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MANIPULATING FOLLICLE AND CORPUS LUTEUM FUNCTION TO IMPROVE EFFICIENCY OF SYNCHRONIZATION OF OVULATION IN LACTATING DAIRY CATTLE

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MANIPULATING FOLLICLE AND CORPUS LUTEUM FUNCTION TO IMPROVE EFFICIENCY OF SYNCHRONIZATION OF OVULATION IN LACTATING DAIRY CATTLE.

By

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Abstract

MANIPULATING FOLLICLE AND CORPUS LUTEUM FUNCTION TO IMPROVE EFFICIENCY OF SYNCHRONIZATION OF OVULATION IN LACTATING DAIRY CATTLE

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The synchronization of ovulation (Ovsynch) program is an effective tool for dairy managers to control calving interval. However, the program may be more widely adopted if conception rate following the program was higher or if Ovsynch required less labor. Objectives of this thesis were to: 1) determine if initiating Ovsynch at mid-cycle increased conception rate, 2) determine if conception rate and synchronization rate after treatment with Ovsynch are depressed when the second gonadotropin releasing hormone (GnRH) and the prostaglandin $F_{2\alpha}$ are administered simultaneously, 3) determine the optimal time between the prostaglandin $F_{2\alpha}$ injection and the second GnRH injection of the Ovsynch protocol for maximal conception rates. To address objective one, a novel program was developed (OVPLUS). OVPLUS succeeded in synchronizing cows in a mid-luteal phase before Ovsynch. However OVPLUS did not increase conception rates. For objective two, a program was tested that administered the prostaglandin $F_{2\alpha}$ injection at the same time gonadotropin releasing hormone was administered. When these two injections are administered simultaneously synchronization rate was similar but conception rate was reduced when compared to administering the two injections 48 h apart. For objective three, four time periods between the prostaglandin $F_{2\alpha}$ and the final GnRH injection of Ovsynch were tested. Conception rate was greatest when prostaglandin $F_{2\alpha}$ was injected 36 h before last GnRH.

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INTRODUCTION

Pregnancy is the greatest stimulator of lactation (*68*). However achieving pregnancy in lactating dairy cows is becoming increasingly difficult. In the 1950's conception rates for lactating dairy cattle were approximately 66 % (*65*). In the 1970's conception rates declined to 50 % (*65*). In the early 1980's conception rates were reported to be 46 % (*21*). Presently, conception rates are at an all time low of approximately 40 % (*49*). During this time period, estrus detection rates have also declined dramatically (*59*). Traditionally artificial insemination of cows requires detection of estrus. To facilitate use of artificial insemination methods have been developed to synchronize estrus in cows (*35*), facilitate detection of estrus behavior (*16*), and synchronize ovulation to circumvent estrus detection and allow for timed insemination. Synchronizing ovulation is useful because it removes the need to detect estrus, thus increasing number of cows inseminated. Currently synchronization of ovulation results in conception rates that are similar to conception rates after estrus detection. However, increasing conception rates after treatment with Ovsynch, or reducing labor needs to perform the hormone injections, may increase the number of dairy producers who adopt Ovsynch. This thesis test adaptations to Ovsynch that may accomplish the two ideas listed previously.

Chapter 1

Review of Literature

Rationale for controlling ovulation

It is possible for a dairy cow to produce milk for up to four years following one parturition. However, at some point milk production drops below a profitable level. There is no profit when the maintenance costs for a low producing cow exceeds the income from milk sold. To maintain a profitable dairy herd, milk production must stop and be restarted. The best method for restarting lactation is a cow completing a healthy gestation followed by parturition (*68*). However achieving a pregnancy in lactating dairy cattle is becoming increasingly difficult. In the 1950's, conception rates for lactating dairy cattle were approximately 66 % (*65*). In the 1970's, conception rates declined to 50 % (*65*). In the early 1980's, conception rates were reported to be 46 % (*21*). Presently, conception rates are at an all time low of approximately 40 % (*49*). Conception rates are affected negatively by shorter days to first service and high milk production (*25*). It is also difficult to detect estrus in lactating dairy cows (*59*). A cow only displays estrus for an average of 7.1 h (*9*). During the 7.1 h period cows have an average of 8.5 occurrences of standing to be mounted (*9*). Consequently the average estrus detection rate in dairy cattle is below 50% (*59*). Failure to detect estrus or false diagnosis of estrus results in an estimated loss of over $300 million to the US dairy industry each year (*59*). Since the inception of artificial insemination (AI), dairy producers rely on estrus detection to know when to AI cows. These low estrus detection rates and conception rates lead to an estimated pregnancy rate (PR) of 20%. (A definition of PR is estrus detection rate x conception rate). Maintaining an efficient dairy operation is difficult with a 20% PR, because cows that do not become pregnant are forced to have long inefficient lactations. Farm management may decide to cull cows instead of

continuing to try to inseminate them. A replacement must then be raised or purchased to maintain lactating cow numbers.

To gain a better understanding of poor reproductive performance in lactating cows, previous research has focused on the basic mechanisms of dairy cattle reproduction. Applied research then focused on ways to employ basic findings to assist producers in achieving pregnancy in dairy cattle. A key application of basic reproduction research is the ability to synchronize ovulation in dairy cattle. Controlled ovulation allows for timed insemination without the need to observe cows for estrus activity. By synchronizing ovulation in cows scheduled for AI, 100% of the cows can be inseminated on the first day of the breeding period without waiting for the cow to display estrus. This is equivalent to a 100% estrus detection rate for first AI, and results in a 100% service rate. When applied to the formula above; a 40% PR can be attained. The benefits from synchronization of ovulation are demonstrated in a 1997 paper from Pursley et al. They demonstrated the power of breeding all cows on a predetermined day. In this study the control cows were inseminated only after visual detection of estrus. The treated cows had ovulation synchronized with a program called Ovsynch. Treated cows received their first insemination by 50 to 57 days in milk (DIM), with an average of 54 d. The control cows averaged 83 DIM at first insemination and ranged from 50 to 160 DIM. The second and third inseminations also occurred earlier and with less variation compared to the control group. The earlier times of AI in the Ovsynch group resulted in a shorter median interval to conception for Ovsynch (99 d) vs. the control group (118 d; *47*).

This literature review will address the basic and applied research that formed the foundation for development of the synchronization of ovulation method. The first section

will summarize the literature on the basic biology of follicular growth, also known as folliclulogenesis. The second section discusses applied dairy cattle reproduction research and current pharmacological techniques used to control estrous cycle and ovulation in cattle.

Bovine Folliculogenesis

In 1672 Regnier de Graaf first discovered and described antral ovarian follicles. However, de Graff thought the whole follicle was the egg, instead of an oocyte within the follicle (*60*). Since the time of de Graff, the knowledge of follicular function and growth has evolved immensely. It is now well understood that bovine follicles grow in wave-like pattern that is under strict hormonal control.

Bovine Follicle Growth

In 1960, Rajakoski did histological studies of bovine ovaries (*50*). Cows and heifers were slaughtered on a known day of an estrous cycle. Ovaries of the slaughtered cattle were collected and examined. All antral follicles were counted, measured, and recorded. Based on these results he proposed that two waves of follicular activity occur during each estrus cycle. Rajakoski reported that a "crop" of follicles appeared at the beginning of the cycle and by day 5 one follicle continued to grow while the other follicles within the cohort became atretic. The second wave appeared on approximately day 12 of the cycle and resulted in recruitment of the ovulatory follicle. In 1972, Dufour et al. (*10*) used a laproscope to mark the largest and the second largest follicle on the ovary with India ink. Follicles on the ovaries of heifers were marked on days 9 through

