

SYNCHRONIZATION OF OVULATION BASED ON STAGES OF FOLLICULAR AND
CORPORA LUTEA DEVELOPMENT IN LACTATING DAIRY COWS DIAGNOSED
NOT-PREGNANT

By

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ABSTRACT

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Cows that fail to conceive to 1st or subsequent inseminations are a major reproductive problem on dairy farms and commonly used re-synchronization programs are not as effective as pre-synch/Ovsynch programs for 1st AI. Ovsynch appears to be most effective when initiated during the 1st follicular wave of an estrous cycle when the dominant follicle is responsive to the 1st GnRH allowing the PGF_{2α} to be administered prior to natural luteolysis. Yet, most cows are in other stages of follicle and CL development at time of a not-pregnant diagnosis. Pre-synchronization programs are designed to synchronize cows that are in these other stages of follicle and CL development to the 1st follicular wave. The objective of this thesis was to test 3 re-synchronization programs (Ovsynch, G6G, and GGPG) tailored to 4 different stages of follicular and corpora lutea development in cows (n = 515) that did not become pregnant at 1st AI in order to synchronize cows to the 1st follicular wave at the start of Ovsynch. Conception rates were 27.8, 36.6, and 45.3% for Ovsynch, G6G, and GGPG, respectively, and were not statistically different. Heart girth measurements indicated that smaller cows had greater rates of luteolysis following the final PGF_{2α} of Ovsynch and a tendency for higher conception rates in cows with a mature CL. This could potentially be an artifact of incorrect dosage of PGF_{2α} and GnRH used in synchronization protocols. In Chapter 4, the relationship between linear type traits and reproduction in 2299 cows was examined. Cows with increased body volume traits (strength and body depth) consistently had poorer fertility, which agrees with our findings in Chapter 3.

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LIST OF ABBREVIATIONS

AI	artificial insemination
CIDR	controlled internal drug release
CL	corpus luteum/corpora lutea
CR	conception rate
CV	coefficient(s) of variation
d	day (s)
DIM	days in milk
DF	dominant follicle(s)
ELISA	enzyme-linked immunosorbent assay
E₂	estradiol
GnRH	gonadotropin releasing hormone
hCG	human chorionic gonadotropin
HG	heart girth
LH	luteinizing hormone
ME	mature equivalency
mL	milliliter
ng	nanogram
pg	picogram
P/AI	pregnancy per artificial insemination
PTA	predicted transmitting ability
P₄	progesterone

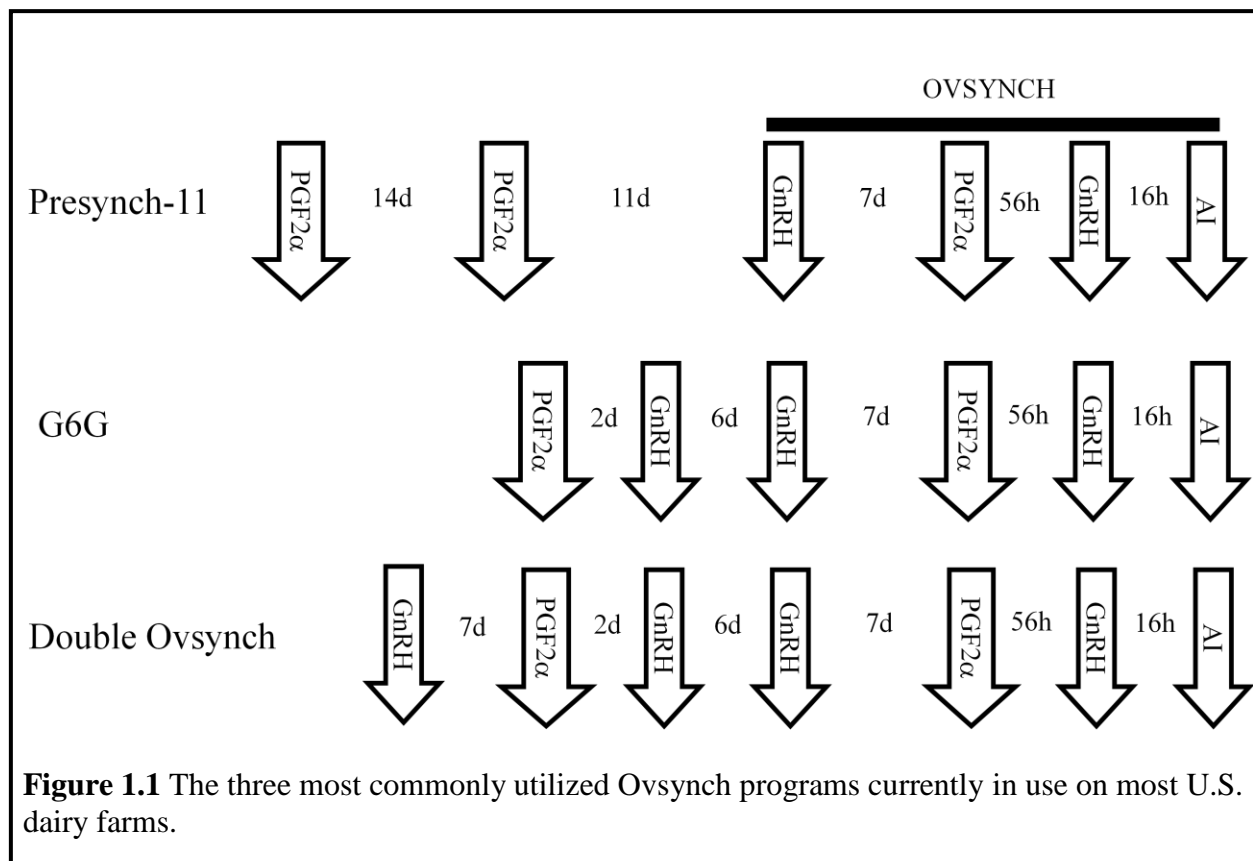
PGF_{2α}	prostaglandin F _{2α}
RIA	radioimmunoassay
TMR	total mixed ration

Chapter 1

INTRODUCTION

Pregnancy rate, the percentage of eligible cows in herds that become pregnant every 21 days, declined from 22% in the late 1970's to currently 12.5% (deVries & Risco, 2005; Campbell et al., 2009). There are two components that make up pregnancy rate: estrus detection rate (percentage of cows eligible to be serviced every 21 days) and conception rate (the number of cows that become pregnant as a proportion of total number of inseminations). Estrus detection rates have declined from about 51% to 42% from 1985 to 1999 and conception rates have dropped from 53% in the 1970's to 30% in the 2000's (Washburn et al., 2002; Norman et al., 2009). This decline in reproduction is partially attributed to an intense selection for increased milk production during this period, particularly since genetics is estimated to account for one-third of the decrease in pregnancy rate (Shook, 2006). Although milk production is negatively correlated with reproduction (Olds et al., 1979; Berger et al., 1981; Laben et al., 1982; Faust et al., 1989; Clay and McDaniel, 2001; VanRaden et al., 2004), other factors such as nutrition, management, physiology, and health are likely factors also responsible for poor fertility. One such factor involved is a metabolic shift due to lactation in high-producing cows that can be illustrated by comparing a nulliparous heifer to a high-producing cow (Wiltbank et al., 2006). Nulliparous heifers have much higher conception rates (60-75%) than lactating cows (25-40%) (Pursley et al., 1997). Fertility continues to decline as cows reach greater parities (Norman et al., 2009). The decline in fertility from nulliparous heifers to high-producing dairy cows may be explained by a model suggesting that elevated feed intake for the high-producing cow leads to increased blood flow to the liver. Subsequently, steroid reproductive hormones are metabolized more quickly and are in lower concentrations in circulation, resulting in decreased fertility (Wiltbank et al., 2006).

Dairy producers can take advantage of opportunities to enhance profit through improving reproductive efficiency of their herds, especially when prices of milk are high. There are numerous ways to enhance reproductive efficiency of dairy herds. This includes enhancing estrus detection with proper timing from accurate detection to AI, inseminator compliance, and Ovsynch programs that allow for timed-AI. Currently, reproductive technologies, such as Ovsynch programs (Figure 1.1) that control follicle and corpora lutea development, increase the



percentage of cows that receive AI during a 21 day period and increase conception rates to nearly 50 % (Souza et al., 2008). Data from Strickland et al., 2010 indicated that Presynch-11/Ovsynch increased conception rates of lactating dairy cows from 30 to 45 % compared to AI following detected estrus. Even though these current synchronization programs do not increase conception rates to that of heifers, the efficiency of reproduction in dairy cattle has the potential to be

significantly enhanced if conception rates can continue to be improved. This is especially true for cows that are re-synchronized following a not-pregnant diagnosis. Conception rates following re-synchronization programs that do not pre-synchronize are generally 20% points lower than in cows that receive a fertility treatment such as G6G/Ovsynch (Bello et al., 2006), Double Ovsynch (Souza et al., 2008), or Presynch-11/Ovsynch (Moreira et al., 2001; Strickland et al., 2010). These programs enhance fertility through greater synchronization of follicle and CL development and through the increase in progesterone concentrations during the time of development of the ovulatory follicle. The key reason for these improvements is through the synchronization of follicle and CL development at the time of the 1st GnRH of Ovsynch. Data indicate that when cows are on day 6 or 7 of the estrous cycle at the time of the 1st GnRH of Ovsynch (Bello et al., 2006; Bello, 2006), the probability of ovulation and initiation of a new follicular wave and accessory CL are dramatically enhanced. These events are necessary to control follicle development and increase progesterone and subsequently to enhance fertility of lactating dairy cows. The primary study of this thesis establishes the impact of this new understanding of pharmacological control of follicle and CL development in cows that are re-synchronized but may be at different stages of follicular and corpora lutea development at time of the initial re-synchronization treatment. Since response to each injection of a synchronization program is vital to its success, heart girth measurements were taken to determine if body size has an impact on response to each injection. Even though this is a separate question, since heart girth measurements were taken on all cows in this study, results on body size will be reported in the chapter on re-synchronization.

