RATES, PROGESTERONE CONCENTRATIONS, AND MILK PRODUCTION IN LACTATING DAIRY COWS

Introduction

Farm management practices have dramatically changed over the past 50 years due to the introduction of AI and other modern technologies, such as synchronization protocols. Coupled with an increase in milk yield, PR in lactating dairy cows have dropped from 55.6% to 39.7% and continues to drop at a rate of 1% per year (Royal et al., 2000). Poor estrus detection greatly impairs reproductive performance and profitability of lactating dairy cows (Pursley et al., 1997b) and as herd sizes continue to grow estrus detection becomes more challenging.

The development of Ovsynch TAI protocol has allowed for the control of ovarian follicular and CL development, decreasing the need for estrus detection (Pursley et al., 1995). While PR in response to Ovsynch TAI are similar to PR obtained by breeding at natural estrus (Pursley et al., 1997ab; Lucy, 2001; Rabiee et al., 2005), rates are still far from satisfactory. Many researchers have attempted to increase PR to Ovsynch TAI by using treatments such as: 1) pre-synchronzation (El-Zarkouny et al., 2004; Navanukraw et al., 2004; Souza et al., 2008; Gordon et al., 2010); 2) post-insemination treatments with P_4 (Larson et al., 2007) or GnRH (Ambrose et al., 2000; Willard et al., 2003; Franco et al., 2006; Gordon et al., 2010); and 3) different hormonal regimes including P_4 supplementation (Stevenson et al., 2006), and pLH (Colazo et al., 2009; Ree et al., 2009) or hCG (Schmitt et al., 1996b; De Rensis et al., 2002) in lieu of GnRH. These strategies have inconsistent success and/or can involve more hormone injections to the animals,

require more labour, increase costs, and are a challenge for farmer compliance. Still, the economic benefit of Ovsynch TAI protocol is based on reduced intervals to first AI, number of days open, and culling of cows for infertility (Vasconcelos et al., 1999; Tenhagen et al., 2004a; Rabiee et al., 2005).

Protein metabolism in the cow

It is common herd management to feed lactating dairy cows a diet high in CP (17-19%) to stimulate and support high milk production (Canfield et al. 1990; Butler, 1998; Zimmerman et al., 2001). Crude protein can be classified into RDP, the protein that can be fermented by microorganisms within the rumen, and ruminally undegradable protein (RUP), the protein which cannot be fermented by microorganisms in the rumen. Through microbial fermentation, RDP provides a source of ammonia for microbial protein synthesis. Dairy cows absorb amino acids available in the small intestine supplied mainly by digestion of microbial protein and RUP (Cyriac, 2009). Excessive intake of protein, particularly RDP, results in high systemic concentrations of ammonia and urea (Ocon and Hansen, 2003; Kenny et al., 2002). When RDP exceeds the capacity of the rumen microbes to digest and assimilate it for themselves, ammonia builds up in the rumen. Excess ammonia produced by microbial fermentation is absorbed through the ruminal mucosa into the blood circulation and is detoxified by synthesis into urea in the liver. In addition, blood urea can be produced from deamination and metabolism of circulating amino acids, which originated from microbial protein and RUP (Butler, 1998). The conversion of ammonia to urea costs the dairy cow energy that could otherwise be used for milk production. This loss of energy can further exacerbate NEB in the cow (Butler,

1998).

Urea circulating in the bloodstream is measured as blood urea nitrogen (BUN). Urea also passes freely from blood into the milk and can be measured as milk urea nitrogen (MUN) (Butler, 2005b). High systemic concentrations of ammonia and urea (> 19 mg/dL) have been associated with reductions in fertility (Canfield et al., 1990; Elrond and Butler, 1993; Butler et al., 1998; Kenny et al., 2002) via negative effects on ovulation, fertilization and the early embryo for up to 20 d post-breeding (Ball and Peters, 2004).

Effect of protein on fertility

Possible mechanisms for reduced fertility from excess dietary CP include impaired embryonic development due to changes in uterine physiology, oviductal environment, or oocyte quality, reduced plasma P_4 concentrations, as well as exacerbation of NEB, which can impair reproduction further (Butler, 1998). Ammonia and urea have been observed to have toxic effects on oocytes (Ocon and Hansen, 2003) and the embryo (Rhoads et al., 2004) through decreasing uterine pH (Elrod and Butler, 1993) and altering mineral composition of the uterine fluid (Jordan et al., 1983). Lactating dairy cows that were fed excess RDP had early degeneration and poor development of embryos (Blanchard et al., 1990). Several researchers have also observed decreased P_4 concentrations during the mid-luteal stage of the estrous cycle in lactating dairy cows fed a high protein diet (> 19%) compared to cows fed a lower protein (12-17%) diet (Jordan and Swanson, 1979; Folman et al., 1983; Sonderman and Larson, 1989).

Canfield et al. (1990) and Barton et al. (1996) fed cows a diet low in CP, from calving to peak lactation and observed increased PR along with decreased milk production. However, Barton et al. (1996) showed that cows fed a 20% CP diet had higher milk production during the first 8 wk after calving than did cows fed a 13% CP diet, but production between the weeks 9 to 15 was similar between diets. Studies have also shown that feeding a diet high in RDP to superovulated cows had deleterious effects on the yield and quality of embryos (Dawuda et al., 2002), which suggest that relatively short-term nutritional manipulation can alter metabolic hormones and the uterine environment, in turn affecting ovarian development in cattle. Changes in insulin and IGF, associated with metabolic stress, can alter the pattern of ovarian follicular growth and development during the early postpartum period, resulting in reduced reproductive function (Gong et al., 2002).

The National Research Council (NRC, 2001) recommends that dietary CP at concentrations between 16.5 and 17.5% of the DM supply the protein requirements of early lactation dairy cows under most conditions and should be equal or below 16.5% as cows advance into the second half of lactation. The current study was designed to compare the effects of feeding a traditional high protein diet vs. a moderately low protein diet during ovulation synchronization TAI and early embryonic development in lactating dairy cows, to compare ovulation synchronization rate, PR, follicular growth, P_4 concentrations, milk composite characteristics, and milk production.

Materials and Methods

Animals, housing, and treatment

This experiment was conducted at the UBC Dairy Education and Research Centre between November 2008 and February 2010. Animals were cared for according to the guidelines outlined by the Canadian Council of Animal Care (1993). A total of 180 multiparous (n = 135) and primiparous (n = 45) cows were used. Animals were housed in free stall pens and milked twice daily at approximately 0600 and 1700h. Cows were fed a standard high protein TMR (Table 5.1 and 5.2) from calving until 61.9 ± 11.5 DIM (mean ± SD). After balancing for DIM, parity, and milk production, cows were randomly assigned to either remain on the high protein diet or switched to a lower protein diet. Animals on both treatments were housed together. Animals were moved to pens containing 12 free-stalls fitted with mattresses (Pasture Mat, Promat Inc., Woodstock, Ontario, Canada) covered with sand. Pens had vulcanized rubber floors in the alleys and crossovers (Red Barn Dairy Mat, North West Rubber Mats Ltd., Abbotsford, British Columbia, Canada) and an electronic feeding system that included six feed bins and one water bin (Insentec, Marknesse, Holland; Chapinal et al., 2007), which had the ability to capture individual animal feed and water intake and assign animals to have access to particular bins. Stocking density within the pens was 12 cows per pen; 6 animals per treatment.