21 of the estrous cycle. Ovaries were then removed 3 days after subsequent estrus and examined. A new corpus luteum surrounded by India ink was an indicator that one of the marked follicles ovulated. They reported that after day 18 postestrus, the largest follicle on the ovary ovulated. However, before day 18, the largest follicle on the ovary was never the ovulatory follicle. While unknown to them at the time, these data helped foster the theory of follicular waves by showing that the ovulatory follicle does not become the largest follicle on the ovary until late in the cycle. The idea of follicular waves was tested again in 1981, when Matton et al. (*38*) published histological data augmenting Rajakoski's theory of two follicular waves. The author used a laproscope to mark the largest follicles in 20 animals on either day 3, 8, 13, or 18 of the estrous cycle. The other 20 of the animals had the largest follicle destroyed and their next largest follicle marked with India ink on the same days of the cycle as above. Heifers in the day 3, 8, and 13 groups were slaughtered 5 days after surgery. Heifers in the day 18 group were slaughtered one day after estrus. Ovaries were examined post-mortem for follicular changes or ovulation. Matton's results supported the theory of rapid follicle turnover. Also, they postulated that continued growth of medium sized follicles occurs only after the largest follicle has been destroyed. In 1983, more data was published that supported the theory of follicular waves (*28*). Ireland and Roche used blood steroid levels, follicular measurements, and follicular fluid steroid levels to argue that two or three waves of growth occurred during each estrous cycle in heifers. Thirty heifers were ovariectomized on day 5, 7, 9, 11, 13, or 15 of the estrous cycle. Follicles larger than 6 mm were categorized as estrogen active or estrogen inactive based on estradiol concentrations within the follicular fluid. They reported that at least two waves of

follicle growth occurred during days 3 to 13 of each estrous cycle. On days 9, 11, and 13 of the cycle only one estrogen inactive follicle was reported for each heifer, all other follicles were estrogen active. On days 5, 7 and 15 of the cycle one follicle was estrogen active while all others were estrogen inactive. Their paper provided endocrinological evidence for follicle waves by demonstrating that a group or cohort of estrogen active follicles emerge at the beginning of each follicle wave. From this cohort of estrogen active follicles a single estrogen active follicle emerges. These data augment the theory that on approximately day 5 of a follicle wave only one follicle is estrogen active. This phenomena is referred to as dominance. Then on approximately day 9 of the wave the dominant follicle becomes estrogen-inactive and with atresia (loss of dominance) another wave of estrogen active follicles begin to grow. The hormonal control of follicle dominance will be discussed further in the section entitled *Endocrinology of Follicle Waves in the Bovine* (p 14).

Prior to the mid-1980's, progress in the study of follicular dynamics was limited because animals had to have invasive surgery or be slaughtered to observe ovarian changes. These techniques can be expensive and make it difficult to acquire large amounts of sequential data. A 1984 publication reported the use of transrectal ultrasonography to monitor growth of ovarian structures in the bovine (*43*). This noninvasive technological discovery allowed researchers to observe and monitor growth and atresia of antral follicles, ovulation, and corpus luteum growth and regression. Ultrasonography of the bovine ovary could be done as frequently as needed to address objectives. In the mid 1980's, the capabilities of ultrasound were expanded. The equipment manufacturers developed ultrasound emitting probes that could display

pictures of 2 mm follicles on the ovaries. Also, users of ultrasound technology became more adept at collecting and analyzing data derived from ultrasound sessions. Articles describing daily tracking of follicle growth flourished. Many of these papers reported that a majority of heifers had 3 follicular waves per cycle (*56,63*). Others reported that the majority of heifers had 2 follicular waves per cycle (*19*). Above and beyond identifying the number of waves per cycle, daily ultrasound observation of ovaries allowed researchers to characterize the follicle turnover that occurs during follicular waves (*19*). Independent of discrepancies in number of waves per estrous cycle, published data agreed on several key points regarding follicle waves. Ultrasound allowed researchers to determine that a new wave starts as a cohort of small follicles near the time of ovulation. Approximately three days into a wave, one follicle will become dominant and the others will become subordinate. Subordinate follicles become atretic. A second wave emerges around day 10 of the estrous cycle. If an animal exhibits three waves, the third and final wave emerges around day 16 of the estrous cycle. The ovulatory follicle develops from the final follicular wave (*17,20*). Ultrasound has also allowed researchers to demonstrate that follicle waves occur during pregnancy (*44*) and that growth hormone and diet influence follicle growth (*36*).

Applications of ultrasound technology for study of folliculogenesis are still expanding. Ultrasound technology has been used to identify atretic follicles by the pixel intensity of the image displayed on the screen (*62*). In addition to allowing researchers the ability to visually characterize follicle waves, ultrasonography has also given researchers a valuable tool to help correlate follicle growth and turnover with changes in serum hormone concentrations.

Endocrinology of Bovine Follicle Waves

The pituitary gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH) play a key role in regulation of follicle growth and atresia. Adams et al. demonstrated in cattle that an increase in plasma FSH is necessary to have a new follicle wave begin. Adams et al. inhibited FSH secretion in a group of heifers treating them with a proteinaceous fraction of bovine follicular fluid. This inhibition of FSH delayed the onset of a new wave of follicle growth. They also reported that an increase in serum FSH is detected at the onset of a new follicle wave (*1*). The increase in FSH causes a cohort of follicles to grow on both ovaries until one of the follicles becomes dominant. Shortly after one of the follicles establishes dominance the other subordinates become atretic (*32,33*). This point in the wave is referred to as deviation or selection of the dominant follicle. Coincident with deviation, the dominant follicle expresses LH receptors, responds to LH and continues to grow. In concurrence with dominance being established, FSH secretion from the pituitary decreases (*1,39*). Estradiol and the endogenous action of inhibin mediate the decline in FSH (*29*). The dominant follicle requires basal levels of LH to continue to grow. Also, once a dominant follicle acquires LH receptors on the granulosa cells it gains the ability to ovulate in response to an LH surge. If the dominant follicle grows in a proestrus wave it will ovulate. If the dominant follicle grows in a wave that occurs during metestrus or diestrus, it will become atretic and regress.

As knowledge of follicle growth continues to grow, a lot is still unknown about it. Mechanisms that dictate which follicles enter the cohort of a wave, and which follicle will become dominant are still unclear.

Pharmacological Control of the Bovine Estrous Cycle

History

The first published reports of successful AI in cattle were in 1902 by a Russian scientist Ivanoff (*76*). Ivanoff adapted techniques done earlier in the horse and human. Semen was collected by placing a sponge in the vagina of a cow. The ejaculate was then squeezed out of the sponge. The semen was then placed into the vagina of another cow. Thirty years later another Russian scientist, Milovanov, demonstrated the power of this technology by artificially inseminating (AI) 19,800 cows using 100 cows per bull. Suddenly one bull could have hundreds of offspring believed to have high genetic merit. The power of AI was expanded when cryopreservation of sperm became possible in 1949. The discovery of glycerol as a cryoprotectant was accidental. But frozen semen could be transported all over the world to expand the genetic impact of superior bulls (*76*).

While AI has many advantages, there are challenges. The most obvious challenge is the need for the farm manager to detect the animals in estrus. As stated earlier, estrus detection in dairy cattle is difficult. It is generally accepted that the estrus detection rate on dairy farms is below 50% (*59*). To address the challenge to detect cows in estrus, there are several non-pharmacological methods to assist dairy managers. These include

(but are not limited to): measurement of electrical impedance in vaginal mucus (*74*), detection of estrus by a trained dog (*23,31,75*), pedometers that measure cow activity (*30*), devices that detect mounting activity in dairy cattle (*9,22*), milk progesterone analysis (*67*), and allowing cows access to dirt lots (*3*). While these non-pharmacological methods benefit the ability of dairy producers to detect cows in estrus, this literature review will not review them in depth. Rather, the following sections will focus on the pharmacological methods that are used to control the timing of estrus, timing of ovulation, and ultimately the timing of AI.

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) programs

In the 1970's Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was discovered. Early in $PGF_{2\alpha}$ research, it became known that exogenous $PGF_{2\alpha}$ causes a 5 day old or older bovine corpus luteum (CL) to regress. On days 1-4 of the bovine estrous cycle, $PGF_{2\alpha}$ will not cause CL regression (*34,55*). CL regression means serum progesterone concentrations decline. Declining progesterone and elevating estradiol produced from the dominant follicle will cause the cow to display estrus. It was reported that estrus occurs between 2 to 4 days after an injection of $PGF_{2\alpha}$ (*34*). With this information, researchers developed programs to control estrus. In 1973 Inskeep reported on the potential uses of $PGF_{2\alpha}$ (*26*). With emphasis on several methods that would cause timed expression of estrus in cattle, Inskeep further forecasted development of methods that would allow for timed AI.

Timing estrus in dairy cattle is advantageous for several reasons. A manager can focus estrus detection during a shorter period and specifically on the animals that were

treated. Also, if more animals are in estrus at one time it is easier to ascertain which animals are in heat, because they will display the signs of estrus with each other (*16*).

In 1974, Lauderdale et al. tested the fertility of cattle after exogenous $PGF_{2\alpha}$. Cattle with a CL were assigned to one of three treatments. Treatment I (controls) were observed for estrus activity twice daily. Cattle detected in estrus were inseminated 12 h after detected estrus. Cattle for treatment II received an injection of $PGF_{2\alpha}$ and were observed for estrus twice daily. They were inseminated 12 h after of estrus. Cattle for Treatment III cattle were time inseminated 72 and 90 h after injection of $PGF_{2\alpha}$. The percent animals pregnant at 35-60 days after AI did not differ among treatments and averaged 54%. Therefore, Lauderdale et al. proposed that $PGF_{2\alpha}$ could be used for timed AI if two inseminations were administered. Lauderdale et al. also reported that cattle would display estrus 1-7 d after $PGF_{2\alpha}$ injection. One to 7 d was longer than the period that he had previously reported (*35*). The 1-7 d window for cattle to exhibit estrus after a $PGF_{2\alpha}$ injection was confirmed in another study in 1978 (*2*).