In addition to the study of control of ovarian function in re-synchronized cows, this thesis determines the impact of conformation traits on fertility. Even though there is potential to

improve fertility of lactating dairy cows, a paucity of data exists that helps to understand the impact of type traits on fertility and the interaction with timed AI and detection of estrus reproductive management programs. Establishing a relationship between these traits and fertility could assist farmers in genetic selection, thereby increasing reproductive efficiency. The objectives of the studies in this thesis are: 1) to determine ways to enhance fertility of lactating dairy cows through greater control of ovarian function with timed AI programs and 2) to determine the relationship between conformation traits and dairy cow fertility.

Chapter 2 reviews the literature that has laid the groundwork for a greater understanding of the pharmacological control of ovarian function and the relationships between conformation traits and reproduction. Chapter 3 describes the key study of this thesis that tested whether initiating the ideal Ovsynch program for various stages of the estrous cycle can enhance synchronization of ovulation and fertility of re-synchronized cows. In addition, the effect of body size on response to Ovsynch injections was analyzed in this study. Chapter 4 describes a study of $n = 2299$ cows with conformation information and the impact of type traits on fertility. Chapter 5 provides conclusions for this thesis in a veterinarian lay article format that will be re-formatted and submitted for publication to an industry magazine.

CHAPTER 2

REVIEW OF LITERATURE

REPRODUCTION IN THE US: THE DECLINE IN FERTILITY

Poor fertility has become a major concern in the dairy industry. Conception rates of high-producing dairy cows has drastically declined, dropping from 53% on average in the 1970's to 30% in the 2000's (Washburn et al., 2002; Norman et al., 2009). Synchronization of ovulation protocols appear to be enhancing conception rates according to recent data (Norman et al., 2009). Nevertheless, as fertility was on a downfall, milk production greatly increased. Although many attribute this increase in milk production as the sole cause of poor fertility, there are other factors involved, such as physiological, management, and nutritional changes.

An antagonistic relationship between reproduction and milk production has been documented in many studies (Olds et al., 1979; Berger et al., 1981; Laben et al., 1982; Faust et al., 1989; Clay and McDaniel, 2001; VanRaden et al., 2004). Genetic merit for milk yield has increased by 120 kg/year while fertility has declined (Animal Improvement Programs Laboratory, 2011); thus, decreased fertility can partially be attributed to this increased selection for elevated milk production (Royal et al., 2002a; Norman et al., 2009). However, there have been disagreements about the possible antagonistic effects of milk production on reproductive efficiency. More than two decades ago, Hillers et al., 1984 did not find a significant negative relationship between fertility and milk yield, but Hansen et al., 1983 found a positive relationship between the two traits. Even though past studies have shown conflicting results, it is generally accepted that there is an antagonistic relationship between reproduction and milk yield in today's dairy industry. Recently, researchers analyzed the relationship between milk production and calving interval using Hierarchical Bayesian modeling and found that higher producing cows tended to have poorer reproductive performance than those with lower milk yields. They did not find a relationship between level of overall herd production and reproduction, so they suggested

that the negative relationship between milk production and reproduction occurs only at the individual cow level (Bello et al., 2010).

Although an increase in milk production has a negative correlation with reproduction in dairy cattle, other factors exist that negatively influence reproduction. One such factor is a shift in the physiology of cattle that can possibly be explained by a model proposed by Wiltbank et al., 2006. The authors compared 27 heifers and 14 lactating cows to assess the differences in fertility using daily ovarian ultrasonography evaluations and hormonal analyses. Heifers had an average peak progesterone of 7.3 ng/mL and peak estradiol of 11.3 pg/mL while cows averaged 5.6 ng/mL of peak progesterone and 7.9 pg/mL of peak estradiol, indicating the obvious differences in hormone concentrations between lactating cows and heifers (Wiltbank et al., 2006). In another study by the same lab, the authors found that blood flow to the liver was higher in lactating cows than in non-lactating cows of comparable age and size. After feed consumption, they saw an instant increase in blood flow to the liver and an increase in metabolism of progesterone and estradiol in both lactating and non-lactating cows. However, estradiol and progesterone metabolism was 2.3 times higher in lactating cows than in non-lactating cows (Sangsritavong et al., 2002; Sangsritavong, 2002). Accordingly, the researchers suggested that high producing dairy cows have increased dry matter intake which then leads to increased blood flow to the liver. As a result of this increased blood flow, reproductive hormones, such as estrogen and progesterone, are metabolized more quickly and are seen in lower concentrations in circulation. Consequently, fertility declines, which can be seen in decreased conception rates, higher levels of embryonic loss, decreased estrus expression, and increased multiple ovulation rate (Wiltbank et al., 2006).

Even though the model proposed by Wiltbank et al., 2006 explains how physiology may be involved in the decrease in fertility, other factors may contribute to the problem. These factors may involve management, nutrition, health, and stressors. For instance, although dairy cows with the highest milk production levels display the most infertility, epidemiological studies indicate other factors are probably involved in decreasing fertility. In herds with higher milk yields, it appears that more intensive management strategies, a greater plane of nutrition, and improved cow health can lead to higher reproductive rates in conjunction with this increased milk production (Nebel and McGilliard, 1993). Over time, increased genetic selection coupled with physiological changes, such as delayed luteolysis, delayed ovulation, and irregular estrous cycles, may alter a cow's vulnerability to other factors, making her increasingly susceptible to lower reproductive efficiency (Lamming and Darwash, 1998). While it is evident many factors are involved in the decline in dairy cattle fertility, researchers have developed synchronization of ovulation protocols to help combat this problem. Even so, since a key determinant of profitability on dairy farms is efficient reproduction, the decline in fertility has caused some dairy producers to suffer substantial losses.

CURRENT STATE OF REPRODUCTION IN US DAIRY CATTLE AND THE NEGATIVE IMPACT ON PROFITABILITY

An illustration of how fertility has been on a decline in the past few decades can be seen when looking at pregnancy rates for Holsteins in Florida and Georgia. Pregnancy rates plummeted from 22% in the 1970's to 12% in the early 2000's (deVries and Risco, 2005). Between 1976 and 1978 in 10 Southeastern states, the number of services per conception was 1.91 and escalated to 2.94 by 1996 for Holsteins and Jerseys (Washburn et al., 2002). In another example, from 1996 to 2007, the number of inseminations per lactation rose from 2.1 to 2.5 in Holsteins and from 2.0 to 2.3 in Jerseys, leading to increased insemination costs. During the

same period, the conception rate for all inseminations dropped from 33% to 30% in Holsteins and 42% to 39% in Jerseys (Norman et al., 2009). An additional study conducted in 10 Southeastern states reported 1976-1978 conception rates were 52% for Jerseys and 53% for Holsteins and decreased to 33% and 32%, respectively, by 1998. The same study indicated days open for Jerseys were 122 days and 124 days for Holsteins between 1976 and 1978 and increased to 152 days and 168 days, respectively, by 1997 to 1999 (Washburn et al., 2002). Based on decreased conception and pregnancy rates as well as increased services per conception and days open, it is evident that fertility has declined over the past several decades.

In addition to less than desirable conception and pregnancy rates, low rates of estrus expression and detection are decreasing reproductive efficiency. In a study performed from data generated from electronic mount detectors (Heat Watch System, DDX Inc., Denver, CO), the average cow had 8.5 stands per estrus with only 7 hours of estrus duration. Almost 25% of the cows had a low intensity (< 1.5 stands per hour) estrus and a short estrus duration of less than 7 hours (Dransfield et al., 1998) indicating the difficulty of detecting cows in estrus. Lopez et al., 2004 found that estrus duration decreases as milk production increases. In addition, the authors found that estradiol concentrations at the time of estrus and intensity of estrus are negatively affected by milk production (Lopez et al., 2004). Thus, it can be difficult to observe cows in estrus, particularly high-producing cows with diminished duration of estrus. If failure to detect estrus occurs frequently, cows will not become pregnant in a timely manner and thus impair profitability.