The TMR were formulated to be isoenergetic, and to meet the NRC (2001) guidelines for lactating dairy cows. The high protein diet (~18.5% CP) was formulated to meet RUP requirements and to exceed RDP requirements. The low protein diet (~16%

CP; Table 5.1 and 5.2) was formulated to meet both requirements (Amino Acids and Metabolizable System Program, Viterra, Chilliwack, BC).

All animals were synchronized for first post-partum insemination using Ovsynch TAI program consisting of two injections of GnRH (Fertiline®, Vetoquinol NA Inc., Lavaltrie, QC, Canada; 100 μg i.m.), 9 d apart with an injection of $PGF_{2\alpha}$ (Lutalyse®, Pharmacia Animal Health, Orangville, ON, Canada; 25 mg i.m.) 48 h before the second GnRH treatment, followed by TAI 16 to 18 h later. Animals were fed the low protein diet starting 1 wk before Ovsynch TAI was initiated and remained on the low protein diet until 32 d after breeding for a total of 7 wk (Figure 5.1). Animals switched to the low protein diet were given a 3-day transition of a mix (50:50) of the high protein and low protein diet. Five cows fed the low protein diet were eliminated from the study because they were taking feed from the high protein feed bins.

Cows were weighed and scored for body condition at the start and end of the experiment. Animals were weighed at the same time on 2 consecutive days and then an average was used as body weight. Body condition score was evaluated using a five point scoring system (Ferguson et al., 1994).

Feed analysis

Fresh feed was provided twice daily at approximately 0600 and 1600 h. Samples of TMR were taken once per week for analysis. Samples were dried at 60 °C for 2 d to determine DM. Dried weekly samples of feed were pooled into monthly samples and sent for nutrient analysis (A & L Canada Laboratories Inc., London, ON) to determine the average CP, ADF, NDF, TDF total digestible nutrient, and NE_L content.

Blood and milk sampling

Blood samples were collected from the coccygeal vein on day -17, -10, -3, 0 (TAI), 7, 14, 21, 25, 28. Beta-hydroxybutyrate was immediately measured from blood (days -17, 7, and 28) using the Abbott Precision Xtra^{TM} Blood Ketone Monitoring System (Abbott Laboratories, Illinois, USA), which had been previously validated for use in dairy cows (Iwersen et al., 2009). Blood samples were centrifuged for 20 min at 4°C. Blood plasma was collected and stored at -20 ˚C until subsequent P_4 analysis using a commercially available solid-phase radioimmunoassay (Coat-A-Count Progesterone, Diagnostic Products, Los Angeles, CA).

Composite milk samples were collected during a.m. and p.m. milking into a plastic vial containing a dissolvable milk fat preservative (BroTab10, Systems Plus Ltd., Ontario, CA) on the day before diets began , on day 7 after insemination, and on day 28 to determine milk fat, milk protein, and MUN. Milk fat and protein and MUN were determined by the Ontario Dairy Herd Improvement Cooperative (Guelph, ON).

Table 5.1 Diet ingredients and composition

Parameter	**Low**	**High**
Ingredient	% DM	
Corn Silage	26.3	21.0
Grass Silage	10.3	19.0
Alfalfa Hay	14.0	10.5
Concentrate	49.5	49.5
Chemical composition		
DM (%)	49.8	50.3
CP	15.8	18.3
Soluble Crude Protein, % of CP	58.3	59.2
RUP, Est. % CP	24.4	23.2
ADF	25.2	20.9
NDF	38.6	36.0
TDF	69.3	72.6
Ca	0.8	0.9
P	0.3	0.3
K	1.5	1.6
S	0.2	0.2
Mg	0.3	0.3
Na	0.3	0.4
Zn, mg/kg	104.0	119.1
Fe, mg/kg	300.0	379.3
Mn, mg/kg	61.5	65.1
Cu, mg/kg	18.0	19.0
NE_L, Mcal/kg	1.6	1.6

Table 5.2 Ingredients in the concentrate

Ingredient formulation of concentrates (kg/tonne as fed)	Low	High
Barley flattened	235.0	270.0
Barley rolled (11% CP)	135.0	174.5
Soybean meal[1] (44% CP)	0.0	106.0
Soybean hulls	100.0	0.0
Canola meal[1] (36% CP)	0.0	76.5
Corn-ground	135.0	60.0
Corn-flaked	75.0	69.0
Amino plus[2]	124.6	68.0
Tex-line	0.0	50.0
Millrun[3]	42.6	34.0
Corn gluten meal (60% CP)	35.0	0.0
Megalac[4]	22.0	15.5
Calcium carbonate	19.0	22.4
Fish meal	17.0	0.0
Molasses	15.0	15.0
Bicarbonate/Soda	12.0	9.5
Dairy premix[5]	10.5	10.5
Salt	7.0	7.0
Farm-pak[6]	6.5	6.5
Dicalcium phosphate	4.6	0.0
Magnesium oxide 58%	3.9	3.2
Urea	0.0	2.1
Smartamine® M[7]	0.35	0.35

[1] Solvent extracted.
[2] Ag Processing Inc., Omaha, NE.
[3] Wheat by-product.
[4] Church and Dwight Co., Princeton, NJ.
[5] Akey, Lewisberg, OH. Dairy premix is a vitamin and mineral complex formulated for stage of lactation.
[6] Alltech, Nicholasville, KY. Farm-pak includes a vitamin and mineral complex with active dry yeast.
[7]Adissio, Alpharetta, GA. Encapsulated methionine.

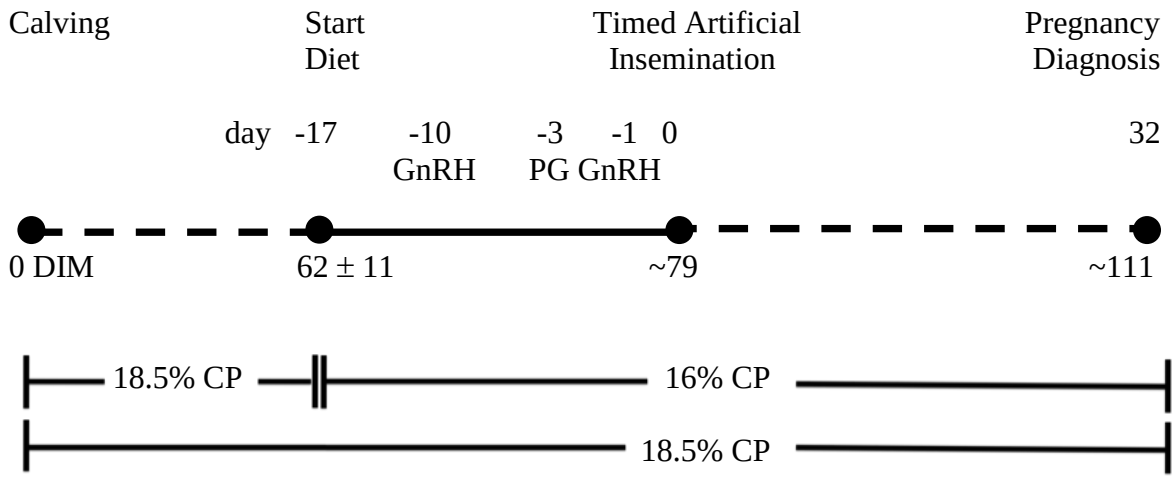

Figure 5.1 Schematic diagram of treatment schedules

All cows were fed standard high protein diet (~18.5% crude protein [CP]) from calving until 62 ± 11 days in milk (DIM). Cows then either remained on the high protein diet or switched to a lower protein diet (~16% CP). All cows were synchronized for first service breeding using Ovsynch TAI (consisting of two injections of GnRH, 9 d apart with an injection of $PGF_{2\alpha}$ 48 h before the second GnRH treatment, and TAI 16 to 18 h later). Cows were fed the low protein diet starting 1 wk before Ovsynch was initiated and remained on the low protein diet until 32 d after breeding for a total of 7 wk.