In 1979, $PGF_{2\alpha}$ became available for commercial use. This spurred a number of researchers to test the applicability of programs that utilized $PGF_{2\alpha}$ on dairy farms. Dailey et al. showed that a single timed AI after a single injection of $PGF_{2\alpha}$ will not yield conception rates comparable to those achieved with estrus detection (*7*). However, two injections of $PGF_{2\alpha}$ administered 10 to 14 d apart resulted in a greater percentage of animals in heat 1-7 d after the second injection than when one injection of $PGF_{2\alpha}$ was used. The greater percentage of animals synchronized happens because at the time of the second injection all animals will have a CL that is responsive to $PGF_{2\alpha}$ and will regress. However, after a timed insemination this treatment regime does not yield conception

rates that are as great as conception rates after detected estrus (*64*). This is because estrus occured over a period of 6 d among treated animals. If estrus is displayed over a period of 6 d, ovulation does not occur over a short enough period of time to allow timed AI. Successful timed AI requires a synchronous ovulation. In spite of timed AI not being efficient with $PGF_{2\alpha}$ injections, $PGF_{2\alpha}$ is still a powerful tool for estrus synchronization. Seguin et al. reported on the applicability of using $PGF_{2\alpha}$ in a milking dairy herd (*58*). Half the cows of three herds were assigned to the control group (traditional estrus detection with no $PGF_{2\alpha}$ injection). The experimental group of cows received an injection of $PGF_{2\alpha}$ if a CL was present. After the injection of $PGF_{2\alpha}$, cows were observed for estrus. Cows in the experimental group received first AI at fewer days in milk when compared to the control (70 d vs 81 d respectively). Cows in the experimental group also conceived an average of 18 days sooner than the controls (93 d vs 111 d). It is also important to realize that with the use of $PGF_{2\alpha}$ to synchronize estrus, managers must avoid common pitfalls that will counteract the benefits of estrus synchronization. These include: thawing too many units of semen at once (*13*), falsely identifying animals in heat due to anticipatory zeal following $PGF_{2\alpha}$ injection (*58*), and relying on untrained help to detect estrus.

To decrease the period in which estrus is expressed after a $PGF_{2\alpha}$ injection, researchers combined $PGF_{2\alpha}$ with other exogenous hormone treatments. If estrus could be synchronized more precisely than over a 6 d period, timed AI may be possible. Estradiol was tested in conjunction with $PGF_{2\alpha}$ in 194 heifers. The hypothesis was that an injection of estradiol would cause a timed LH surge. A timed LH surge would lead to a timed ovulation, and thus a timed insemination would be possible. All heifers with a

CL present in an ovary were injected with $PGF_{2\alpha}$. Half the heifers were treated intramuscularly with 400 μg of estradiol benzoate 40 to 48 h after $PGF_{2\alpha}$. Timed insemination occurred at 72 or 80 h after the injection of $PGF_{2\alpha}$. Control animals were inseminated 12 h after detected estrus. Exogenous estradiol did not improve the number of heifers that were observed in estrus (86.7% for $PGF_{2\alpha}$ only vs 84.3% for $PGF_{2\alpha}$ and estrogen) or reduce the variability in time to estrus. In Dailey's study, conception rates were lower for animals that were inseminated at 72 h post-$PGF_{2\alpha}$ than controls (*7*).

Exogenous progesterone has also been hypothesized to assist in synchronization of estrus. Progesterone can be administered by way of a progesterone releasing intravaginal device (PRID). PRIDs allow for a sustained release of progesterone over the period of a week or more. This allowed for the maintenance of serum progesterone levels at a constant concentration. Fogwell et al. inserted PRIDs into heifers for 10 d. Seven d following PRID insertion, heifers were treated with $PGF_{2\alpha}$. On d 10 the PRID was removed. Treated heifers had a more homogeneous display of estrus, and a more synchronous LH surge. All treated animals inseminated at estrus did not have reduced fertility when compared to animals bred without progesterone manipulation (*12*). It was suggested from these findings that PRID's with $PGF_{2\alpha}$ may allow for a timed insemination of dairy heifers, but this program was not tested in this study. Other researchers tested the applicability of combining progesterone with $PGF_{2\alpha}$ injections. Smith et al. inserted PRIDs for 7 d and administered $PGF_{2\alpha}$ one day before PRID removal. Smith et al. also demonstrated reduced variability in occurrence of estrus after exogenous $PGF_{2\alpha}$. Heifers were either time inseminated at 84 h after $PGF_{2\alpha}$ or inseminated after detected estrus. Conception rate in heifers that received timed AI was

66 % and did not differ from the control group that was inseminated 12 h after detected estrus. These studies demonstrated that progesterone in conjunction with $PGF_{2\alpha}$ allow for successful timed insemination of heifers. However, PRIDs have not received FDA approval for use in the United States. Therefore, this option is not legally or commercially available to cattle managers at this time.

Using GnRH to Synchronize ovulation

Gonandotropin releasing hormone (GnRH) is FDA approved for use in lactating dairy cattle. GnRH has been proven to be effective in the treatment of ovarian follicular cysts (*8*). When GnRH is injected into cattle, a surge of LH begins within an hour (*6*). An LH surge causes a dominant follicle with LH receptors on the granulosa cells to ovulate (*47*). In 1995 a method (Ovsynch) was developed that uses GnRH to synchronize ovulation in cattle. The method was based partially on the theory that lactating dairy cows display two follicular waves per estrous cycle. Pursley et al. used ultrasound to monitor daily growth and atresia of follicles in lactating dairy cows and heifers. An injection of GnRH was administered at a random time of an estrous cycle. If a cow had an LH responsive follicle, it would ovulate, a new CL would form, and a new follicle wave would begin. Ninety percent of lactating dairy cows injected randomly with GnRH will ovulate a follicle (*47*). If a cow did not have an LH responsive follicle, the injection would do nothing. Seven d after the GnRH injection, an injection of $PGF_{2\alpha}$ was given to regress any CL present in the ovaries. Two d after the $PGF_{2\alpha}$, a second injection of GnRH was given. Cows that responded to the first injection of GnRH should now have a follicle that is 8 to 9 d old. If the animal did not respond to the first GnRH injection, she

is between d 10 to 12 of a follicle wave. Regardless of the earlier response an animal should have a dominant follicle that would ovulate to the second GnRH injection. Ovulation occurred 28 hours after the second GnRH injection, with a range of only 4 hours. This time to ovulation is similar to what is seen after estrus (*73*). In a study published by Pursley et al, 20 of 20 cows had a synchronous ovulation after treatment with Ovsynch. However in heifers, only 18 of 24 had a synchronous ovulation after treatment with Ovsynch (*47*). Because ovulation was synchronized, a timed insemination was possible after cows were treated with Ovsynch. In 1997, data was published that demonstrated applicability of Ovsynch to reproductive management on commercial dairy farms (*46*). At calving, cows were assigned to either a control (reproductive management protocol for each farm) or treatment (Ovsynch) group. Cows in the Ovsynch group could only be inseminated after treatment with Ovsynch. Ovsynch reduced days to first AI (54 treatment vs 83 controls) compared to controls. Days to second and third AI were also reduced. Conception rates for the first AI were the same for control and treatment, in spite of the fact that Ovsynch cows were bred at an average of 19 days sooner. Ovsynch led to more cows pregnant at 60 d post-partum (37% vs 5%) and more cows pregnant at 100 d post-partum (53% vs 35%) compared to controls. Another study published in 1997 (*49*) substantiated the previous reported results for lactating cows, but found unacceptable pregnancy rates in Ovsynch treated heifers (35.1% for Ovsynch vs 74.4% for controls). A Florida lab has further substantiated Ovsynch to be beneficial in controlling PR in lactating dairy cattle (*5*). The same lab has also reported that Ovsynch yields lower conception rates when used on heifers (46% Ovsynch vs 61% for AI 12 h after estrus)

(*57*). So while Ovsynch was a viable alternative for reproductive management of lactating cows, it should not be used for management of heifer reproduction.