Since reproduction plays a crucial role in on-farm profitability, the decline in fertility and reduction in estrus detection can wreak financial havoc on dairies. In 1979, it was estimated that reproductive disorders account for 21% of health costs on dairy farms and insemination expenses

comprise another 19% of health costs (Shanks et al., 1981), indicating the dire need for efficient reproduction, particularly given today's rising costs. There is a direct relationship between profitability and reproduction via milk yield, number of available replacements, and involuntary and voluntary culling costs (Britt, 1985). Lower herd milk yield may result from fewer cows in peak milk production due to increased calving intervals that may be due to poor fertility and estrus detection rates (Norman et al., 2009). Poor fertility may lead to higher insemination costs and inevitability may increase involuntary culling (Norman et al., 2007). Inadequate reproductive performance in the first three lactations is a primary reason for culling dairy cows (Norman et al., 2009), further illustrating the importance of reproduction on profitability.

The economic importance of reproduction combined with unfavorable fertility, the antagonistic relationship between milk yield and fertility, and poor estrus detection, serve as powerful motivations to develop effective, practical methods to improve dairy cattle reproduction. One such program is Ovsynch technology, which was developed 16 years ago to allow for timed artificial insemination to enhance reproductive efficiency through control of time to 1st and subsequent artificial inseminations of dairy operations. Since then, research has improved the way Ovsynch technology controls ovarian function and consequently has improved conception rates of lactating dairy cows by utilizing understanding of the mechanisms involved in follicular dynamics and CL development.

OVSYNCH TECHNOLOGY

Utilizing the understanding of follicular and CL growth, development, and regression, Ovsynch (Pursley et al., 1995) was developed to pharmacologically control the estrous cycle. Ovsynch, which utilizes GnRH and PGF_{2α}, was developed to control follicular growth and CL

regression and synchronize ovulation so that cows can be timed-inseminated without the need for detection of estrus.

In cattle, $\text{PGF}_{2\alpha}$ is the most practical way to induce luteolysis (Beal, 1996). After administration of $\text{PGF}_{2\alpha}$, concentration of plasma progesterone decreases rapidly within 6 hours to less than 1 ng/ml within 48 hours (Louis et al., 1974). Cows typically begin estrus between 60 to 72 hours following $\text{PGF}_{2\alpha}$ administration (Beal, 1996).

Administering GnRH causes a surge of LH within 8 hours that induces ovulation (Pursley et al., 1995). In the Ovsynch protocol, ovulation to first GnRH determines the success of the protocol (Vasconcelos et al., 1999). Although stage of the estrous cycle is often unknown, previous experiments indicated that starting Ovsynch between days 5-9 of the cycle is most effective (Vasconcelos et al., 1999; Moreira et al., 2000). More recently, Bello et al., 2006 determined that beginning Ovsynch on day 6 of the estrous cycle was most effective. As a result, G6G was developed to increase the ovulatory response to the 1st GnRH of Ovsynch and subsequently improve synchronization rates. This program synchronizes the majority of cows to be on day 6 when the 1st GnRH of Ovsynch is administered utilizing an injection of $\text{PGF}_{2\alpha}$ followed by GnRH 2 days later and then beginning Ovsynch 6 days later (Bello et al., 2006).

STRATEGIES AND OBSTACLES OF RE-SYNCHRONIZATION

If cows were to restart their estrous cycle 21 days following an unsuccessful insemination, it would be assumed that 27 or 28 days following AI would be the best time to reinitiate a timed AI program (Vasconcelos et al., 1999; Moreira et al., 2001). However, it was found that 16-22% of cows did not have a CL 33 days after AI (Fricke et al., 2003; Sterry et al., 2006). Moreover, approximately 55-65% of cows fail to conceive after 1st AI (Bisinotto et al., 2009). Of those cows, less than 45% returned to estrus 20-24 days following AI (Dewey et al.,

2010). This suggests there is a large amount of variation of follicular and corpora lutea development between cows, possibly due to normal variation among cows, occurrence of pregnancy loss, and lack of synchrony of ovarian function of a timed AI program (Silva et al., 2007). In order to successfully synchronize and then resynchronize ovulation, proper timing and control over follicle development and luteal function must be established (Pursley et al., 1997). As a result, comprehensive research is warranted to develop a re-synchronization strategy that is most effective in getting cows re-inseminated, especially in relation to their stage of follicular and corpora lutea development.

Strategies for re-synchronizing cows that did not conceive upon 1st AI have seen mixed results. For example, while it is advantageous to re-inseminate cows as soon as possible when diagnosed not-pregnant, Sterry et al., 2006 found that postponing the first injection of GnRH of Ovsynch for re-synchronization until 33 days after timed AI resulted in a higher pregnancy rate than waiting only 26 days. In contrast, Fricke et al., 2003 found that pregnancy rate showed little difference between initiating Ovsynch at 33 days after timed AI (38%) versus 26 days (34%), but was lower at 19 days (23%).

In a more aggressive approach, administration of GnRH 7 days before pregnancy diagnosis was shown to expedite the re-synchronization process because it synchronizes follicle development. Then, upon pregnancy diagnosis, cows that are not-pregnant will continue with Ovsynch and it will not harm those diagnosed pregnant (Fricke et al., 2003; Chebel et al., 2003). This aggressive approach to re-synchronization was also examined in a study by Dewey et al., 2010. They found that administration of GnRH 7 days prior to pregnancy diagnosis followed by Cosynch-72 for non-pregnant cows resulted in increased pregnancy per AI. They found that cows that received the additional GnRH had higher ovulation rates to the 1st GnRH of Cosynch-

72, resulting in more CL than the control group. The additional GnRH caused more cows to be on day 7 of the estrous cycle when initiating a re-synchronization program, increasing their synchrony (Dewey et al., 2010). Another study compared the administration of a CIDR and GnRH prior to pregnancy diagnosis followed by completion of Ovsynch to cows that received only Ovsynch upon not-pregnant diagnosis. They found comparable conception rates between the two treatments; however, those that were treated with GnRH and a CIDR prior to pregnancy diagnosis became pregnant 3.2 days sooner than the Ovsynch group, reducing the number of days open (Thompson, 2010). Based on these studies, the effectiveness of this aggressive strategy of re-synchronization is unclear.

Different combinations of hormonal treatments in re-synchronization protocols have been extensively studied, particularly protocols that use an intravaginal progesterone releasing device (CIDR), but results have been inconclusive. In one study, cows without a CL when diagnosed open were treated with a CIDR and had pregnancy rates similar to cows with a CL at not-pregnant diagnosis (Sterry et al., 2006). Chebel et al., 2006 found that utilizing a CIDR in a re-synchronization program reduced pregnancy loss and increased pregnancy rate at 60 days. Bisinotto et al., 2009 conducted a study examining the effects of supplementing progesterone via a CIDR insert in a 5-day re-synchronization protocol at 34 days after 1st AI. It was found that cows with progesterone supplementation had increased pregnancy per AI for cows with a CL present but not for those without a CL. However, overall, they saw low ovulation rates of 35.6%, probably due to the day the synchronization program was initiated (Bisinotto et al., 2009). These results were confirmed in yet another study that found supplementation of a CIDR insert increased pregnancy per AI versus the control in a Cosynch-72 re-synchronization program (Dewey et al., 2010). Similar results were seen in a study comparing use of a CIDR prior to

initiating Ovsynch on day 23 following AI. Use of a CIDR increased pregnancy per AI only for cows that had a CL at not-pregnant diagnosis but decreased pregnancy per AI for cows inseminated to estrus prior to pregnancy diagnosis. The authors surmised that using a CIDR was not a cost effective method and did not improve overall pregnancy per AI (Bartolome et al., 2009). Thus, use of a CIDR in a re-synchronization protocol has shown mixed results.

Other studies have been conducted that examine the use of estradiol cypionate and hCG in re-synchronization programs. One such study examined the effects of three re-synchronization programs: 1) re-synchronization program when diagnosed not-pregnant; 2) CIDR days 14-21 after AI followed by AI based on estrus or initiation of re-synchronization program if not re-inseminated; 3) same as treatment 2 plus a dose of estradiol cypionate upon CIDR removal, on conception rates for cows of unknown pregnancy status. The researchers found that overall reproduction were not different between the 3 treatments (Galvão et al., 2007). Another trial compared the use of GnRH, hCG, and saline as a mechanism to induce ovulation 7 days prior to pregnancy diagnosis with not-pregnant cows receiving PGF_{2α} at pregnancy diagnosis followed by GnRH and timed AI 72 hours later. They hypothesized that the hCG would cause ovulation in more follicles and subsequently increase conception rates. Due to a treatment by herd interaction, treatment effects on conception rate were not interpretable but it appeared that cows in the hCG group had more pregnancy loss than cows in the GnRH and saline groups (Buttrey et al., 2009).

Researchers (Silva et al., 2009) compared conception rates between two strategies of re-synchronization: 1) administering Ovsynch at 25 days after timed AI with pregnancy diagnosis using pregnancy associated glycoprotein concentrations in the blood (ELISA test) at 27 days after AI or 2) administering Ovsynch at 32 days following timed AI with pregnancy diagnosis at 39 days with ultrasonography. No differences were found for conception rates between the two

treatments; however, average concentrations of progesterone were greater in cows started on day 32, suggesting that more cows in this group were in the later part of the estrous cycle (Silva et al., 2009). Other researchers discovered that 16-22% of cows did not have a CL at 33 days following timed AI, which further implies that cows are at various stages of the estrous cycle at pregnancy diagnosis (Fricke et al., 2003; Sterry et al., 2006). A similar 2011 study compared conception rates of two re-synchronization strategies: 1) administering CIDR-Ovsynch on day 18 after timed AI with pregnancy associated glycoprotein blood test pregnancy diagnosis on day 25 and 2) CIDR-Ovsynch on day 25 following timed AI and pregnancy diagnosis via transrectal ultrasonography on day 32 after timed AI. No differences in pregnancy rate were detected between the two treatments at 1st or 2nd insemination. The researchers suggested that initiating CIDR-Ovsynch on day 18 can be an effective way to rapidly resynchronize cows without compromising conception rates (Green et al., 2011).