Uterine and ovarian examination

Animals were subjected to transrectal ultrasound examination using a scanner equipped with a 7.5-MHz linear-array transducer (Aloka-SD500, Aloka Co., Tokyo, Japan) to determine the presence of follicular or luteal tissues on the ovaries on day -10, -3, and -1. Pregnancy was determined via transrectal ultrasound examination 32 d post-TAI.

Analysis of data

Synchronization response to treatment was based on animals with P_4 concentrations < 1 ng/mL on the day of TAI. Animals were determined to be cycling if at least one P_4 sample (day -17 or day -10) was ≥ 1 ng/mL. Pregnancy loss was determined if an animal aborted between days 32 to 60 post-insemination. Pregnancy data were analyzed by Chi-square using an ordinal logistic procedure using JMP®. Covariates considered in the analysis were treatment, parity, group, and DIM and BCS at TAI. Body condition score, DIM, and parity were considered continuous variables. Pregnancy rates comparing parity and treatment were also analyzed by dividing cows within parity (first, second and third lactation or greater). Mean plasma concentrations of P_4 were analyzed using a standard least squares model for repeated measures to compare treatments, synchronized, and pregnant to non-pregnant animals. Percentage of cows with ≤ 1 ng/mL or > 1 ng/mL plasma P_4 were treated as categorical data and were analyzed by Chi-square test; this was used for synchronization response to treatment, and cycle status.

Overall average daily milk production and milk components (% protein and fat, and MUN) data were analyzed using standard least squares for repeated measures. Parity,

week, treatment, and week*treatment were in the model. Fat corrected milk was determined using the following formula to convert milk components: 3.5% FCM = (0.4324 x kg of milk) + (16.216 x kg of milk fat). The percent milk fat analyzed from milk samples collected was used to determine the kg of milk fat. Milk production was also divided into three periods: pre-treatment (5 wk prior to start of experiment), during treatment, and post-treatment (5 wk following the end of experiment). Average daily dry matter intake (DMI) was analyzed using standard least squares. Parity, body weight, day, and treatment were included in the model. Significant α level was set at $P \leq 0.05$, and P values > 0.05 but ≤ 0.10 were considered a tendency towards difference.

Results

Animals

Average DIM, BCS, body weight, and parity at TAI (mean ± SEM) were 78.5 ± 0.9, 3.0 ± 0.02, 643.5 ± 5.1, and 2.7 ± 0.1, respectively. Overall average DMI was greater for cows fed the high protein diet vs. cows fed the low protein diet (23.8 ± 0.05 kg vs. 22.8 ± 0.05 kg, respectively; $P < 0.0001$; data not shown).

Pregnancy results

Overall PR was 24.4% and 34.1% for high and low protein diets, respectively ($P = 0.16$). Parity tended to affect PR ($P = 0.09$). Pregnancy rates for 1st and 2nd lactation animals were 27.7% and 42% ($P = 0.10$) for high and low protein groups, respectively (Table 5.3). Pregnancy rates for cows greater in their 3rd or later lactation were 20.9% and 22.9% ($P = 0.53$) for high and low protein diet groups, respectively. Pregnancy rates were

greater for 1st and 2nd lactation cows in the low protein group compared with cows in their 3rd or greater lactation ($P = 0.05$), but no differences were observed between 1st and 2nd and 3rd or greater lactation cows in the high protein group ($P = 0.46$).

Based on P_4 concentrations, there were no differences ($P = 0.59$) in the proportion of animals cycling at the start of Ovsynch between treatments (81.0% vs. 76.2% for high and low diets, respectively). There were no differences ($P = 0.53$) in synchronization rates to TAI between treatments (88.1 % vs. 83.3% for high and low protein diets, respectively).

When animals were followed through to other services, there were no differences ($P = 0.56$) in the number of services required for a cow to get pregnant between the high (2.5 ± 0.2) and low (2.4 ± 0.2) groups. Pregnancy loss between day 32 and 60 post-insemination was 3.3% (3/90) and 4.7% (4/85) for high and low groups, respectively ($P = 0.64$)

Milk production and components

Overall 305 d average daily milk production ($P < 0.0001$) and 3.5% FCM production ($P < 0.0001$) was higher in cows fed the lower protein diet compared with cows in the high protein group (Table 5.4). Milk production increased as parity increased ($P < 0.0001$). During treatment cows on the low protein diet had lower average daily milk production compared to cows on the high protein diet (46.6 ± 0.1 kg vs. 47.7 ± 0.1 kg, respectively; $P < 0.0001$). The low protein group had greater average daily milk production compared to the high protein group both pre-treatment (51.4 ± 0.3 kg vs. 48.2 ± 0.3 kg, respectively; $P < 0.0001$) and post treatment (46.2 ± 0.2 kg vs. 45.3 ± 0.2 kg,

respectively; $P = 0.007$). There were no differences in milk fat % between cows fed the high and low protein diets on day -17, 7, or 28 (Table 5.5). There were no differences in milk protein % between treatments before diets started on day -17 ($P = 0.85$) or on day 7 ($P = 0.13$). Cows fed the low protein diet had lower milk protein % on day 28 ($P = 0.02$) (Table 5.5) while on treatment compared to cows fed the high protein diet. There were no differences in MUN levels ($P = 0.78$) between treatments before diets started on day -17. Cows fed the low protein diet had lower MUN levels on day 7 and 28 (Table 5.5) while on treatment compared to cows fed the high protein diet.

Blood progesterone and BHBA concentrations

There were no differences in P_4 concentrations on days -17, -10, -3, 0, 7, and 14 between cows fed the high and low protein diets. There were also no differences in P_4 concentrations between treatments in pregnant cows (Data not shown). There were no differences in BHBA levels between high and low protein groups on day -17 (0.80 ± 0.07 vs. 0.72 ± 0.07 mmol/L; $P = 0.44$). There were also no differences between the high and low protein groups when BHBA levels were greater than 1.1 mmol/L (15.6% vs. 17.6%; $P = 0.62$; data not shown).

Discussion

Since it is important to supply the animal with high quality protein sources especially during the first few weeks after calving (peak lactation), producers often feed above the NRC guidelines resulting in excess ammonia that the body must metabolize

and excrete (Butler, 1998). However, diets high in protein have become associated with reduced PR, (Visek 1984; Butler, 1998; Ocon and Hansen 2003).

In this study we did not observe any differences in PR between lactating cows fed a high protein diet vs. lactating cows fed a low protein diet (both subjected to an Ovsynch TAI protocol). However, PR tended to be greater for primiparous and 2nd lactation cows fed the lower protein diet compared to 3rd or greater lactation cows. Possible reasons for better PR in primiparous and 2nd lactation cows include a reduced risk of metabolic disorders in early lactation (Gröhn and Rajala-Schultz, 2000) and better fertility (Stevenson and Phatak, 2005; Balendran et al., 2008) which could have interactive effects with the diet. Canfield et al. (1990) observed lower first service PR in cows fed a high protein diet (19% vs. 16%), regardless of parity, when diets were fed from day of calving until 20 d after breeding. Whereas both Kaim et al. (1983) and Bruckental et al. (1989) reported that high CP diets reduced PR of cows in 4th or greater lactation, but had no effect on 2nd or 3rd lactation cows. Bruckental et al. (1989) also observed lower PR in primiparous cows fed a high CP diet. While Tenhagen et al. (2004b) and Stevenson and Phatak (2005) reported that primiparous cows have greater PR than 2nd or greater lactation cows after synchronization following Ovsynch, Jobst et al. (2000) reported greater PR in multiparous cows compared with first lactation cows. The variation and differences in PR between primiparous cows and older cows could be associated with differences in management of primiparous cows compared to multiparous cows and differences among farms (Tenhagen et al., 2004b).