The economic advantage of Ovsynch over traditional estrus detection remains controversial. One study demonstrated that Ovsynch provides economic advantages over traditional estrus detection (*4*). However, a 1998 paper on the use of synchronized estrus using $PGF_{2\alpha}$ injections and the Ovsynch protocol argued otherwise (*41*). They reported that the mean cost of a pregnancy using the $PGF_{2\alpha}$ program with a 40% estrus detection rate was $29.61. This was substantially lower than the mean cost of pregnancy from synchronization of ovulation, which was reported to be $46.10. The majority of the cost for the Ovsynch protocol was the GnRH injections. The average cost for a 100μg injection of GnRH was $7.27. To assist in making Ovsynch more economical, Fricke et al. tested the efficacy of using 50μg (half dose) of GnRH for each injection (*18*). They found that a half dose of one form of GnRH yielded the same synchronization rate and conception rate as a full GnRH dose. Now Ovsynch can be utilized at a decreased cost. Further work with modifying the Ovsynch protocol has not demonstrated that changing the sequence of or time between the injections yields conception rates higher than those of Ovsynch (*42,66*). Also there is no reported advantage in using hCG to ovulate a follicle instead of GnRH (*57*). Publications show that timing AI after treatment with Ovsynch may allow for alterations in the gender ratio and decreased pregnancy loss (*48*). Further research needs to be done on greater numbers of animals to substantiate these results.

Summary

Low estrus detection rates, coupled with low conception rates have led to difficulties managing reproduction of lactating dairy cows. To combat low fertility ultrasound and endocrinology research was done to gain a greater understanding of follicle growth. Years of work and many researchers have contributed to the knowledge of follicle waves in the bovine. Basic discoveries allowed researchers to define when a follicle would become responsive to an LH surge. With basic knowledge of follicle growth, CL formation and regression, and ovulation, there is an informed rationale for applied research to synchronize estrus and ovulation. Synchronization of estrus is a powerful management tool to control reproduction in cows and heifers. Synchronization of ovulation is a management tool that dairy mangers can use to control reproduction in lactating cows. Ovulation synchronization allows managers to control cow reproduction, and is a key to proactive versus reactive management.

Chapter 2

Starting Ovsynch in the mid-luteal phase of the estrous cycle failed to improve fertility of lactating dairy cows

Introduction

Synchronization of ovulation (Ovsynch) is a management tool to control time to first artificial insemination (AI) in dairy cattle (*46*). This in turn allows farm management to have more control of calving interval. Ovsynch accomplishes control of calving interval by allowing for timed insemination (*47*). Ovsynch creates the opportunity for managers to inseminate at a preset time with no need to observe cows for estrus. Compared to Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) programs, Ovsynch is more advantageous because the need to observe cattle for estrus can be eliminated and service rate is 100 %. Even with diligent visual observation for estrus after synchronization of estrus with $PGF_{2\alpha}$ some cows will still not be detected in estrus (*58*). Thus, service rate after $PGF_{2\alpha}$ is almost always less than 100%. Within a herd, conception rate following treatment with Ovsynch is similar to inseminations after observed estrus (*4,46,49*). Improving synchronization and conception rates after Ovsynch would elevate the percent of cows that become pregnant to an insemination. In lactating dairy cows conception rates were increased when progesterone (P_4) levels were increased 10-12 d prior to insemination (*14,15,52*). From these data it appears that if lactating cows were synchronized into the luteal stage of the estrous cycle (d 5 to 17) at the start of Ovsynch conception rates may be increased. Also, synchronization rates after Ovsynch are highest when Ovsynch is initiated d 1 through 12 of the estrous cycle (*71*). Increasing synchronization rate after Ovsynch could increase the number of cows conceiving after an AI. This study tested whether lactating dairy cows in luteal phase (d 5 to 179) of an estrous cycle at the time of the first injection of the

Ovsynch program will have greater fertility than cows that begin Ovsynch at a random stage of an estrous cycle.

Materials and Methods

From February to December 1997 lactating dairy cows (n=530) from two commercial dairy farms in Michigan were utilized. All cows were housed in freestall barns. All cows between 49 to 55 d in milk were assigned to either the control or the treatment group. All cows received AI at 69 to 75 d in milk. Only cows receiving their first postpartum AI were used in the study. The control cows received the traditional Ovsynch program

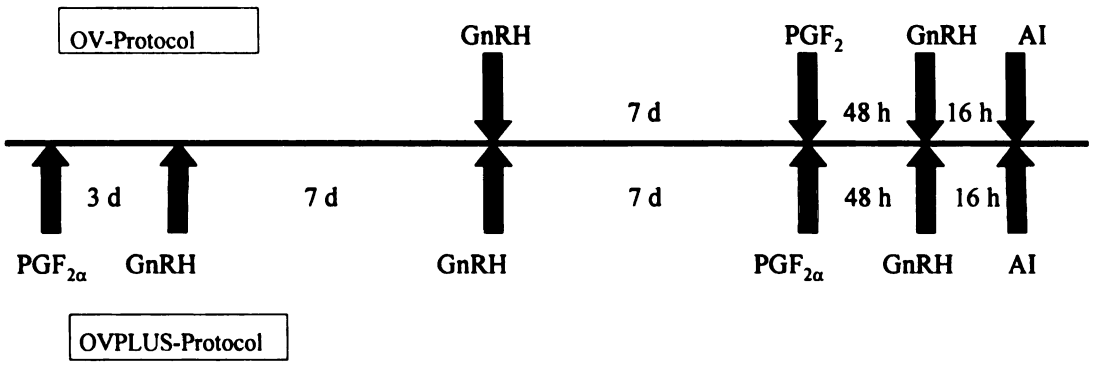

Figure 1. Description of the timing for each of the injections used in the OV group (control) and the OVPLUS group.

(Figure 1). The treated cows received $PGF_{2\alpha}$ 10 d and GnRH 7 d before Ovsynch (Figure 1). This program was labeled Ovsynch plus (OVPLUS). Laboratory staff administered all injections. The GnRH injections were Cystorellin® (Merial, Ltd., Iselin, NJ) administered at 100 μg/dose. The $PGF_{2\alpha}$ was Lutalyse® (The Pharmacia-Upjohn Co., Kalamazoo, MI) administered at 25mg/dose. Farm personnel performed all AI. Control and treated cows received injections in sequences that allowed for AI on the same

calendar date and the same week postpartum. The herd veterinarian diagnosed pregnancy at 36 d post AI by rectal examination of uterus for a conceptus. Conception rate data were analyzed using Chi Square analysis. Within treatments, a randomly selected subset of cows (n=84) had blood sampled from a coccygeal vessel. Blood was sampled at three times: at the time of the first GnRH of Ovsynch, 3 d after the first GnRH of Ovsynch, and at the time of the final $PGF_{2\alpha}$. Progesterone (P_4) in serum was quantified by radioimmunoassay (P_4 Coat-a-count kit, Diagnostic Products Cooperation, Los Angeles, CA). Intra- and interassay coefficients of variation were 5.6 % and 9.1 %, respectively. Milk production data were available from one of the farms used in this study. The average milk production nearest time of AI (milk weight measurement taken every two weeks) within each parity was determined to compare conception in rates in cows above the mean versus conception rates in cows producing below the parity mean. The average milk production was 75 pounds/day for first pairity, 109 pounds/day for second parity, and 110 pounds/day for third and greater parities. Cows that had an infirmity that negatively impacted milk production were removed from the analysis. Conception rates within milk production groups were examined by Chi Square analysis for effects of treatment and parity.

Results

There was no effect of farm on conception rates therefore data were pooled. Conception rates for OVPLUS (39.7 %) did not differ from Ovsynch (39.1 %). Conception rates by parity did not differ between treatments (Table 1). However, conception rates for first parity cows (48.2 %) were greater than cows in parity three or greater (33.9 %, p = 0.008). Effects of milk production on conception rates are reported in Table 2.

Table 1. Conception rates in lactating dairy cows following Ovsynch initiated at a random stage of the estrous cycle (control) or during the mid-luteal phase of the estrous cycle (OV+).