Additional studies focusing on PGF_{2α} were conducted, aiming to improve reproduction. One study tested the use of cloprostenol versus dinoprost as the PGF_{2α} of Ovsynch in a re-synchronization program, and found that dinoprost increased luteolysis rates but no differences were detected in conception rates (Stevenson and Phatak, 2010). Similar results were also confirmed by Martins et al., 2011 who found no effect on conception rates between the two analogues of PGF_{2α} in an Ovsynch re-synchronization program. In a different trial, treating cows with PGF_{2α} 12 days before initiating a re-synchronization regimen resulted in more pregnancies and less pregnancy loss from 31 to 66 days after timed AI. However, it remains unclear whether this regimen is economically viable due to the extended period of re-synchronization (Silva et al., 2007).

Although various regimens have been developed for re-synchronizing ovulation, it remains unclear which protocols are the most effective at quickly re-inseminating cows, particularly since stage of the estrous cycle is unknown and extremely variable. Because of the economic implications of dairy cattle reproduction, it is crucial to the producer to determine how to maximize pregnancy rate and in turn, boost profits. Few studies have examined stage of the estrous cycle when placing cows on a specific re-synchronization program, and thus research is warranted to determine the most appropriate protocols for re-synchronization at various stages of the estrous cycle in order to improve conception rates.

ADVANTAGES OF SYNCHRONIZATION PROGRAMS

Synchronization protocols have many advantages. Ovsynch provides economic advantages, such as reducing the number of days open and the number of cows culled for poor fertility (Risco et al., 1998) and reduces reliance on estrus detection (Sterry et al., 2006; Tenhagen et al., 2004). A study of over 1 million herds in the US divided cows by potential synchronization status based on parameters such as time between inseminations (Norman et al., 2009). Herds were then divided into the following categories: not synchronized (n = 527,549), possibly synchronized (n = 89,109), probably synchronized (n = 344,470) and synchronized (n = 70,272). Synchronized Holstein herds inseminated in 2006 expressed 18 fewer days to first service than non-synchronized herds utilizing estrus detection. Synchronized herds also exhibited a shorter calving interval by 7 days (n = 38,352) over herds (n = 265,274) using only estrus detection (Norman et al., 2009). Moreover, synchronization programs provide management advantages since there is reduced reliance on estrus detection and a specific schedule of injections.

Although synchronization programs have many advantages and have been widely accepted in the dairy industry, there is room for improvement of these protocols. A portion of cows do not undergo complete luteolysis when treated with Ovsynch, which can be a limiting factor of the protocol (Pursley et al., 1997) This was further illustrated in a study comparing cloprostenol sodium to dinoprost tromethamine in which only 94% of first parity cows underwent complete luteolysis and 81% of second or more parity cows underwent complete luteolysis, indicating that cows fail to undergo complete luteolysis 20-30% of the time (Martins et al., 2011).

RELATIONSHIP BETWEEN TYPE TRAITS AND REPRODUCTION

Producers often utilize type traits in their breeding goals. However, the relationship between reproduction and type traits has not been conclusively studied. Farmers frequently utilize current measures of fertility, including nonreturn rate, days open, and calving interval, which are often documented poorly or inaccurately and have low heritabilities (Royal et al., 2002a). These traditional fertility measures are influenced by management and because of their low heritabilities, it is difficult to make genetic progress through selection for these traits (Darwash et al., 1998). Since improving reproduction through genetics alone can be very difficult, establishing relationships between conformation and fertility may allow producers to select for certain type traits and thereby indirectly improve fertility. As opposed to using traditional reproductive measures, a more effective approach would be to utilize dimensions of endocrine function, such as commencement to luteal activity, as an indicator of fertility which expresses higher heritability and exhibits less management bias. Producers would be able to make more knowledgeable breeding decisions if endocrine measures of fertility were placed in a fertility index which would increase the accuracy of breeding value prediction of reproduction

(Royal et al., 2002a). Establishing relationships between linear type traits and reproductive measures may conceivably lead to a more intensive selection process and avoidance of inadvertent selection for poor fertility while possibly improving reproduction.

Linear type traits refer to a variety of observable conformation features of animals (Berry et al., 2004). Recording for type traits in Holsteins has been in place since 1929 (Short & Lawlor, 1992) and emphasis on particular traits has become a substantial part of breeding programs used today. Selection programs often focus on increased milk production, placing emphasis on milk production conformation traits. While these traits are economically imperative, emphasis on functional traits, including fertility, longevity, and udder health is becoming increasingly critical in boosting overall farm profitability (Rupp and Boichard, 1999). While many studies have concentrated on the relationship between type traits and various other measures, few have been conducted to determine the effects of type traits on reproduction.

Previous studies examining type traits and their correlation with measures of fertility have shown confusing and conflicting results. For example, Berry et al., 2004 indicated that cows that were taller, wider, deeper, more angular, and had higher pins needed more services and displayed a decreased genetic merit for pregnancy.

However, the study by Berry et al., 2004 contradicts Pryce et al., 1998, who indicated that narrow-chested cows had lower conception rates and taller cows exhibiting angularity expressed a longer calving interval. Nevertheless, another study by Pryce et al., 2000 more closely mirrored the results of Berry et al., 2004, when they found that the body volume traits of stature, body depth, and chest width had positive genetic correlations with calving interval (0.33, 0.26, 0.28, respectively), indicating that taller, deeper bodied, and wider chested cows had a longer calving interval. However, Berry et al., 2004 disagreed with the direction of correlations

in a study by Royal et al., 2002a that proposed that cows possessing traits associated with frailty (such as angularity, thinness, narrow-rumped, or narrow-chested) have decreased fertility as indicated by increased time from parturition to 1st ovulation. In contrast, cows that were stronger and deeper had improved fertility. A negative correlation was found between body condition score and commencement to luteal activity in this study. Due to the moderate heritability of commencement to luteal activity and the fact that it is measurable in all animals, developing a more accurate sire PTA for fertility may be possible. In addition, genomic selection, which estimates breed values based on thousands of molecular markers rather than performance or family information, is becoming increasingly popular. Genomic selection allows for a shorter generation interval as genetic change increases two-fold over progeny testing (Schaeffer, 2006), so mating programs can allow for improved fertility based on genetic markers. Overall, endocrine measurements dealing with fertility, such as commencement of luteal activity and length of first luteal phase, have been found to have higher heritabilities than traditional reproductive traits (Royal et al., 2002b). This higher heritability, along with genomic selection, has the potential to help improve fertility.

Most studies examining type traits in relation to fertility have discovered that linear traits related to fertility were often volume traits, such as angularity, body depth, or stature. Also, the majority of genetic correlations between traits not associated with body volume and fertility were not significant (Wall et al., 2005). Unfortunately, information on genetic relationships between traditional and endocrine reproductive measures and linear type traits often used in the selection process is not readily available (Royal et al., 2002a). As the majority of studies that have been conducted in this area are more focused on the genetic side of reproduction, physiological data is necessary to discover the impact of conformation traits on reproduction. Therefore, the

relationship between type traits and endocrine reproductive measures has not been well established in the industry and further research is required. Furthermore, due to the conflicting nature of these genetic-based research projects and lack of physiological data, a comprehensive study focusing on type traits in relation to stage of follicular and corpora lutea development in conjunction with hormonal concentrations needs to be conducted.

Most research involving linear type traits have focused more on genetic based relationships with traits other than reproduction, such as locomotion, milk production, somatic cell score, and disease incidences. Research that focused on reproduction utilized traditional measures of fertility rather than utilizing stage of follicular and corpora lutea development. Due to the economic importance of reproduction in breeding goals, the associations between fertility and conformation traits merit more substantial further research.

SUMMARY

It is evident that fertility in the lactating dairy cow has drastically declined in the past several decades. Even though this decline is often attributed to the antagonistic relationship between milk production and fertility, other factors, including physiological, nutritional, and management factors can also hinder fertility. Synchronization of ovulation protocols have been developed to aid in combating this problem, but there is still room for reproduction to improve. When unfavorable reproduction results from low conception rates and diminished duration of estrus, producers can suffer economic losses due to the financial implications of reproduction on profitability.

With the increasing understanding of the mechanisms involved in the estrous cycle, great strides have been made to improve fertility of the lactating dairy cows. Synchronization of ovulation has been developed to eliminate reliance on estrus, control follicular growth and CL

lifespan, and allow for timed inseminations. This management tool can improve pregnancy rates; however, utilizing current synchronization methods as a strategy to re-inseminate cows that did not become pregnant on 1st service have seen mixed results. This is partially attributed to cows being in various stages of the estrous cycle when a re-synchronization protocol is administered, so further research is warranted to help producers improve conception rates for repeat inseminations.