While both Canfield et al. (1990) and Barton et al. (1996) reported lower milk production in cows fed a lower protein diet beginning at calving, Barton et al. (1996)

observed no differences in milk production between cows fed a high and low protein diet after the first 8 wk (Barton et al., 1996). In the current study, animals were not switched to the lower protein diet until approximately 8 wk after calving and were only kept on the low protein diet for 7 wk, with the objective of not affecting milk production. Overall, cows fed the lower protein diet had higher milk production compared to cows fed the high protein diet. However, during the 7 wk treatment low protein cows did have lower milk production compared to the cows that remained on the high protein diet. Davidson et al. (2003) fed diets of varying CP to lactating cattle from 21 to 120 DIM milk demonstrated moderate CP levels (16.5%) did not affect milk yield and minimized nitrogen excretion. Improvements in diet modeling and consistency of predicting metabolizable protein and amino acid requirements allows for the formulation of lower protein diets with less risk to reduce milk production (Tylutkai and Van Amburgh, 2010). In recent years, as diet modeling has become more efficient, the diets from previous studies using a high protein diet would not have been formulated as well to predict metabolizable protein and amino acid profiles as the high protein diets that are fed today on commercial dairy farms. As a result, the high protein diet in this study has been formulated in such a way that there was more efficient diet balancing for limiting amino acids and this may have resulted in less of an effect in cows on the low protein diet.

In a study comparing diets formulated to provide different concentrations of RDP fed to cows in mid-lactation for 3 wk, milk fat and protein concentrations increased by 0.16% units for cows fed 11% RDP compared with 6.8% RDP (Kalscheur et al., 2006). In the current study, the lower protein diet was formulated to provide approximately 8.8% RDP and though no differences were observed in milk fat % a 0.10% decrease in milk

protein was seen compared to the high protein diet, which contained approximately 11.4% RDP.

Concentrations of urea in blood and milk reflect the quantity and degradability of dietary protein and also the balance between RDP and ruminally fermentable energy intake (Kenny et al., 2002). Cows fed the lower protein diet achieved lower MUN concentrations compared with cows fed the high protein diet. However, the MUN concentrations for cows fed the high protein diet were at levels that have been shown not to have negative effect on PR. Several researchers have observed a relationship between high BUN and MUN (> 19 mg/dL) as a consequence of excess protein feeding and reduced fertility in cattle (Jordon et al., 1983; Elrond and Butler, 1993; Butler et al., 1996). Sinclair et al. (2000) demonstrated that cows that consumed diets that generated high levels of plasma ammonia also had high levels of ammonia in follicular fluid. A further study reported that cows with MUN concentrations below 10 mg/dL were almost two and a half times more likely to be confirmed pregnant than cows with MUN concentrations above 15.4 mg/dL (Rajala-Schultz et al., 2001). A more recent study has reported that PUN concentrations through the range of 12 to 24 mg/dL can exert direct effects on uterine pH (Rhoads et al. 2004). Any deviations within the uterine environment can be determental to the development and survival of the oocyte and early embryo because they rely on uterine secrections until implantation (Barnes, 2000). Canfield et al. (1990) and Barton et al. (1996) also observed higher PUN in cows fed a high protein diet compared to cows fed a lower protein diet. However, the urea nitrogen concentrations in the cows fed the high protein diets in these studies were substantially higher (> 19 mg/dL) than the concentrations observed in cows fed the high protein diet in the current

study. This provides further evidence of how improvements in diet formulation and consistency of predicting metabolizable protein and amino acid requirements over the years has allowed for the formulation of diets that are more efficient for nutrient utilization (Tylutkai and Van Amburgh, 2010).

While researchers who fed lower CP diets to cows have observed higher P_4 concentrations than in cows fed high CP diets (Jordan and Swanson, 1979; Folman et al., 1983; Sonderman and Larson, 1989; Spicer et al., 1990), others have not observed any differences (Barton et al., 1996). When cows are fed excess RDP during early lactation, NEB is exacerbated and P_4 clearance is increased (Butler 1998). These authors began feeding the low CP diets at calving rather than later in lactation; whereas in this study cows were not switched to the low protein diet until about 62 DIM.

The possibility of interactive effects between protein intake and lactation induced NEB may confound the interpretation of some studies on pregnancy (Butler et al., 1998). As a result, blood BHBA, an index of NEB, was measured in the present experiment. It is difficult to determine the specific effects of dietary protein or systemic concentrations of ammonia and urea, alone, in lactating dairy cows (Kenny et al., 2001b). Cows which have BHBA concentrations ≥ 1.2 mmol/L are considered to have sub-clinical ketosis, a sign of NEB and concentrations of ≥ 2.1 mmol/L indicate acute ketosis (Iwersen et al., 2009). In this study there were no differences in the BHBA concentrations between treatments at the start of the experiment. Moreover, there were no differences between treatments in the proportion of cows with BHBA concentrations ≥ 1.2 mmol/L. However, it is not known what possible deleterious interactive effects individual cows experienced prior to the start of the experiment, early in the post-partum period. It has been observed that

cows with circulating BHBA concentrations ≥ 1.0 mmol/L in the first week post-partum or ≥ 1.4 mmol/L in the second week post-partum were less likely to be diagnosed pregnant after first AI (Walsh et al., 2007). Negative energy balance during the early post-partum period might exert residual effects on the follicular development of preovulatory follicles that will appear later during the breeding period (Butler 1998).

Overall DMI was about 1 kg lower in the cows fed the low protein diet. Increasing protein concentration in the diet stimulates food intake in cattle (Grings et al., 1991). The reduced DMI may also have been a result of reduced palatability of the diet as five cows on the low protein diet had to be eliminated, due to their taking feed from neighbouring Insentec bins containing the high protein diet. This 1 kg/d difference in feed intake may account for the differences observed in milk production of cows fed the lower protein diet.

As estrus detection is increasingly difficult on dairy farms due to growing herd sizes, many producers now rely on using synchronization TAI protocols, such as Ovsynch for breeding. With this management of breeding large groups of animals together, feeding a moderately lower protein diet would not be difficult to implement. The tendencies for increased PR were observed only in the primiparous and 2nd lactation cows fed the lower protein diet. Measuring NEFA concentrations and BHBA concentrations of the animals through their early post-partum period and through the entire study may provide more information to better understand the results of the current study. Moreover, the possibility of a carry-over affect of the high protein diet fed earlier on the follicular development of preovulatory follicles may warrant feeding the lower protein diet for longer than 1 wk before cows began synchronization. In addition to

affecting fertility, overfeeding CP increases feed costs and decreases the efficiency of nutrient utilization and as a result, produces environmental waste from ammonia loss into the air and potential nitrate contamination of surface and ground water (Tamminga et al., 1992).