Parity[a]	OV	OV+	Total[c]
First (%)	43.5 (n=69)	52.8 (n=73)	48.2 (n=142)
Second (%)	40.0 (n=50)	37.9 (n=58)	38.9 (n=108)
Third + (%)	33.3 (n=90)	34.5 (n=87)	33.9 (n=177)
Total[b] (%)	38.3 (n=209)	41.5(n=218)	39.9 (n=427)

[a]No difference in parity*treatment ($P = 0.332$)
[b]No difference in treatments ($P = 0.87$)
[c]First parity different than third+ ($P = 0.008$), all others similar ($P > .14$)

Table 2. Effect of milk production on conception rates in lactating dairy cows.[a]

Parity[b]	High	Low	Total
First[c] (%)	59.7 (n=67)	37.3 (n=75)	48.2 (n=142)
Second[d](%)	35.6 (n=55)	43.4 (n=53)	38.9 (n=108)
Third +[e] (%)	42.4 (n=92)	24.7 (n=85)	33.9 (n=177)
Total[f]	45.8 (n=214)	33.8 (n=213)	39.9 (n=427)

[a] Data grouped by peak daily milk production above the parity mean (High) or below parity mean (Low). Parity milk production means:

First parity < 75 pounds/day = Low
≥ 75 pounds/day = High
Second parity < 109 pounds/day = Low
≥ 109 pounds/day = High
Third+ parity < 110 pounds/day = Low
≥ 110 pounds/day = High

[b]Treatment data pooled since no difference in treatments
[c]High and low producers different within parity ($P < 0.01$)
[d]High and low producers trend towards difference within parity ($P < 0.10$)
[e]High and low producers different within parity ($P < 0.005$)
[f]High and low producers different ($P < 0.025$)

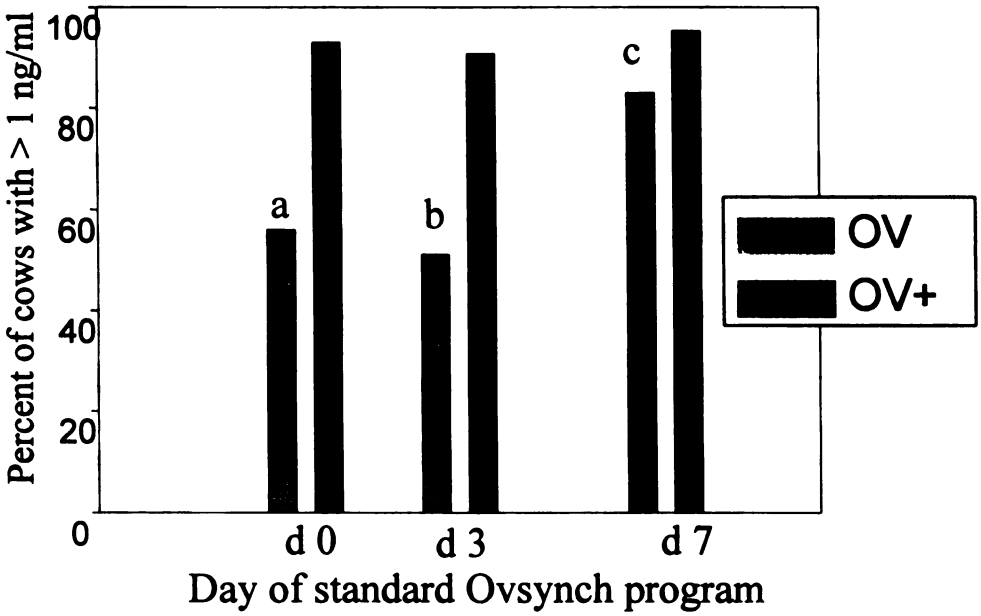

Figure 2. Proportion of lactating dairy cows with P_4 concentrations > 1 ng/ml during the Ovsynch protocol at time of the 1st GnRH of the standard Ovsynch protocol (d 0), 3 d after 1st GnRH (d 3), and at the time of $PGF_{2\alpha}$ of the standard Ovsynch protocol (d 7).

[a]Day 0 treatments different ($P = 0.022$)
[b]Day 3 treatments different ($P = 0.013$)
[c]Day 7 treatments similar ($P = 0.352$)

Independent of parity, conceptions rates of cows that had higher milk production around the time of AI (45.8%) had a greater conception rate compared to cows that produced below the parity mean (33.8%, $p < 0.025$). High producing first parity cows (59.7 %) had higher conception rates than low producing cows (37.3 %, $p < 0.01$). Low producing second parity cows tended ($p < 0.10$) to have greater conception rates than high producing cows (43.4 % vs 35.6 %). Conception rates for high producing cows (42.4 %) in third plus parity was greater ($p < 0.005$) than conception rates in low producing cows (24.7 %) of the same parity. Percent of cows with a functional CL are summarized in Figure 2. At first injection of GnRH during Ovsynch, percentage of cows with greater than 1 ng/ml serum P_4 was greater for OVPLUS than Ovsynch (93.0 % vs 56.1 %, $p = 0.02$). Three days after the first injection of GnRH during Ovsynch, percentage of cows with greater than 1 ng/ml serum P_4 was greater for OVPLUS (90.7 %) than Ovsynch (51.2 %, $p = 0.013$). On the d of $PGF_{2\alpha}$ the percentage of cows with greater than 1ng/ml serum P_4 did not differ for OVPLUS (82.9 %) and Ovsynch (95.3 % , $p = 0.352$). The OVPLUS group had a higher percentage of cows greater than 2 ng/ml serum P_4 compared to Ovsynch (95.3 % vs 63.4 %, $p = 0.049$) on d of $PGF_{2\alpha}$.

Discussion

This study was designed to test if increasing the number of cows in the mid-luteal phase of the estrous cycle at commencement of Ovsynch program would improve fertility in lactating dairy cows. To have cows in mid-luteal phase at the beginning of treatment with Ovsynch, cows were injected with $PGF_{2\alpha}$ 10 d and GnRH 7 d before start of Ovsynch. Gonadotropin releasing hormone was injected three days after the first $PGF_{2\alpha}$

to ensure that all cows would ovulate and start formation of a CL at least 6 d prior to the initiation of Ovsynch. Cows responding to the $PGF_{2\alpha}$ (CL regression) should have either had an estrus induced endogenous LH surge or a follicle large enough to ovulate at the time of the GnRH (*45*). Interestingly compared to Ovsynch, OVPLUS increased the percentage of cows above 1 ng/ml serum P_4 at the time of first GnRH during Ovsynch and three days later. OVPLUS also increased the number of cows with more than 2 ng/ml serum P_4 at the time of the final injection of $PGF_{2\alpha}$ during Ovsynch. The percent of cows with greater than 1 ng/ml serum P_4 in the Ovsynch group at first GnRH was similar to a previous study (*49*). Therefore, differences in Ovsynch and OVPLUS are due to increase by OVPLUS and not spurious decrease in cows treated with Ovsynch. The increase in number of cows in the mid-luteal phase of the estrous cycle did not affect conception rate following treatment with OVPLUS. Conception rates in this study were 39 % for OVPLUS and Ovsynch.

Previous studies established that lactating cows with serum P_4 greater than controls 11 to 13 d prior to AI have increased conception rates (*14,15,52*). Reasons for the differences in our data and previous findings could be in other studies serum P_4 was increase with exogenous sources (*14*). Folman et al used progesterone releasing intravaginal devices (PRID) to elevate the serum P_4 concentrations prior to insemination. This treatment increased conception rates 27 %. Other studies reported higher serum P_4 levels were the result of a CL from an endogenous LH surge (*15,52*). While OVPLUS did not elevate serum P_4 concentrations in the treated animals, OVPLUS did synchronize cows to a mid-luteal phase of the estrous cycle.

Starting Ovsynch at specific stages of an estrous cycle increases the percentage of synchronized ovulations (*71*). Therefore, OVPLUS may have elevated synchronization rate as well as increased percentage of cows in the mid-luteal phase of the estrous cycle. Previous reports indicate that beginning Ovsynch 5 to 9 d postestrus resulted in 96 % of the cows ovulating a follicle to the first GnRH of Ovsynch (*71*). Sixty six percent of the cows treated with OVPLUS should be d 5 to 9 postestrus. The other one third would be 10 to 15 d postestrus. Vasconcelos et al. also reported that cows 1 to 12 d postestrus at the time of the first GnRH of Ovsynch had a higher percentage of synchronized ovulations than cows 13 to 20 d postestrus. (*71*). Approximately 90 % of the cows in the OVPLUS treatment would be between 5 to 12 d postestrus. Vasconcelos et al. also reported that cows 5 to 9 d postestrus at the commencement of Ovsynch had the higher average serum P_4 concentrations at the time of $PGF_{2\alpha}$ than cows that were in any other stage of the estrous cycle (*71*). This is consistent with the findings of the current study.

These data demonstrate that conception rates decline as parity increases. This trend is consistent with literature reports where synchronization of ovulation was not used (*25*). However the effect of parity on conception rate in this study is at variance with previous data on synchronization of ovulation (*48*). In the previous report in which synchronization of ovulation was used, second parity cows had a higher conception rate than first or $\geq$3 parity. The reason for the disparity in my data and the other published reports is unclear.

The positive association of milk production with conception rate in the current study is contrary to previous reports. When cows are inseminated following a detected estrus, it has been reported previously that milk production negatively affects conception

rates (*11,15,25,65*). In my data, after treatment with Ovsynch cows that produce more milk in their first parity or third and greater parity are more likely to conceive compared to cows with lower milk yield. However this positive effect of yield on fertility is not seen in second parity animals. A difference between experiments is that cows in the present study were all inseminated without regard for estrus following synchronization with Ovsynch. If high yield cows experienced higher synchronization rate, this would be consistent with greater conception rate. However, this experiment was not designed to test whether a difference in synchronization rates exists between low and high producing cows. No data exists regarding the effect of milk yield on follicle growth and subsequent synchronization rates following Ovsynch. If low producing cows have a lower synchronization rate, they would have a lower conception rate following treatment with Ovsynch. An additional possible explanation for the effect of milk yield on conception rate observed in this study is that the high milk producing cows may be healthier at the time of AI. These cows may have experienced less body condition loss after calving and experienced fewer metabolic problems and greater health should support greater fertility.