Another potential aid in improving fertility lies in elucidating the relationship between type traits and fertility measures as it has not been conclusively studied. While some studies have examined the effect of type traits on fertility measures, many utilized traditional reproductive measures which can be biased and inaccurate. Thus, comprehensive research is warranted to reveal the relationship between type traits and fertility. Due to the economic implications of reproduction on profitability, it is increasingly vital to develop ways to help producers improve on-farm reproduction.

CHAPTER 3

SYNCHRONIZATION OF OVULATION BASED ON STAGES OF FOLLICULAR AND CORPORA LUTEA DEVELOPMENT IN LACTATING DAIRY COWS FOLLOWING A DIAGNOSIS OF NOT-PREGNANT

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ABSTRACT

Ovsynch appears to be most effective when initiated during the 1st follicular wave of an estrous cycle when the dominant follicle is responsive to the 1st GnRH allowing the PGF_{2α} to be administered prior to natural luteolysis. Thus, pre-synchronization programs, mainly utilized for 1st AI, are designed to synchronize cows to the 1st follicular wave; however, most cows are in other stages of follicle and CL development at time of a not-pregnant diagnosis. Therefore, the objective of this descriptive study was to examine three re-synchronization protocols adapted for four stages of follicular and corpora lutea development at a diagnosis of not-pregnant. Cows were placed on one of four re-synchronization protocols based on their estimated stage of follicular and corpora lutea development during an estrous cycle using transrectal ultrasonography: 1) Early cycle with small follicles (< 10 mm) and CL (< 20 mm) – Recheck 1 week later; 2) 1st wave dominant follicle (> 10 mm) and CL (> 20 mm) – Ovsynch (GnRH – 7d PGF_{2α} – 2d GnRH – 16 hr AI); 3) 2nd follicular wave with at least n = 1 follicle > 10 mm and new growing follicles or 2 follicles > 10 mm and CL > 20 mm – G6G (PGF_{2α} – 2d GnRH – 6d Ovsynch); 4) Pro-estrus or anovular condition with at least n = 1 follicle > 10 mm and either regressing (< 20 mm) or no CL – GGPG (GnRH – 6d Ovsynch). Conception rates were 27.8, 36.6, and 45.3% for Ovsynch, G6G, and GGPG, respectively, and were not statistically different. Heart girth measurements recorded at time of treatments indicated that smaller cows had greater rates of luteolysis following the final PGF_{2α} of Ovsynch and a tendency for increased conception rates in cows with a mature CL. This may be an artifact of ineffective dosage of PGF_{2α} in larger cows. In summary, cows in later estrous cycle that received re-synchronization with G6G and GGPG had greater ovulation rates to the 1st GnRH of Ovsynch than cows receiving Ovsynch

during the 1st follicular wave. This difference may be due to better control of the age of the follicle at time of 1st GnRH of Ovsynch. Cows in the 1st follicular wave group treated with Ovsynch likely had follicles that ranged from d 5 to d 10 of the cycle compared to cows that had follicles that were primarily on d 6 of the estrous cycle.

INTRODUCTION

Fertility of dairy cattle has drastically declined in the recent past, with conception rates (CR) reaching an alarmingly low rate of 30% on average (Norman et al., 2009). Boosting reproduction is crucial to increasing farm profitability. New pre-synchronization/Ovsynch programs enhance conception rates to 1st AI (Souza et al., 2008). Yet, cows that do not get pregnant on the 1st service are creating a huge financial problem for producers because of their subsequent low conception rates. As a result, farmers are seeking more effective re-synchronization protocols to reduce the number of repeat inseminations and boost CR.

Particularly in recent years, high producing dairy cows have exhibited diminished duration and expression of estrus, due to decreased circulating estradiol concentrations (Lopez et al., 2004; Galvão et al., 2007). As a result, hormonal protocols have been developed to decrease reliance on estrus detection (Sterry et al., 2006). Ovsynch, for example, is a regimen of injections of GnRH and PGF_{2α} to synchronize ovulation and control follicular growth and CL regression (Pursley et al., 1995; Peters & Pursley, 2002). This protocol eliminates the need for estrus detection completely because the cows will be inseminated at a certain time and date within the program (Pursley et al., 1995). However, for maximum synchrony, Ovsynch should be initiated at day 6 of the estrous cycle (Bello et al., 2006).

When cows do not conceive upon first service, there are several re-synchronization protocols readily available to get cows re-inseminated; however many of these programs are not

as effective as pre-synch/Ovsynch programs for 1st AI. In the high producing Holstein, AI CR were reported at 40% or less (Pursley et al., 1997) and have since been reported at 30% (Norman et al., 2009); therefore, with 60-70% of cows failing to conceive, an effective re-synchronization approach must be initiated for subsequent AI (Fricke et al., 2003). Moreover, nearly 55-65% of not-pregnant cows fail to conceive when re-inseminated, which causes an elongated period from calving to conception and increased number of days in milk (Bisinotto et al., 2009). If cows were to restart their estrous cycle following an unsuccessful insemination, it would be assumed that 32-33 days following AI would be the best time to reinitiate a timed AI program (Vasconcelos et al., 1999; Moreira et al., 2000). However, it was found that 16-22% of cows did not have a CL 33 days after AI (Fricke et al., 2003; Sterry et al., 2006) most likely due to these cows being anovular or in the follicular phase of the estrous cycle. This suggests there is a large amount of variation of follicular and corpora lutea development between cows, possibly due to normal variation among cows, occurrence of pregnancy loss, and lack of synchrony of ovarian function of a timed AI program (Silva et al., 2007). In order to successfully synchronize and then re-synchronize ovulation, proper timing and control over follicle development and luteal function must be established (Pursley et al., 1997). As a result, comprehensive research is warranted to determine which re-synchronization protocol is most effective in getting cows re-inseminated, especially in relation to their follicular and corpora lutea development.

Transrectal palpation can be an accurate technique to diagnose pregnancy; however, it is a poor method of detecting ovarian follicles (Pieterse et al., 1990). In contrast, ultrasonic imaging is a very accurate, quick way to assess structures on the ovary (Griffin and Ginther, 1992). Moreover, transrectal ultrasonography can be practically used to diagnose pregnancy status during early gestation (Fricke, 2002) and use is becoming more prevalent among bovine

practitioners. Many times, veterinarians scan the uterus during examinations and ignore examining the ovaries; however, ovarian status can be very useful in aiding in the diagnosis of reproductive status and appropriate reproductive interventions (Fricke, personal communication).

Few studies have examined stage of the estrous cycle via transrectal ultrasonography when placing cows on a specific re-synchronization program. This research is necessary to determine the most appropriate protocols for re-synchronization at specific stages of the cycle in order to improve CR. If effective, our approach to re-synchronization can help producers improve their on-farm profitability by putting cows on the re-synchronization protocol that will be most effective and potentially boost CR.

Moreover, it appears that body size may play a role in reproductive capacity. Berry et al., 2004 found that cows that were taller, wider, deeper, more angular, and had higher pins needed more services and displayed a decreased genetic merit for pregnancy. This suggests that larger, wider cows have decreased fertility. A study by Pryce et al., 2000 found that the body volume traits of stature, body depth, and chest width had positive genetic correlations with calving interval (0.33, 0.26, 0.28, respectively), indicating that taller, deeper bodied, and wider chested cows had a longer calving interval. These data suggest that cows with larger body volume traits have decreased reproductive capacity. Therefore, it may be possible that larger cows are not as responsive to each injection of a synchronization protocol as smaller cows. Response to each injection is vital to a synchronization protocol's success, so it is important to determine if body size is negatively impacting the success of these programs. Thus, our research will analyze the relationship between body size, measured by heart girth, and response to the injections of the re-synchronization programs in this study.

Thus, the objectives of this study are: 1) to examine three re-synchronization protocols adapted for four stages of follicular and corpora lutea development at not-pregnant diagnosis, 2) to aid in the development a reference guide for veterinarians to utilize ultrasonography to recommend the most effective synchronization protocol on an individual cow basis, and 3) to determine the effect of body size, measured by heart girth, on ovulation, luteolysis, and conception rates.

MATERIALS AND METHODS

The first portion of the study was conducted at a commercial dairy farm (Farm 1) in Elsie, MI between March and June 2010. Lactating Holstein cows were housed in free-stall barns with constant access to water. Cows were fed a TMR composed mostly of corn silage balanced to meet or exceed nutrient recommendations for lactating dairy cows (NRC 2001). Cows (n = 131) were milked three times a day. Pregnancy diagnoses were performed via transrectal palpation of uterine contents from 36 to 42 days after AI.

The second portion of the project was completed at a commercial dairy farm (Farm 2) in St. Johns, MI from July to December 2010. Lactating Holstein cows (n = 384) were housed in free-stalls with free access to water. Cows were fed a TMR composed mostly of corn silage balanced to meet or exceed nutrient recommendations for lactating dairy cows (NRC 2001). Cows were milked three times a day. Pregnancy diagnoses were performed via ultrasonography from 35 to 41 days after AI. Due to logistics, ovarian status was diagnosed 3 days following pregnancy diagnosis. Heart girth (HG) measurements were taken on all cows on the day before AI.