Table 5.3 Effect of parity on pregnancy rates in lactating dairy cows

	Treatment		
	High CP[+]	**Low CP**	***P value***
Overall	24.4% n = 90	34.1% n = 85	0.16
Parity 1	27.3% n = 22	43.5% n = 23	0.20
Parity 2	28.0% n = 25	40.7% n = 27	0.25
Parity 1 & 2	27.7% n = 47	42% n = 50	0.10
Parity ≥ 3	20.9% n = 43	22.9% n = 35	0.53
P value	0.46	0.05	

[+] CP = crude protein

Table 5.4 Average daily milk production

All cows were fed a high crude protein (CP) diet from calving. Half of the animals were switched to the low protein diet at 62 ± 11 DIM and continued on the the diet for 7 wk. a) Overall milk production (kg/day) and 3.5% FCM (kg/day) is based on the whole lactation period (305 DIM) b) Average daily 3.5% FCM production is based on the period 5 wk before treatment (pre), during the 7 wk of treatment (during), and the 5 wk following the end of treatment (post).

a)

305 DIM	**High CP**	**Low CP**	**SE**	***P value***
Overall (All parities)	37.3	37.9	0.05	< 0.0001
3.5 % FCM (All parities)	42.6 n = 90	43.7 n = 85	0.07	< 0.001
3.5 % FCM Parity 1	35.2 n = 22	35.5 n = 23	0.10	0.04
3.5 % FCM Parity 2	43.1 n = 25	46.0 n = 27	0.13	< 0.0001
3.5 % FCM Parity ≥ 3	49.2 n = 43	50.0 n = 35	0.11	< 0.0001

b)

3.5% FCM	**Period**	**High CP**	**Low CP**	**SE**	***P value***
All parities	**Pre**	48.2	51.4	0.28	<0.0001
	During	47.8	46.6	0.14	<0.0001
	Post	45.3	46.2	0.23	0.007
Parity 1	**Pre**	36.0	36.1	0.35	0.90
	During	37.1	35.1	0.19	< 0.001
	Post	36.6	37.6	0.30	0.02
Parity 2	**Pre**	51.4	57.7	0.55	< 0.0001
	During	50.2	49.3	0.25	0.01
	Post	46.3	47.9	0.46	0.01
Parity ≥ 3	**Pre**	59.0	60.6	0.44	0.009
	During	56.2	55.5	0.26	0.05
	Post	52.7	53.1	0.40	0.44

Table 5.5 Milk composition

Individual composite milk samples (pooled twice daily milk samples) were collected from all lactating dairy cows on each treatment (high crude protein (CP) group, n= 90; low CP, n= 85) during milking on days (relative to day of artificial insemination) -17 (before treatment was introduced), 7, and 28 to measure the percent of milk fat and protein and milk urea nitrogen (MUN) concentrations.

	Day	**High CP**	**Low CP**	**SE**	***P value***
Milk Fat %	-17	4.33	4.47	0.11	0.37
	7	4.39	4.42	0.09	0.77
	28	4.43	4.51	0.08	0.46
Milk Protein %	-17	2.92	2.91	0.03	0.85
	7	3.07	3.02	0.03	0.13
	28	3.14	3.04	0.03	0.02
MUN (mg/dL)	-17	11.09	10.95	0.37	0.78
	7	10.32	9.35	0.32	0.02
	28	10.86	9.86	0.32	0.02

CHAPTER 6: SUMMARY, GENERAL DISCUSSION AND CONCLUSIONS

In this dissertation I first set out to conduct a benchmark field study on the reproductive performance of lactating dairy cows from local dairy farms. This action was important in order to determine how best to apply reproductive management strategies to improve PR. Ovsynch TAI is a management tool that dairy producers commonly use because it reduces the need for estrus detection, therefore the research following the field study focused on how to improve Ovsynch TAI to increase PR. I proposed that either a pre-synchronization treatment before or a post-insemination treatment after Ovsynch TAI would increase PR in lactating dairy cows and heifers. As well, I examined the use of two different hormones as replacements to GnRH in an Ovsynch TAI protocol to increase PR through increased synchronization rates and P_4 concentrations. Finally, I examined the effects of feeding a lower protein diet to cows during Ovsynch TAI and early embryonic development to increase PR while minimizing effects on milk production.

Summary of experiments

The results of the field study conducted on local dairy farms in the Fraser Valley of British Columbia (Chapter 2) indicate a loss of reproductive performance due to inaccurate estrus detection and/or breeding a cow that is not in estrus, fertilization and ovulation failure, and/or embryonic mortality as the major reproductive problems. Post-partum cyclicity also had an impact on subsequent first service PR. Dairy producers state that poor reproductive performance is one of the top reasons for culling cows and reproductive disorders are one of the top health concerns. Gröhn et al. (2003) have also

reported that reproductive status is the most important influence on culling decisions. Many producers are more frequently using estrus synchronization and ovulation synchronization TAI (Ovsynch) protocols within their herds. Reproduction information from USDA data base collected from 1995 to 2007 reveal that the implementation and use of ovulation synchronization TAI programs appears to have lowered CR, but reduced days to first breeding, days open, and calving interval (Norman et al., 2009). Ovsynch TAI is becoming widely used for the induction and synchronization of ovulation because it offers potential freedom from estrus detection. With the results of the field study and the benefits of Ovsynch the main objective of the experiments in Chapters 3, 4, and 5 was to improve Ovsynch TAI protocol using some modifications to increase PR.

In lactating dairy cows the Ovsynch TAI protocol has yielded comparable PR to those observed in cows bred at natural estrus; yet, these PR still are considerably lower than those achieved over 50 years ago, resulting in significant economic losses (Rabiee et al., 2005). Some cows may not be at the proper stage of the estrous cycle to respond when Ovsynch is initiated (El-Zarkouny et al., 2004) and embryonic mortality still significantly contributes to lower PR (Vasconcelos et al., 1999; Lucy, 2001, Ambrose et al., 2006b). Pregnancy rates in heifers obtained following Ovsynch TAI have been considerably lower than what is achieved with breeding following natural estrus (40% vs. 70%, respectively) (Pursley et al., 1997a; Ambrose et al., 2000). In the first experiment reported in this dissertation (Chapter 3) synchronization rates, PR, and P_4 concentrations were compared in three treatments: Ovsynch TAI, Presynch + Ovsynch TAI, and Ovsynch TAI + PostAI GnRH at 6 d after TAI. No differences in PR were observed between Ovsynch, Presynch + Ovsynch, and Ovsynch + PostAI GnRH in either lactating

dairy cows or heifers. However, lactating dairy cows had lower PR if cows were bred before 76 DIM in the Ovsynch group. Pursley et al. (1997a) and Stevenson and Phatak (2005) also reported that breeding lactating cows that are < 75 DIM following Ovsynch TAI protocol yields lower PR. This is very useful information for a dairy producer when making management decisions to breed their post-partum cows. For example, the average VWP before inseminating post-partum cows was 68 DIM in the field study (Chapter 2) conducted on local dairy farms and 58% of the farms used a form of estrus synchronization for breeding their animals, with the majority of those farms using Ovsynch. For those producers that breed their animals before 75 DIM and use Ovsynch, adding Presynch or a post-AI treatment with GnRH has the potential to increase their PR.

Pregnancy rates in heifers in this study were high compared to what others have observed in heifers bred to Ovsynch TAI (Pursley et al., 1997a; Rivera et al., 2005), even though there was a high rate of heifers that came into estrus before TAI. Results from this study demonstrated poorer PR in heifers bred less than 14.6 mo of age or weighing less than 380 kg. This again is valuable information for a producer, when deciding when to start breeding heifers. Often producers push to breed their heifers as early as possible so that these animals can begin producing milk earlier in their lifetime. However, body weight and age are important factors in the reproductive maturity of heifers and breeding too soon, when heifers are smaller, can also result in increased calving difficulties, decreased milk production, and increased days open in first lactation service breeding (Heinrichs, 1993). Finally, while synchronization rates were greater in lactating cows, heifers had greater PR, which was likely due to better functional CLs at day 7 post-insemination and lower embryonic losses. In a recent study in our lab, lactating dairy

cows had reduced expression of genes regulating angiogenic, steroidogenic, and luteotropic factors in the CL compared to heifers. These factors play a critical role in the development and survival of embryos (Pretheeban et al., 2010).