In summary, the OVPLUS protocol increased the percentage of cows in luteal phase of the estrus cycle before starting Ovsynch. However timing cows into a luteal phase did not increase conception rates after treatment with Ovsynch.

Chapter 3

Effect of various schedules to inject Prostaglandin $F_{2\alpha}$ and second GnRH during Ovsynch on conception rate, ovulatory follicle size, and subsequent progesterone

Introduction

Detecting estrus in lactating dairy cows is a difficult and labor intensive responsibility for dairy producers (*59*). Average success to detect estrus is only 50 % and severely limits the use of Artificial Insemination (AI) and reproductive performance of dairy cows. To assist with this problem, a program to synchronize ovulation (Ovsynch) was developed (*47*). Lactating cows were inseminated prior to an ovulation that was controlled by exogenous hormones injected during a 10 d period before AI. The specific schedule of injections for Ovsynch is inject gonadotropin releasing hormone (GnRH) –wait 7 d-inject Prostaglandin$F_{2\alpha}$ ($PGF_{2\alpha}$)-wait 2 d-inject GnRH-wait 24 h-AI. Ovsynch is effective to manage pregnancy rate (*5*) and to decrease days to first and subsequent AI (*46*). However, the Ovsynch program requires that dairy farm managers must perform injections on three separate occasions. A program for synchronization of ovulation with two injections is appealing because there would be fewer times to handle animals and less farm labor. Because it is possible to increase efficiency, this advantage may increase the adoption of synchronized breeding. Furthermore, the optimal time between the Prostaglandin$F_{2\alpha}$ ($PGF_{2\alpha}$) injection and the second gonadatropin releasing hormone (GnRH) for maximal conception rates is unknown. Two experiments were conducted to test whether synchronized ovulation could be achieved when cows receive GnRH and $PGF_{2\alpha}$ simultaneously and to determine the optimal time between $PGF_{2\alpha}$ and the final GnRH of Ovsynch. Experiment 2 of this thesis was designed to test whether the second GnRH injection of the Ovsynch program can be moved and given concurrently with $PGF_{2\alpha}$ and allow to similar conception rate compared to Ovsynch. Experiment 3 of this

thesis was designed to test for the optimal time between the injection of $PGF_{2\alpha}$ and the second GnRH during Ovsynch to maximize fertility.

Materials and Methods

Experiment 2

To evaluate the effects on conception rate of administering $PGF_{2\alpha}$ and the second GnRH of the Ovsynch protocol (Figure 3) simultaneously, 218 non-pregnant lactating dairy

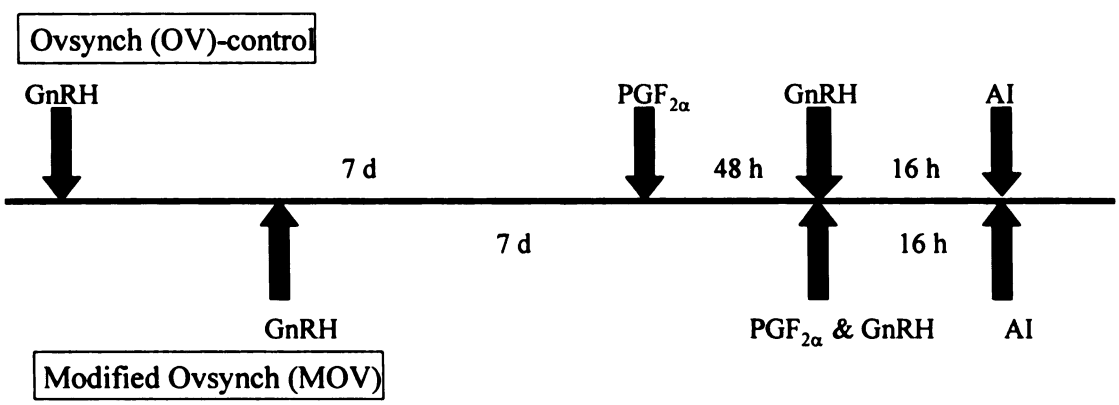

Figure 3. Description of the timing for each of the injections used in the Ovsynch and the modified Ovsynch protocol.

cows >60 d in milk were assigned randomly to the control or treatment group. The study occurred February through May 1998. All cows were located at Nobis Dairy (St. Johns, MI) and the rolling herd average is greater than 23,000 pounds of milk. All cows were housed in freestall barns with free access to feed and water. Controls were treated with the standard Ovsynch program (OV) and the treatment group received a modified version of Ovsynch (MOV--Figure 3-1). All GnRH injections were 100 μg/dose of Cystorellin® (Merial, Ltd., Iselin, NJ) and all $PGF_{2\alpha}$ injections were 25mg/dose of Lutalyse®

(Pharmacia-Upjohn Co., Kalamazoo, MI). The farm owner performed all AI. The herd veterinarian diagnosed pregnancy 36 d after AI by rectal examination of the uterine horns and ovaries. Synchronization rates were analyzed in a randomly selected subset of cows (n=85). To determine synchronization, serum progesterone (P_4) levels and ovarian ultrasonography data were collected. An ovulation was categorized as synchronized if the CL regressed (indicated by serum P_4 concentration decreasing to below 1 ng/ml) and if the dominant ovulatory follicle disappeared (indicated by ultrasound). To determine CL regression, blood was sampled from a coccygeal vessel from the subset cows concurrent with and two days after injection of $PGF_{2\alpha}$. Progesterone concentration was quantified in blood serum by RIA Coat-A-Count kits from Diagnostic Products Corporation (Los Angeles, CA). Intra- and interassay coefficients of variation were 5.6 %and 9.1 %, respectively. Ultrasonography was performed at the time of the injection of GnRH and 48 h post-injection to determine ovulation of the dominant follicle(s). All ovarian structures were measured and mapped using a Pie Medical 200 ultrasound machine with a 7.5 MHz transducer. Disappearance of a follicle and appearance of a new CL in the location of the antecedent follicle by ultrasound were considered an ovulation. In previous experiments, ovulation occurred 24 to 32 hours after the second injection of GnRH (*47*). Differences in pregnancy and synchronization rates were tested for significance using Chi square analysis. Size of the ovulatory follicle at the time of the second injection of GnRH and P_4 concentrations were analyzed using Student's T-test.

Experiment 3

This experiment was conducted to determine the optimal period of time between the injection of $PGF_{2\alpha}$ and the second injection of GnRH to maximize conception rates after Ovsynch. During May and June of 1999, 457 cows from five Michigan dairy farms were

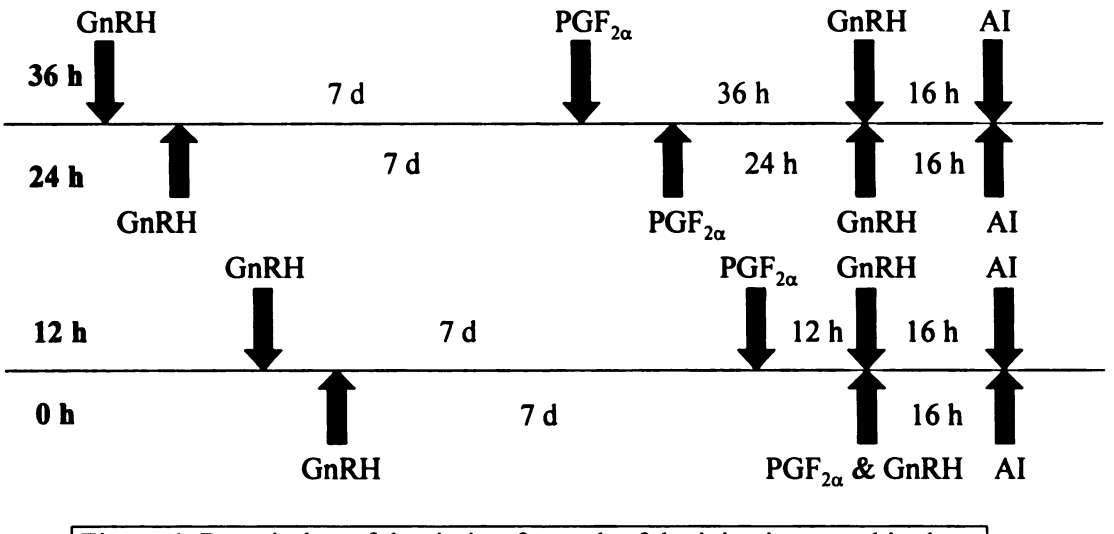

Figure 4. Description of the timing for each of the injections used in the 36 h (control), 24 h, 12 h, and 0 h treatments.