Treatments

At ovarian diagnosis, cows were assigned to 4 stages of development. Based on stage of follicular and corpora lutea development, cows were assigned to treatment. Stage 1: Cows in this group were in early stages of the estrous cycle in which neither the CL nor follicles were likely mature enough to respond to hormonal treatments (CL was < 20 mm in diameter and all follicles were < 10 mm in diameter). Cows in this group were rechecked one week later and assigned to treatment at that time. Stage 2: Cows in this group were in the 1st follicular wave of the estrous cycle with 1 large dominant follicle > 10 mm in diameter and at least 1 CL > 20 mm in diameter. Cows in this group were assigned to receive Ovsynch the following day [100 µg GnRH (Fertagyl, Intervet-Schering Plough Animal Health, DeSoto, KS 66018) - 7d 500 µg cloprostenol (Estrumate, Intervet-Schering Plough Animal Health, DeSoto, KS 66018) - 100 µg GnRH (G2) - 16 h AI]. Stage 3: Cows in this group were in the 2nd follicular wave of the estrous cycle with 2 large follicles, a non-responsive dominant follicle from the 1st wave that was greater than 10 mm in diameter and a dominant follicle from the 2nd wave, or 1 large follicle and a new growth of follicles and a mature CL > 20 mm in diameter. Cows in this group were treated with G6G/Ovsynch [500 µg cloprostenol – 2 d later - 100 µg GnRH (preG) - 6d later - Ovsynch (see above)]. Stage 4: Cows in this group were either in proestrus with at least 1 large dominant follicle >10 mm in diameter and either a regressing CL (less than 20 mm) or no visible CL, or anovular including cows with follicular cysts. Cows in this group were treated with GGPG [100 µg GnRH (preG) - 6d later - Ovsynch (see above)].

Transrectal ultrasound examinations were conducted at each injection using a real time, B-mode, SonoSite MicroMaxx ultrasound machine with a 10.0-MHz linear array probe (SonoSite, USA). Height and width of the maximal size of CL and the antrum of each follicle >

4 mm in diameter were measured with built-in calipers. Follicular and luteal measurements were recorded in an ovarian map for each cow with date and time of examinations. Mean follicular diameter (d) was calculated by the average of height and width ($d = H+W/2$) of each follicle. Blood samples were collected by coccygeal venipuncture using tubes without anticoagulant additive (BD Vacutainer, Preanalytical Solutions, Franklin Lakes, NJ) at stage of follicular and corpora lutea development diagnosis and each injection of Ovsynch. Samples were refrigerated and centrifuged at $3,000 \times g$ for 20 minutes within 24 hours. Serum was collected and placed in freezer at -20°C for later progesterone (P_4) and estradiol (E_2) analysis.

Cows were inseminated by farm technicians using normal operating procedures for each particular farm. Semen was purchased and designated to each cow by farm managers. Pregnancy diagnoses were performed by farm veterinarians. Cows designated not-pregnant were utilized in this experiment.

Cows with clinical signs of illness were excluded from the experiment. Cows placed on the wrong program, as determined by ultrasound diagrams and later P_4 analyses, were excluded from this analysis. Animal handling procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Michigan State University.

Hormonal Assays

Serum P_4 concentrations (ng/mL) were quantified using RIA (Coat-A-Count Progesterone, Siemens Diagnostics, Los Angeles, CA). Sensitivity of the assay was 0.04 ng/mL. Intra-assay and inter-assay CV were 8.7 and 12.1%, respectively for the low quality control and 6.3 and 5.1%, respectively for the high quality control.

Serum E_2 concentrations (pg/mL) were quantified from blood collected on the day of final GnRH and the day of AI. Serum samples were ether extracted in duplicate and measured

using a modified version (Prendiville et al., 1995) of RIA MAIA kit (Polymedco Inc., Cortland Manor, NY). Intra-assay and inter-assay CV were 11.9 and 18.3%, respectively for the low quality control which averaged 0.468 pg/mL and 14.3 and 11.2% for the high quality control which averaged 4.39 pg/mL. Sensitivity of the assay was 0.19 pg/mL.

Statistical Analysis

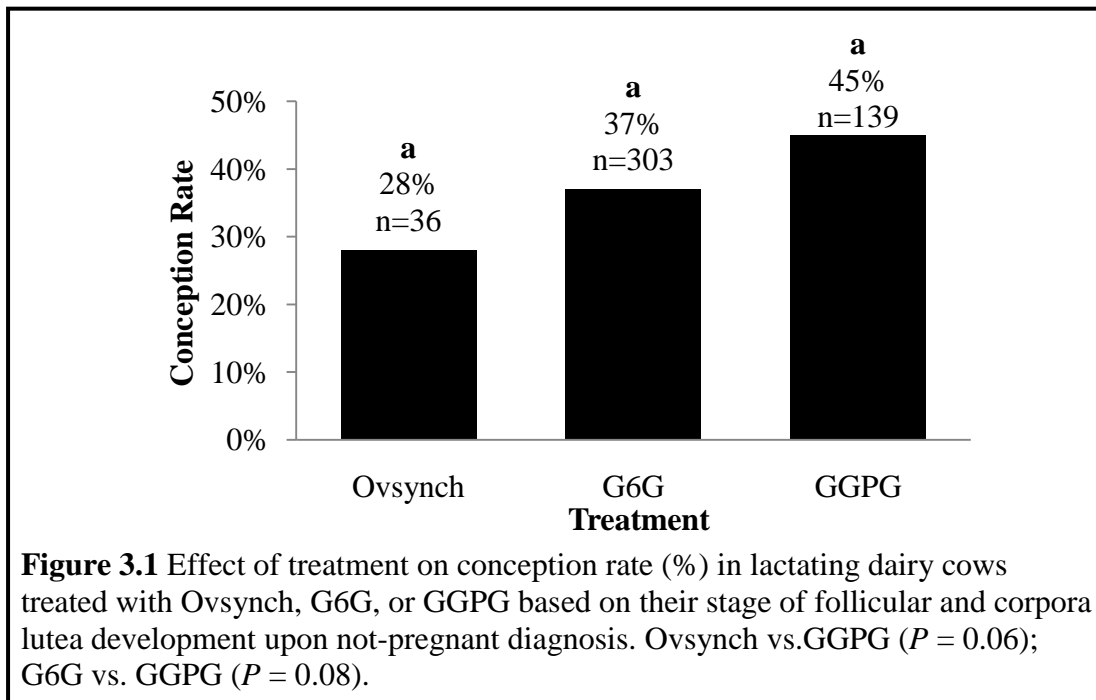
Data were analyzed using the GLM procedure in SAS (Version 9.2, SAS Institute, Cary, NC). Response variables measured were conception rates, luteolysis rates, synchronization rates, serum E₂ concentrations, and serum P₄ concentrations. The final model considered treatment, parity, and their interactions as fixed effects and cows as a random effect. There was no treatment by farm ($P = 0.9$) or milk production ($P = 0.16$) interaction. Binomial data were analyzed using chi-square analysis. The variables measured were pair-wise differences between treatments for conception rates, luteolysis rates, and ovulation rates.

RESULTS

Effect of Treatment on Conception Rate and Response to Injections

Conception rates were 27.8%, 36.6%, and 45.3% for Ovsynch, G6G, and GGPG, respectively (as seen in Figure 3.1) and not significantly different.

Parity had a significant effect on conception rates ($P = 0.005$) with 1st parity cows having greater conception rates. Cows placed in the Recheck 1 week group were reassigned to new treatments one week later. The number of cows that became pregnant for their new groups were (2/4), (6/18), and (7/14) for Ovsynch, G6G, and GGPG, respectively.



Ovulation rate to the 1st GnRH of Ovsynch was less for Ovsynch compared to G6G and GGPG (Figure 3.2). Percentage of cows with complete luteolysis following PGF_{2α} of Ovsynch was greater for GGPG compared to G6G (Figure 3.3). Percentage of cows that responded to all injections of Ovsynch was less ($P < 0.05$) for Ovsynch (47%) compared to 67% for G6G and 72% for GGPG.

Accuracy of analysis of luteal function was determined by P₄. Cows that were designated to the correct group based on P₄ was 100% for Recheck 1 week, 74% for Ovsynch, 95% for G6G, and 87% for GGPG, with a total of 91% overall.

Cows with greater E₂ concentrations at G2 of Ovsynch ($4.5 \text{ ng/mL} \pm 1.5$ for pregnant cows vs. $3.8 \pm 1.6 \text{ ng/mL}$ for non-pregnant cows) had greater CR ($P < 0.0001$). In synchronized cows, cows that became pregnant had an average E₂ concentration of $4.6 \pm 0.11 \text{ ng/mL}$ at G2 versus $4.1 \pm 0.12 \text{ ng/mL}$ for cows that did not become pregnant ($P = 0.001$).