High P_4 concentrations of have been shown to have an inhibiting affect on LH release from the pituitary gland in response to exogenous GnRH (Robinson et al., 2000). Treatment with pLH or hCG acts directly on the ovary, has longer stimulatory effects, and has been shown to result in increased P_4 concentrations after treatment. In Chapter 4, the effects of pLH and hCG in lieu of GnRH in an Ovsynch TAI protocol on ovulatory response, synchronization rates and PR, and P_4 were compared in lactating dairy cows. No differences were observed in ovulatory response, synchronization rate, or PR between treatments. Progesterone concentrations in pregnant animals were greater for the hCG group on days 7, 11, and 14, and greater for the pLH group on day 11 than in the GnRH group. Since Colazo et al. (2009b) has already demonstrated that replacing the second GnRH treatment with pLH can increase PR, there may also be potential for increasing PR when the second GnRH treatment is replaced with hCG. Further investigations are needed to determine the optimal time of insemination when using pLH or hCG in a TAI program and regarding the influence of elevated LH concentrations during the peri-ovulatory period on oocyte maturation and post-fertilization development.

Chapter 5 incorporated the effects of diet on fertility with the advantages of using Ovsynch TAI. Feeding high protein diets to lactating cows causes high systemic ammonia and urea concentrations, which result in decreased uterine pH and P_4 concentrations, poor oocyte, embryo, and CL development, and exacerbation of NEB, thereby reducing fertility (Butler, 1998). Milk production was on average almost 1 kg

lower during the 7 wk that cows were fed the lower protein diet, which may be partially explained by the palatability of the low protein diet. With improvements and consistency in diet modeling programs, the formulation of lower protein diets allows for less risk to reduce milk production (Tylutkai and Van Amburgh, 2010). While the cows fed the high protein diet had higher MUN compared with the cows fed the low protein diet, these MUN concentrations were still low. This may indicate the use of a well-formulated diet on the farm where the study was conducted. Higher PR were achieved in primiparous and 2nd lactation cows fed the lower CP diet. In the US the average number of lactations in dairy cows declined from 3.2 in 1980 to 2.8 in 1994 (Hare et al., 2006b) and in Canada the average number of lactations ranged from 2.3 to 3.4 lactations in 1998 (Jairath et al., 1998); therefore a significant portion of cows could benefit from feeding a lower protein diet.

Strengths and Limitations of Dissertation

This dissertation emphasizes the importance of improving PR in lactating dairy cattle. The field study (Chapter 2) provides further evidence of the loss of reproductive performance due to post-partum cyclicity, estrus detection, ovulation and CL development failure, and embryonic loss. This dissertation also advances the current knowledge of using ovulation synchronization protocols to improve PR in cattle. The current research confirms what other researchers (Pursley et al., 1997a; Stevenson and Phatak, 2005) have also demonstrated: lactating dairy cows bred to an Ovsynch TAI protocol have lower PR when bred < 75 DIM. Also, this dissertation demonstrates that age and weight may be a factor in the success of pregnancy in heifers that are bred to a

synchronization protocol; this has not been examined in other research. Evidence from this research indicate that feeding a lower protein diet to lactating cows has the potential to increase PR with minimal effects on milk production, as there was a tendancy for first and second lactation cows to have greater PR. Feeding a lower protein diet also results in a decrease in nitrogen excretion, which is important for environmental concerns.

One limitation to a $PGF_{2\alpha}$-based pre-synchronzation protocol is that follicular and luteal stages are still not precisely synchronized, due to the variability in time to estrus/ovulation following $PGF_{2\alpha}$ treatments (Souza et al., 2008). As well, Presynch cannot induce cyclicity in anovular cows because of the lack of the presence of a CL. Bicalho et al., (2007) added a CIDR supplementation to a Presynch protocol in hopes of inducing anestrous and anovulatory animals, which do not respond well to GnRH-$PGF_{2\alpha}$-based synchronization protocols. No increase in PR was found, although the incorporation of the CIDR inserts into a Presynch protocol reduced the proportion of cows expressing estrus after the second $PGF_{2\alpha}$ treatment, and consequently improved timing of initiation of the Ovsynch protocol (Bicalho et al., 2007). A more recent pre-synchronzation system, Double-Ovsynch, used Ovsynch as the presynchronization method to increase cyclicity of anovular cows prior to beginning Ovsynch and found improved first service PR compared to Presynch-Ovsynch (Cunha et al., 2008; Souza et al., 2008). Pre-synchronization of cows with Double-Ovsynch increased the percentage of cows with high circulating P_4 concentrations at the last $PGF_{2\alpha}$ treatment (Souza et al., 2008).

Luteal insufficiency is one of the causes for pregnancy failure in dairy cattle (Ambrose et al., 1998). A delay in the normal rise in P_4 concentrations around days 4 to 6

post-insemination have been associated with negative effects on embryo survival (Ambrose et al., 1998; Changes e Silva et al., 2002; Starbuck et al., 2004; McNeill et al., 2005; Stronge et al., 2005). A review by Lewis et al. (1990) reported that post-insemination treatments with GnRH increase PR by about 5%, while Howard et al. (2006) found no differences in PR. High producing cows in early lactation have a high rate of metabolism and the continuous high plane of nutrition for lactating cows appears to chronically elevate liver blood flow and metabolic clearance rates of steroid hormones (Starbuck et al., 2004). Moreover, NEB is exacerbated and P_4 clearance increased when excess CP is fed (Butler 1998). A post-insemination intervation may be too late to prevent embryonic loss, as the oocyte or embyo may already be compromised. Many other factors are also associated with embryonic mortality, including genetic abnormalities, nutrition, stress, infectious causes, early conception, endocrine, uterine environment, and the age of the animals (Gordon, 2004). Most genotypic abnormalities, such as expression of lethal genes or inappropriate gene expressions, will cause death of the embryo within the first 2 wk of pregnancy (King, 1990; Bilodeau-Goeseels and Kastelic, 2003). Our lab recently observed differential expression of some developmentally important genes in embryos and in the endometrium and CL of heifers vs. lactating cows (Pretheeban et al., 2009; 2011). Moreover, our lab has also observed differences in embryo quality between heifers and 2^{nd} and 3^{rd} parity lactating cows (unpublished data). Further research on the expression of genes within the endometrium or ovary that important for the maintenance of pregnancy in relationship with nutrition and diseases may provide more thorough understanding into the poor fertility observed in lactating dairy cows.

While each of the experiments reported in this dissertation consisted of a large number of animals; PR are highly variable. When animal numbers are broken down into smaller proportions to further analyse for particular factors it can be challenging to expand on the biological relevance beyond the population of values. For example, in Chapter 3 significant differences were detected in heifers that were bred at a younger age and smaller weight. More studies are needed to confirm the findings before recommendations can be made to producers about when to breed their heifers. While other researchers have reported that breeding cows to Ovsynch before 75 DIM results in lower PR, further studies should be completed to confirm that Presynch or a post-AI treatment increase PR to cows bred earlier than 75 DIM.