used for this study. All non-pregnant cows that were beyond the voluntary waiting period were assigned randomly to one of four treatment groups (Figure 4). Each farm was allowed to use its own VWP. The 36 h treatment served as our control because it provided conception rates similar to AI 12 h after detected estrus (*49*). The other three treatments had different periods of time (24 h, 12 h, and 0 h) between the $PGF_{2\alpha}$ and the second GnRH of Ovsynch (Figure 4). Interval from first injection of GnRH to $PGF_{2\alpha}$ and interval from second GnRH to AI were identical for all treatments. All injections were scheduled so AI for all groups would occur on the same calendar date. Laboratory personnel administered all injections. Pregnancy diagnoses were performed using ultrasound at 28 d after AI. Differences in pregnancy rate among groups were examined

by Chi Square analysis within the Proc GENMOD function of SAS (*27*). Cows from two farms in the study were selected for determination of synchronization rate (n=102). This subset of cows was prescribed to the same schedule to sample blood and to examine ovaries as described in experiment 2. In this experiment an Aloka SSD-900 ultrasound machine with a 7.5 MHz transducer was used. Also, a group of cows (n=63) that ovulated in response to the second GnRH injection had blood sampled on 4, 6, 8, 11, 13, 15, and 18 d postestrus of the cycle subsequent to induced ovulation. All serum samples were analyzed for P_4 by the RIA method described in experiment 1. Differences in size of ovulatory follicle were examined by ANOVA using the general linear model procedure of SAS. Synchronization rate was examined for differences among groups using Chi Square within the Proc GENMOD function of SAS (*27*). Serum P_4 levels during the subsequent cycle were analyzed using the Proc MIXED function of SAS (*27*).

Results

Experiment 2

Results of experiment 2 are shown in Table 3. Synchronization rate and percent of cows <1 ng/ml P_4 two days following $PGF_{2\alpha}$ were not different ($p > 0.1$) between OV and MOV. Diameter of the ovulatory follicle at the time of second GnRH was greater in OV than MOV ($p < 0.05$). Conception rate in OV was greater than for MOV ($p < 0.025$).

Experiment 3

As in experiment 2, synchronization rate was not different among all groups ($P > 0.28$; Figure 5). Size of the ovulatory follicle had a significant linear effect. As time increased between $PGF_{2\alpha}$ and the second GnRH follicle size increased ($p < 0.05$; Figure 6).

Table 3--Comparison of synchronization rate, CL regression, follicle size and pregnancy rate between OV (control) and MOV[1] groups			
	OV	MOV	P value
Synchronization rate (%)	89 (n=45)	85 (n=40)	> 0.10
Regress CL to $PGF_{2\alpha}$ Injection (%)	97.5 (n=45)	100 (n=40)	> 0.10
Average Ovulatory Follicle Size (mm)	14.5 (n=45)	13.3 (n=40)	< 0.05
Conception Rate (%)	31.3 (n=109)	14.7 (n=109)	< 0.025
[1]MOV =GnRH—7d—$PGF_{2\alpha}$ and GnRH—16 h--AI			

However, the ovulatory follicle size at 0-hour was significantly smaller than the follicle size in the 36-hour group ($p < 0.05$). All other pair comparisons of follicle size were not different within the linear trend ($p > 0.17$). Pregnancy data had a significant linear trend ($p < 0.0001$; Figure 7). As time between $PGF_{2\alpha}$ and GnRH injection increased conception rate increased, with 36 h having the highest conception rate. Pregnancy rates were different when the 0 h group was compared to 24 and 36 h ($p < 0.01$); significant difference was also detected between the 12 h and 36 h treatment group ($p < 0.01$). Serum P_4 levels following AI are reported in Figure 8. Cows in the 0 and 36 h treatment groups had similar mean serum P_4 throughout the estrous cycle subsequent to AI. On d 11, 0 h group was different than 12 and 24 h groups, and 36 h group was different than 24 h group ($p < 0.05$). On d 13, 0 and 36 h groups had significantly greater serum progesterone concentrations than 24 h group ($p < 0.02$).

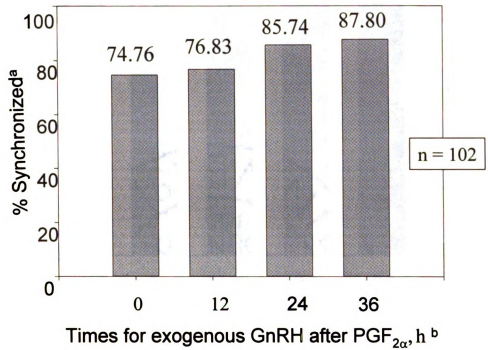

Figure 5. Synchronization of ovulation rates in lactating dairy cows treated with GnRH at specific times after $PGF_{2\alpha}$.

[a]Synchronization=CL regression and disappearance of dominant follicle

[b]No differences in treatments ($P>0.24$) and no significant linear trend ($P>0.16$)

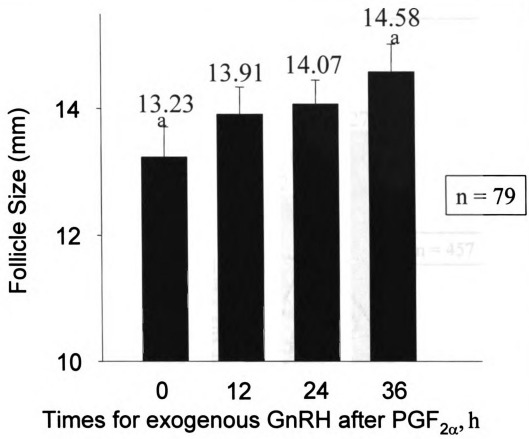

Figure 6. Size of the ovulatory follicle in lactating dairy cows at time of final GnRH at specific times after $PGF_{2\alpha}$[b].

[a]Treatments different from each other ($P < 0.05$)
[b]Data has significant linear effect ($P < 0.05$)

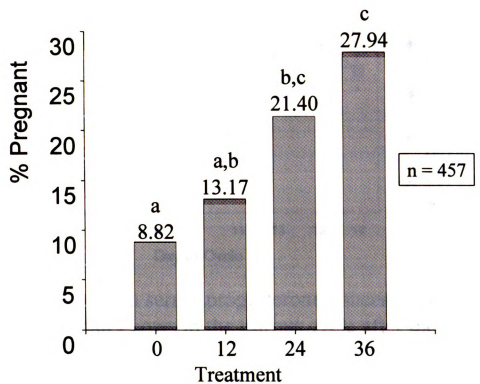

Figure 7. Conception rates in lactating dairy cows following treatment with GnRH at specific times after $PGF_{2\alpha}$[b].

[a]Bars with different superscript letters are significantly different ($P < 0.01$)
[b]Data significantly linear ($P < 0.0001$)

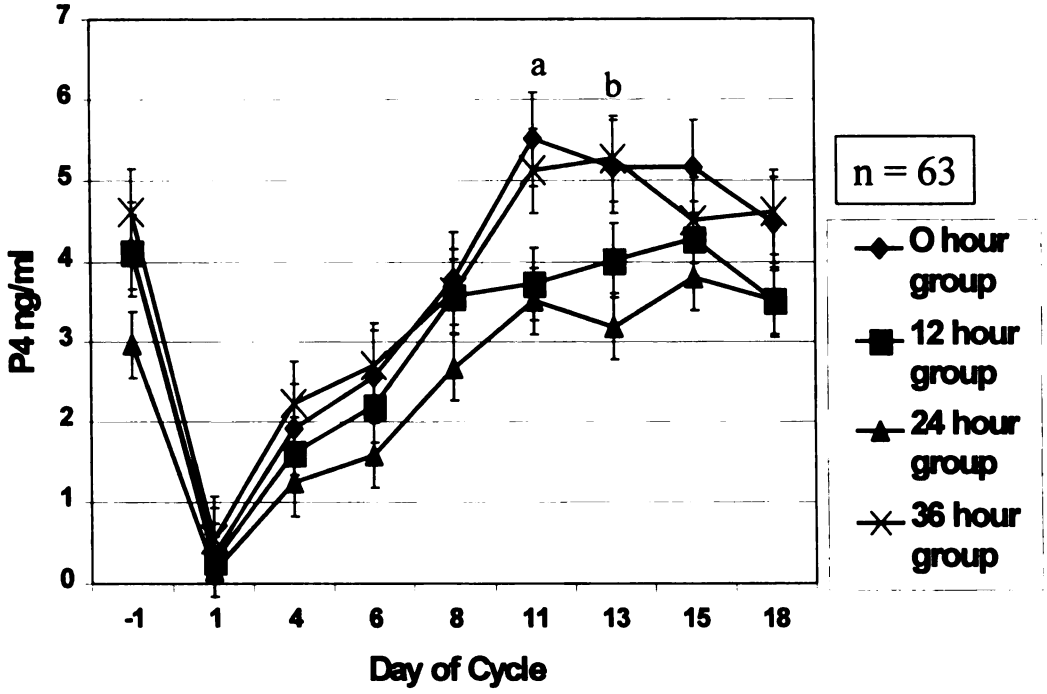

Figure 8. Mean serum progesterone concentrations in lactating dairy cows in the estrous cycle after modified versions of Ovsynch. The modified versions involved injections of GnRH at specific times (0, 12, 24, 36 h) after exogenous $PGF_{2\alpha}$.