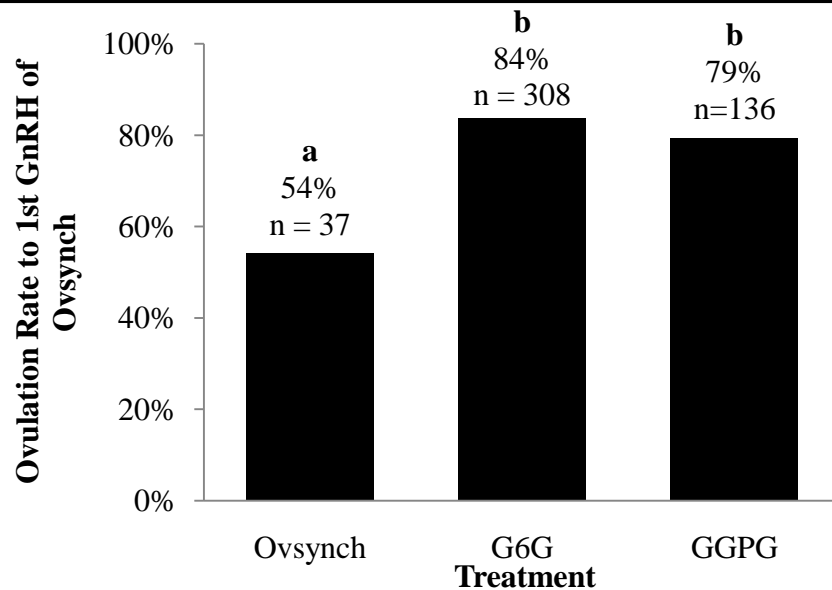


Figure 3.2 Effect of treatment on ovulation rate (%) at time of 1st GnRH injection of Ovsynch in lactating dairy cows treated with Ovsynch, G6G, or GGPG based on their stage of follicular and corpora lutea development at not-pregnant diagnosis. Bars without common letters were significantly different ($P < 0.05$).

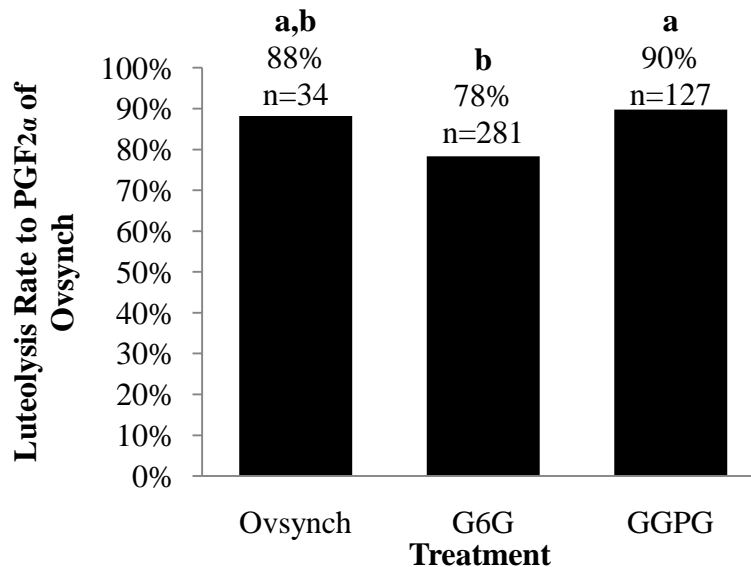
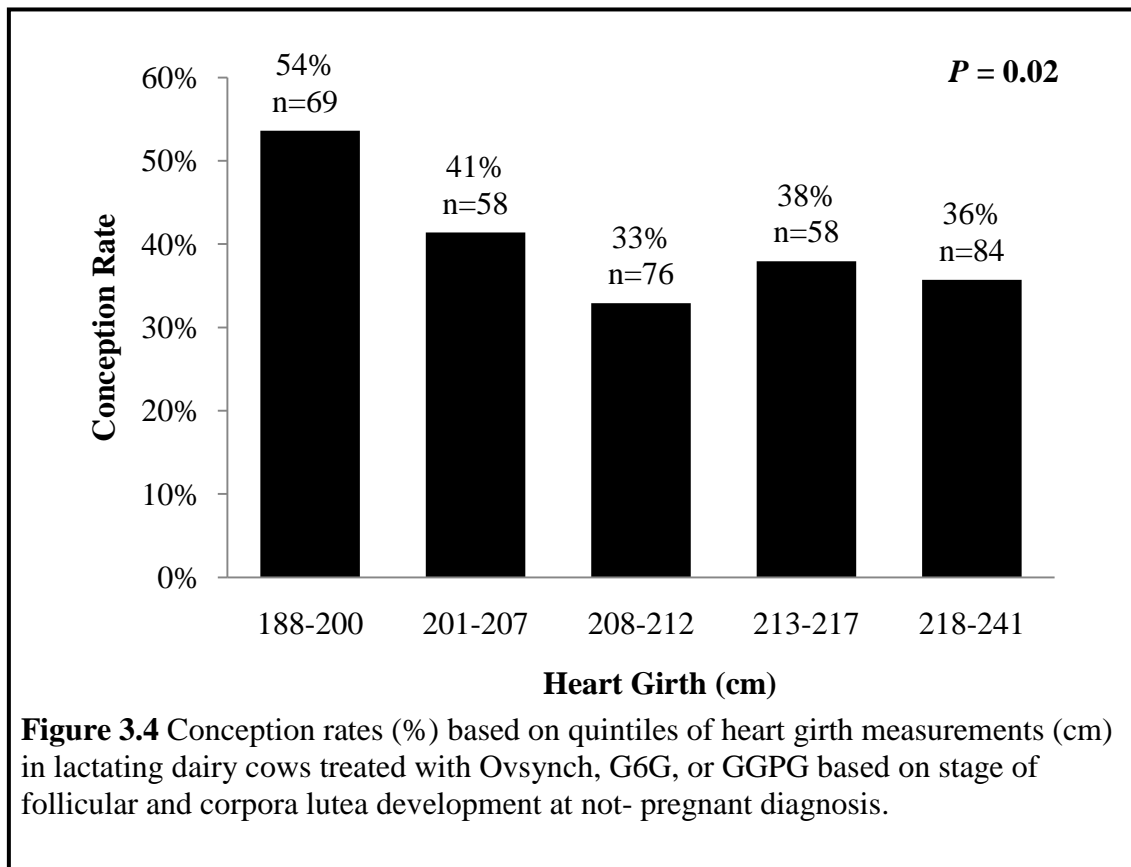


Figure 3.3 Effect of treatment on luteolysis rate¹ (%) to PGF_{2α} of Ovsynch for cows treated with Ovsynch, G6G, or GGPG based on stage of follicular and corpora lutea development at not-pregnant diagnosis. Bars without common letters are significantly different ($P < 0.05$). Bars with common letters are not significantly different.

¹Complete luteolysis = $P_4 < 0.5$ ng/mL 50 and 66 h after PGF_{2α} injection for cows with a functional CL at time of PGF_{2α}.

Based on heart girth measurements at time of treatment, smaller cows had increased CR (Figure 3.4). Larger cows had a tendency for lower rates of luteal regression (Figure 3.5). Average heart girth measurements in pregnant vs. non-pregnant cows are illustrated in Table 3.1. Average P_4 at $PGF_{2\alpha}$ for cows with 2 CL was 5.9 ng/mL and average CL volume was 13557 mm³. Concentrations of P_4 in circulation per CL volume (average 0.00046 ng/mL P_4 per mm³ luteal tissue) was highly insignificant in relation to size of cow at time of $PGF_{2\alpha}$ of Ovsynch ($P = 0.96$; $n = 161$).



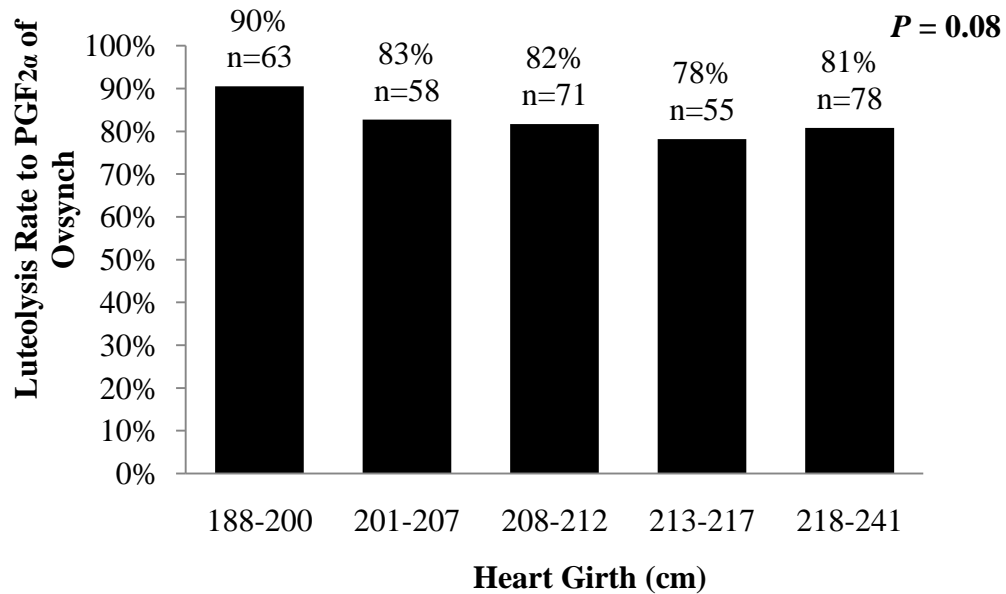


Figure 3.5 Rate of luteolysis¹ (%) on quintiles of heart girth measurements (cm) in lactating dairy cows treated with Ovsynch, G6G, or GGPG based on stage of follicular and corpora lutea development at not-pregnant diagnosis.