In the experiment in Chapter 4, it would have been useful to determine cyclicity of the cows before the experiment began. Although anolvular cows have been found to be well synchronized by an Ovsynch protocol, they have reduced fertility to the TAI protocol (Gumen et al., 2003). Human chorionic gonadotropin or pLH may have beneficial affects on anovluar cows because of the possible benefits on the competence of oocytes and CL development. As well, timing of insemination relative to last treatment was not pre-determined before the experiment and this could have had an affect on timing of ovulation. In the future it would be of interest to investigate the timing of ovulation using pLH or hCG to induce ovulation following a timed AI program and further investigate the mechanisms behind oocyte and embryo competence following treatment with pLH or hCG.

In chapter 5, issues with palatability of diet to cows may have been the cause of the reduced intake and therefore the lower milk production observed during treatment.

Formulation of diet for forage content could be improved, since this can result in increased rumen fill and therefore more saitey to hunger. It is difficult to determine whether the lower milk production was due to a decrease in intake or the specific diet. Since NEB can have interactive effects with diet and milk production it would have been useful to determine the NEB of cows during the early post-partum period. As well, it would have been valuable to determine uterine pH and to examine what its effects were on the oocyte and embryo development between cows fed the high or low protein diets. High urea concentrations can affect protein receptors and expression of genes within the embryo, such as INFτ, and HSP. Also, the lower protein diet was introduced only 1 wk before Ovsynch was initiated. Hendrickson et al. (2003) proposed that subordinate and small follicles from a previous follicular wave can join the next wave to compete for dominance. If this is the case, then it may be beneficial to begin feeding the lower protein diet earlier. Future studies to address these issues are pertinent.

Closing Discussion

While the dairy industry has made tremendous advances with genetics, feed efficiency, and milk production, they have been achieved at a huge cost. Post-partum health disorders and poor reproductive performance of cows have become and remain the lead concerns for dairy producers. Treatment of these problems is costly and can directly affect long-term milk production profits.

Advances in reproductive technologies have lead to the widespread use of AI, increasing the need for accurate detection of estrus, and the development of estrus and ovulation synchronization protocols. While these protocols can be extremely beneficial in

an industry with increasing herd sizes coupled with minimal labour time to watch for animals in heat, and with dairy cows which are not expressing estrus as strongly or frequently, these protocols still do not resolve the issue of the drastic decline in PR. The lactating dairy cow in the 1950's had first service CR of about 60%; the modern dairy cow now only has CR of about 20% (Butler, 1998; Norman et al., 2009). Meanwhile, dairy heifers have not experienced this decrease and fertility remains high in these animals.

The Ovsynch protocol and other protocols alike were developed as management tools for producers to reduce the problems faced with estrus detection and to increase the number of animals subjected to AI. More and more protocols, including pre-synchronzation and resynchronization regimes are being developed to try to increase PR. However, these synchronization protocols can involve several hormone injections to the animals, which increase costs and require labour and farmer compliance, detracting from the actual benefits of these protocols. While some of these protocols improve PR, these rates are still not considered satisfactory. The benefit of these protocols also depends on individual farms. With so many variations in synchronization protocols available for a producer to use, they may not know which is most suitable to their farm. Recently, more economic modeling software has been developed to address these issues (Giordano et al., 2010).

Even with the use of these synchronization protocols, the dairy industry is still faced with poor reproductive performance. With the selection of cows for high milk production, nutrition and diet have become very important to stimulate and support this feat. High-producing dairy cows are fed specially formulated diets high in energy and

protein. The modern dairy cow is a highly efficient animal converting its feed into milk. So efficient, that the physiological mechanisms in the body increase the partitioning of nutrients towards producing milk. As a result, the dairy cow is more susceptible to an array of metabolic and health disorders, such as milk fever, ketosis, NEB, metritis, mastitis, and lameness – all of which can exert residual effects on fertility and even milk production.

A major challenge for dairy producers continues to be maintaining healthy dairy cows during the transition period centered around the time of calving. During this time there are many physiological, metabolic, and endocrine challenges related to calving and the onset of lactation that increase the cows' susceptibility to disease. Dairy cows must also adapt to numerous management challenges such as social regroupings and changes in diet. Despite the research in the area of transition cow health and management the high incidence of health disorders around calving continues to negatively affect cow reproductive performance and milk production (Mulligan and Doherty, 2008). Recently, researchers have shown that reduced feed intake prior to calving increased the risk of cows developing metritis (Hammon et al., 2006; Huzzey et al., 2007) and sub-clinical ketosis (Goldhawk et al., 2009). It is important to be able to prevent or diagnose infections and health disorders early because of their profound influence on fertility and milk production. Cows with metritis have increased days between first service after calving and conception (Fourichon et al., 2000). Moreover, Wittrock et al. (2011) demonstrated that multiparous cows diagnosed with metritis had reduced feed intake after calving, lower long-term milk yield, and increased chance of culling.

A common feeding practice is to switch dairy cows to a low forage, energy dense diet 3 wk pre-partum in hopes of increasing dietary energy intake for milk production. However, this practice has been criticized because it may lead to the overconsumption of energy and increase the risk of postpartum metabolic disease (Dann et al., 2006). In a recent study, Vickers (2011) observed fewer animals diagnosed with sub-clinical ketosis, a tendency for fewer cows diagnosed with metritis, and a tendency for more cows to be pregnant by 120 DIM for cows fed a high forage versus those fed a lower forage pre-partum diet. Future progress in the area of post-partum health must combine our understanding of nutrition, metabolism, physiology, immunology, and behaviour to determine the relationships between these stressors and disease and to develop management strategies that will reduce the incidence of disease after calving and thereby improve fertility (Huzzey et al., 2007).

Other nutritional management strategies that researchers are focusing on include examining the effects of soybean on reproductive performance in cattle. Soybean meal is a very common feed ingredient in dairy cattle rations. However, more evidence points to negative effects of soybean on fertility because of its high phytoestrogen content. Phytoestrogens are capable of exerting estrogen-like effects when fed in high quantities (Maggiolini et al., 2001). Phytoestrogens can increase $PGF_{2\alpha}$ secretion from the endometrium in cattle (Woclawek-Potocka et al. 2005ab) and inhibit LH-stimulated P_4 secretion (Piotrowska et al., 2006). Mlynarczuk et al. (2011) demonstrated that phytoestrogens affect the synthesis of oxytocin in follicles and CL. Oxytocin plays an essential role in the growth and development of ovarian follicles (Okuda et al. 1997), supports luteinization of the granulosa and theca interna cells (Tallam et al. 2000), and

stimulates the synthesis of P_4 (Miyamoto and Schams 1991). Adams et al. (1995) observed that cows fed soybean diets suffered from irregular estrus, nymphomania and anestrus. Most recently, Kowalczyk-Zieba et al. (2011) demonstrated that cows inoculated with lipopolisacharide to induce mastitis and metritis had increased daidzein and genistein (isoflavone phytoestrogen compounds) absorption, biotransformation, and metabolism due to the acute activation of the enzyme β-glucuronidase from the immune system mobilization. This provides evidence that when cows are already immunocompromised, isoflavone concentrations in the blood may influence the secretory functions of the reproductive system and impair fertility further.