[a]0 h mean concentration different than 12 and 24 h mean concentration, and 36 h mean concentration different than 24 h mean concentration ($P < 0.05$)
[b]0 and 36 h mean concentration different than 24 h mean concentration ($P < 0.02$)

Discussion

This study was designed to determine the effect of different intervals from CL regression to the LH surge on reproductive performance in dairy cows. To our knowledge, this is the only study that directly compared the effect of different intervals from $PGF_{2\alpha}$ to LH surge on conception rates in lactating dairy cattle. This study tested a GnRH-induced LH surge at 0, 12, 24, or 36 h following $PGF_{2\alpha}$ induced CL regression. This was facilitated by the Ovsynch program (*47*). Ovsynch will synchronize follicle growth and luteal function using GnRH and $PGF_{2\alpha}$. From previous data after Ovsynch, the first injection of GnRH ovulates a follicle and initiates a new follicle wave 90 % of the time (*47*). The injection of $PGF_{2\alpha}$ regressed the CL 97 % of the time (*49*). The second injection of GnRH caused ovulation 24 to 32 hours after injection. In previous studies, synchronization rate was 83 to 100 percent of lactating cows (*18,47*). In the current experiments, synchronization rates for different treatment groups ranged from 75 to 89 percent. In previous studies with Ovsynch, the second injection of GnRH occurred at 36 to 48 h after $PGF_{2\alpha}$. In previous studies, conception rates after Ovsynch were not different from conception rates achieved with AI 12 h after detected estrus (*46,48*). Thus the basic Ovsynch program with modifications provides an ideal opportunity to manipulate occurrence of an LH surge at various times after luteal regression. Our question was whether different timing of an LH surge will affect conception rates. To avoid bias between treatments, follicle growth was synchronized to provide a follicle of similar age in all groups at the time of $PGF_{2\alpha}$. Another procedure to avoid bias was that cows in all groups were inseminated on the same calendar date, at the same time of day, and the inseminator did not know treatments assigned to individual cows. Selection of 36

h as our control group was based on similar conception rates for cows inseminated 12 h after standing estrus to conception rate in cows inseminated after Ovsynch when $PGF_{2\alpha}$ was injected 30 to 36 h before the second injection of GnRH (*49*). It is also reported that 48 h between $PGF_{2\alpha}$ and second GnRH injection provided similar conception rates to cows bred from a standing estrus (*46*). Based upon these data we were confident that 36 and 48 h would have similar results. In previous Ovsynch studies, the time of 36 to 48 h between $PGF_{2\alpha}$ and the second GnRH was selected because this was the maximum amount of time that could pass after $PGF_{2\alpha}$ before cows begin to display signs of estrus and to exhibit endogenous spontaneous preovulatory secretion of LH (*35,69*). Thus, any modifications of Ovsynch that still allow AI without estrus must shorten, not lengthen, the interval between $PGF_{2\alpha}$ and second GnRH injection of Ovsynch. That is why all intervals tested in Experiment 3 were greater than or equal to 36 h.

Previous studies evaluated the fertility of lactating dairy cows following synchronization of ovulation. Conception rates varied from 27 to 39 percent (*5,46,48*). However, none of these studies directly compared different time periods between $PGF_{2\alpha}$ and second GnRH injection. Data from our study proves that shortening the interval between $PGF_{2\alpha}$ and the second injection of GnRH to less than 36 h will reduce conception rate.

This study demonstrates that the negative impact of increasing time between $PGF_{2\alpha}$ and the second GnRH injection on conception rates was not due to decreased P_4 production from the CL resulting from a smaller ovulatory follicle. This is contrary to published reports from sheep (*40*) in which decreased P_4 production from CL formed from a smaller ovulated follicle of the ewe. Our ultrasound scans demonstrate that the 0

h group had a smaller follicle. However, the P_4 concentrations after AI did not indicate any form of luteal insufficiency for the smallest follicle group (0 h). On d 11, 0 h group actually had higher serum P_4 than the 12 h or the 24 h group. At all times during the subsequent cycle the 0 h cows had serum P_4 concentrations similar to the 36 h group. From these data, there is no evidence that follicle size (13.23 mm to 14.58 mm) at the time of the LH surge affects subsequent serum P_4 levels.

For cows in 0 h group, $PGF_{2\alpha}$ began CL regression at the same time an LH surge was being induced. In contrast, when cows were injected with $PGF_{2\alpha}$ and a GnRH agonist at the same time CL regression was delayed by 3 to 6 d (*37*). In this study, all cows injected with $PGF_{2\alpha}$ and GnRH concurrently had serum P_4 levels at sub-luteal levels within 48 h. Concurrent injection of GnRH with $PGF_{2\alpha}$ did not block luteal regression induced by exogenous $PGF_{2\alpha}$.

In the current study, decreased time between decline of P_4 until the LH surge decreased conception rates. It is not clear why conception rates were different between treatments. Conception rates are not associated with P_4 levels in estrous cycle subsequent to AI. Possible explanations for the difference in conception rates are impaired oviduct contractility (*53,54*), impaired oocyte transport (*72*), impaired sperm transport (*24*), and a less than ideal endometrial environment (*61*).

In addition, decreased fertility in the treatment groups with shorter time between $PGF_{2\alpha}$ and the final GnRH may be a result of ovulating a smaller less mature follicle. This is contrary to previous reports in which ovulating a smaller follicle resulted in higher conception rates (*70*) and a more competent oocyte (*51*). Perhaps, a critical time exists in the dominant follicle growth pattern when oocytes reach maximal fertility potential.

In conclusion, ovulation can be synchronized when the GnRH and the $PGF_{2\alpha}$ injections are administered together. However, injecting GnRH 24 h or less after $PGF_{2\alpha}$ will reduce conception rate. Luteal insufficiency does not cause the decreased conception rates in the treatment groups with less than 36 h between the $PGF_{2\alpha}$ and the final GnRH.

CHAPTER 4

General Discussion

General Discussion

The results presented in this thesis add to our understanding of synchronization of ovulation in lactating dairy cattle. The modifications of the Ovsynch program tested in this thesis did not increase conception rates compared to standard Ovsynch. Manipulating lactating dairy cows into a mid-luteal phase of the estrous cycle at the beginning of Ovsynch provided conception rates that were similar to cows that were started on Ovsynch at a random time of the cycle. Another option to increase conception rates after treatment with Ovsynch is to use exogenous progesterone before beginning Ovsynch. Exogenous progesterone sources during the Ovsynch program has not been reported.

When the last two injections of Ovsynch were given concurrently conception rate was reduced and there was no effect on synchronization rate. The final experiment in this thesis tested the time between the $PGF_{2\alpha}$ and the final GnRH of Ovsynch. Results demonstrate that as time between the $PGF_{2\alpha}$ and the final GnRH increase, the conception rate increases with 36 h between $PGF_{2\alpha}$ and the final GnRH of Ovsynch yielding the highest conception rates in my study. The lowered conception rate with less than 36 h between the $PGF_{2\alpha}$ and the second GnRH was not due to luteal insufficiency. Cows with less time between the $PGF_{2\alpha}$ and the second GnRH did ovulate a smaller follicle and this is possible cause for the reduced fertility. The final two experiments of this thesis provide insight into the physiological mechanism needed to establish pregnancy. It is now established that to achieve acceptable conception rates progesterone concentrations must be declining before an ovulatory follicle releases the oocyte. This thesis did not address why serum progesterone concentrations must be declining prior to ovulation, but

the discussion in chapter 3 presented some plausible reasons. Testing the reasons mentioned in chapter 3 of this thesis may provide insight into the problems with dairy cattle fertility.

Synchronization of ovulation is an effective method to control reproduction of lactating dairy cattle, but current methods are not perfect. This thesis pursued some opportunities to increase success and applicability of synchronized ovulation. However, the modifications of Ovsynch tested in this thesis were not effective and should not be employed by dairy producers.

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