Researchers have demonstrated that the inclusion of omega-3 polyunsaturated fatty acids in diets have the potential to improve PR (Ambrose et al., 2006a; Petit and Twagiramungu, 2006) and milk and plasma fatty acids, which can provide health benefits to humans through value added consumption, in lactating dairy cows (Lock and Bauman, 2004). Much research has centered on feeding flax, rich in α-linolenic acid, and fish meal/oil, rich in docosahexaenoic acid and eicosapentaenoic acid, to dairy cows (Santos et al., 2009). Docosahexaenoic acid and eicosapentaenoic acid are also found in very high concentrations in marine algae, which offers an alternative renewable resource to declining fish stocks. There is evidence that the biological effects of various omega-3 fatty acid sources and the balance of specific omega-3 fatty acids can affect lipid metabolism and PG synthesis differently, however this has not been thoroughly investigated (Wamsley et al., 2005). It is proposed that omega-3 fatty acids reduce embryonic mortality in part by suppressing $PGF_{2\alpha}$ production and by affecting the expression of specific genes and proteins (such as estrogen, oxytocin, and progesterone

receptors) in the uterus associated with embryo development and maintenance of pregnancy (Bilby et al., 2006). Marei et al. (2009) demonstrated that omega-3 fatty acid supplementation affected the molecular mechanisms controlling bovine oocyte maturation *in vitro*, thereby improving embryo development. Colazo et al. (2009a) also demonstrated that cows fed diets enriched in linoleic or linolenic fatty acids had a decreased incidence of ovarian cysts and ovulated sooner with no effect on energy balance or PR.

A high incidence of early embryonic mortality, which generally occurs during the period of maternal recognition, significantly contributes to the low PR observed in dairy cows. Maternal recognition is accomplished through a series of signals between the uterine environment and the embryo, inhibiting the expression of endometrial estrogen, oxytocin, and progesterone receptors, thereby preventing the release of $PGF_{2\alpha}$ (Mattos et al., 2000, 2002). Studies of mRNA and protein expression in the endometrium and ovary of many species reveal quantitative and qualitative changes in the expression of key regulatory factors at different stages of the estrous cycle and during pregnancy. It is important to analyze the endometrial, oviductal, and ovarian mRNA and protein expression levels of candidate genes and proteins as they can be influenced by diet and the uterine environment. Pretheeban et al. (2011) observed greater amounts of mRNA for factors related with steroidogenesis (3-beta-hydroxysteroid dehydrogenase), angiogenesis (fibroblast growth factor-2, vascular endothelial growth factor, and IGF-I), and luteal maintenance (interlukin 1α) in the CL obtained from heifers compared to lactating dairy cows. These differences in mRNA expression may also be evident in cows that are fed different diets, such as diets with lower protein content, have the inclusion of omega-3

fatty acids, or have no soybean meal. This can provide for evidence into the full comprehension of the influence of difference parameters on fertility.

Genetic selection for fertility has been difficult due to the low heritability of traits and the dairy industry's strive for high milk production (Veerkamp and Beerda, 2007). Recent advances with the use of genomics and proteomics in the study of reproduction will generate much greater information regarding the selection of cows with superior fertility (Moore and Thatcher, 2006). Already genomics is being used in selection of sires for AI. With genome mapping and the discovery of genetic markers that are related to reproduction, fertility, and health, there is the tremendous potential to improve reproductive performance in lactating dairy cows in the future. Understanding the differences in gene and protein expression under different management conditions will enable researchers to differentiate between good or poor fertile cows and identify those genes that regulate receptors and proteins important for embryo development and maintenance of pregnancy. Recently, Verbyla et al. (2010) demonstrated that genomic selection could be used to select for energy balance and the potential use of genomic selection of this trait and others could be incorporated into selection programs.

Finally environmental conditions can have a major influence on a cow's ability or tendency to exhibit signs of estrus. Overcrowding, the presence of slippery floor surfaces, and hoof problems can restrict mounting activity. Future research should also focus of the use of estrus detection aids that measure activity of cows. The recent NAHMS (2009) summary observed only a 21.1% use of these aids. Data from Chapter 2 field study also indicated a low rate of use. Estrus detection aids, such as electronic activity monitors (e.g. pedometers, HeatWatch®) and pressure sensitive markers (e.g. Kamar®, Estrotect ™)

can be useful management tools to help overcome the problems faced with estrus detection, which may help to move in a direction of using less hormone applications. The use of hormone regimes in the dairy industry may become a contentious issue in the future.

Successful management strategies that integrate the disciplines of reproduction, nutrition, post-partum health, and genetics are imperative for optimizing both milk and reproductive performance in lactating dairy cows. In the meantime, costs associated with synchronization treatments, should be considered before implementation of programs (Tenhagen et al., 2004a, b). Such costs may offset benefits to reproduction in herds with good estrous detection rates (Tenhagen et al., 2004a, b). Moreover, labour time and farmer compliance need to be considered when implementing treatments.

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APPENDIX

Appendix A: Field Study Questionnaire

DAIRY FARM QUESTIONNAIRE:

A Field Study on the Current Reproductive Performance of Dairy Cows in the Fraser Valley Area.

Part 1: Farm Management

1. Please indicate the number of cows you are currently milking.
   _____
2. Please indicate the number of current dry cows in your herd.
   _____

3. Please indicate the breed(s) of cow you are milking.
   _____

4. What type of barn are your cows housed in?
   a) Free Stall
   b) Stanchions
   c) Both
   d) Other _____

5. What type of milking parlour do you have? How many cows can be milked at one time? _____

6. How many times a day are your cows milked? _____

7. At what time of day are your cows milked? _____

8. Do you intend to reduce, expand or maintain quota levels for your operation over the next 4 years?_____

9. Are you a member of DHI? Yes / No

10. Do you use a dairy computer program? Yes / No.
    If so please indicate which program _____

11. Please indicate the major source of your replacement animals
    _____

Part 2: Reproductive Management

12. Is artificial insemination employed on your farm? Yes / No
    If so, please circle those that apply
    a. Inseminations are performed only by farmers
    b. Inseminations are performed only by AI technicians
    c. Inseminations are performed by both farmers and AI technicians
        i. What is the proportion (%) of AI conducted by farmers? _____

13. Is AI used in combination with a bull? Yes / No
    If so, what percentage of the herd is serviced by the bull?
    _____
    What is the purpose of the bull?
    a. Clean up bull
    b. To breed heifers
    c. To detect estrus
    d. Other _____

14. Do you have a voluntary waiting period before you breed your post-partum cows? Yes / No. If yes, how long? _____

15. Do you have regular herd health checks with a veterinarian? Yes / No. (If yes, proceed to parts a,b,c)
    a) How often are your herd health checks? _____
    b) Normally, how many days in milk are your cows when a vet first examines them after calving? _____

c) Are your postpartum cows treated with anything? If so what and why? _____
_____
_____
_____

16. Please describe what your farms routine is for observing/detecting estrus? _____
_____
_____
_____

17. Please indicate the signs your farm uses to detect whether a cow is in heat (other than estrus detection aids described in question 21) _____
_____

18. Does your farm rely on any forms of estrus detections aids to help determine time of breeding? Yes / No
Please list aids used:
    a. Pedometers
    b. High activity monitoring
    c. Chalk marker
    d. Heat mount detector
    e. None
    f. Other, please specify _____

19. Who is assigned estrus detection duties? _____

20. Are there particular times during the day that most estrus detection is done? _____

21. Do you use any estrus synchronization on your cows? Yes / No
If yes, please indicate what your protocol is: _____

_____
_____
_____
_____

22. What is your current herd pregnancy rate? _____
    and/or
23. What is your current herd non-return rate? _____

24. What is the average days open of your cows on your farm?
    _____

25. What is the percentage of cows that require 2 or more breedings? _____

26. Indicate the three most common reasons for culling animals over the last year.
    1. _____
    2. _____
    3. _____

27. Indicate the three most frequent health concerns:
    1. _____
    2. _____
    3. _____

28. Does your farm use any other reproductive technologies?
    a. Embryo transfer
    b. Sexed embryos
    c. Sexed sperm
    d. Ultrasonography
    e. Other _____