¹Complete luteolysis = $P_4 < 0.5$ ng/mL 50 and 66 h after PGF_{2α} injection for cows with a functional CL at time of PGF_{2α}.

Table 3.1 Average heart girth measurements based on pregnancy status and CL regression in lactating dairy cows treated with Ovsynch, G6G, or GGPG based on stage of follicular and corpora lutea development at not-pregnant diagnosis.

	Cows with 1 or more mature CL ¹		All Cows	
	Average HG (in.)	<i>P</i> -value	Average HG (in.)	<i>P</i> -value
Not-pregnant	83.19 ± 0.29	0.067	83.43 ± 0.27	0.015
Pregnant	82.35 ± 0.35		82.38 ± 0.33	
Incomplete Regression	84.03 ± 0.59	0.038	83.91 ± 0.55	0.078
Complete Regression ²	82.77 ± 0.25		82.88 ± 0.24	

¹Only cows that ovulated to preG were considered making the CL 13 days old and thus mature

²Complete luteal regression = $P_4 < 0.5$ ng/mL 50 and 66 h after PGF_{2α} injection for cows with a functional CL at time of PGF_{2α}.

DISCUSSION

Determining morphological stage of follicular and corpora lutea development with ultrasound was not always clear cut and caveats exist. Stage 1 was the most predictable and had the greatest agreement with regards to P_4 concentrations. All cows in this stage had no follicles > 10 mm in diameter and no CL > 20 mm in diameter. All cows placed in this stage were clearly in Stage 2 or 3 a week later (mostly Stage 3). Since Ovsynch treatment in Stage 2 was relatively unsuccessful, our data would suggest that cows in this stage should be automatically assigned to a G6G treatment 1 week later. Cows in Stage 2 were generally difficult to predict based on CL > 20 mm in diameter and a dominant follicle > 10 mm in diameter. As a result, there were fewer cows than predicted for this group. Theoretically, this accounts for approximately 1/4 of cycling cows and we only had 7.5 % of the total in this group. Prediction of stage of the estrous cycle could sometimes fall into what could be seen as two different stages. This was particularly the case at the transition between Stage 3 and Stage 4 when it was difficult to assess the functionality of corpora lutea. There were a small % of cows that had < 20 mm CL that were functional based on P_4 concentrations. There were also a small % of cows that had > 20 mm CL that had low P_4 . In this trial, the majority of cows fell into stages 3 and 4 of the estrous cycle. This may have been due to the timing of pregnancy diagnosis (d 35 to 42) relative to the expected start of the new cycle. If the cows did not become pregnant and restarted their estrous cycle around d 21, they would mostly be in stage 3 or 4, which is what we observed.

The ovulation rate for Ovsynch was lower than desired and expected which led to decreased conception rates albeit numbers of cows in this group make it difficult to assess each of these parameters. The percentage of cows correctly diagnosed in the Ovsynch group was also lower than anticipated due to the high variation in follicular and corpora lutea development at

this stage. As a result, it may be more beneficial to utilize only 3 treatments when pregnancy diagnosing 35 to 41 d after AI: 1) Recheck 1 week for cows with immature CL and follicles; 2) G6G for cows with a CL; 3) GGPG for cows without a CL.

Although we showed evidence of improved conception rates, a limiting factor of these protocols is the percentage of cows failing to undergo complete luteolysis (12% for Ovsynch, 22% for G6G, and 10% GGPG). We defined complete luteolysis as cows < 0.5 ng/mL P_4 2 d following $PGF_{2\alpha}$ based on research that suggested cows with P_4 greater than 0.5 ng/mL had a very low chance of becoming pregnant (Martins et al., 2011; Souza et al., 2007; Brusveen et al., 2009). Data from the present study agree that cows with P_4 intervals greater than 0.5 ng/mL at AI had conception rates of 12.5% or below. Therefore, it is vital to induce complete luteolysis in order to increase conception rates.

Our study indicates that larger cows with a mature CL fail to undergo complete luteolysis a greater percentage of the time and had a tendency to have lower conception rates than smaller cows that had a mature CL. Similarly, Berry et al., 2004 found that cows that were genetically taller, wider, and deeper had lower pregnancy rates to 1st service and required more services. This is potentially an artifact of incorrect dosage in timed AI programs. Currently, all cows are recommended to receive the same dose of GnRH and $PGF_{2\alpha}$, regardless of size. We speculate that larger cows need to receive a higher dose of $PGF_{2\alpha}$ or series of doses to increase chance of complete luteolysis and subsequent chance of pregnancy.

In summary, utilizing ultrasonography to determine stage of follicular and corpora lutea development and subsequently placing cows on a re-synchronization program tailored to that stage can produce favorable synchronization rates. Conception rates seen in this study were acceptable for re-synchronization protocols. However, since Ovsynch did not provide acceptable

synchronization rates, it may be more beneficial to have only 3 treatments: 1) Recheck 1 week for cows with immature CL and follicles; 2) G6G for cows with a CL; 3) GGPG for cows without a CL. Data from this study and others would indicate a second injection of $\text{PGF}_{2\alpha}$ may be necessary in larger more mature cows, e.g., 2+ lactation cows.

CHAPTER 4

THE IMPACT OF TYPE TRAITS ON FERTILITY OF LACTATING DAIRY COWS ON A 3000 COW DAIRY

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ABSTRACT

There are a paucity of studies that examined the relationship between type traits and fertility. Relationships between linear type traits and fertility were utilized from Holstein Association classification records of 2299 lactating dairy cows at a registered / commercial dairy farm for a one year period. Twenty traits (Stature, Strength, Body Depth, Dairy Form, Rump Angle, Thurl Position, Rump Width, Rear Legs Side View, Rear Legs Rear View, Foot Angle, Fore Udder Attachment, Rear Udder Height, Rear Udder Width, Udder Cleft, Udder Depth, Front Teat Placement, Teat Length, Udder Tilt, Rear Teat Placement, and Body Condition) measured on a scale of 1-50 were recorded. Two studies were performed. In Study 1, 1st service cows were divided into quartiles based on each type trait score. Pregnancy per AI (P/AI) was calculated for each quartile group and compared utilizing chi-square analysis. In Study 2, cows were placed into 3 fertility groups based on reproductive performance through the data collection period: 1) high fertility, conceived on 1st service; 2) medium fertility, conceived following 4 or more services; and 3) low fertility, did not conceive following 4 or more services. In Study 1, the only traits that appeared to have an effect on fertility in both 1st and 2nd + parity groups were Strength and Body Depth. In Study 2, the only traits that appeared to impact fertility in both 1st and 2nd + parity groups were Strength, Body Depth, Foot Angle, and Udder Width. Cows with lower Strength and Body Depth scores had greater fertility for both 1st and 2nd + parity groups. Cows with lower Foot Angle scores and narrower Udder Width scores appear to be more fertile. Cows with higher Rump Angle (more sloped) and Thurl (lower position) scores had greater fertility. In summary, traits that may be more profitable in terms of genetic selection for fertility appear to trend towards smaller, narrower cows.

INTRODUCTION

Linear type traits refer to a variety of observable biological features of animals (Berry et al., 2004). Recording for type traits in Holsteins has been in place since 1929 (Short & Lawlor, 1992) and emphasis on particular traits have become a substantial part of breeding programs used today. Selection programs often focus on increased milk production, placing emphasis on milk production conformation traits. While these traits are economically imperative, emphasis on functional traits, including fertility, longevity, and udder health is becoming increasingly critical in boosting overall farm profitability (Rupp and Boichard, 1999). While research has been conducted that examined the relationship between type traits and various measures, such as genetics, production, longevity, and health, research investigating the relationship between reproduction and conformation traits has not been extensively studied.

Since improving reproduction through genetics alone can be very difficult, establishing relationships between conformation and fertility can allow producers to select for certain type traits and thereby indirectly influence fertility. The few studies conducted examining type traits in relation to fertility have discovered that linear traits related to fertility were often volume traits, such as angularity, body depth, or stature. Also, the majority of genetic correlations between traits not associated with body volume and fertility were not significant (Wall et al., 2005). Most of the research involving linear type traits has focused more on genetic based relationships with traits other than reproduction, such as locomotion, milk production, somatic cell score, and disease incidences. Thus, information on genetic relationships between traditional reproductive measures and linear type traits often used in the selection process is not readily available (Royal et al., 2002). Due to the economic importance of reproduction in breeding goals, the associations between fertility and conformation traits merit substantial further

research (Onyiro, 2008). The objective of Study 1 was to: 1) determine the effect of linear type trait scores on P/AI. The objective of Study 2 was to: 2) determine differences in average linear type trait scores in high, medium and low fertility groups.

MATERIALS AND METHODS

Calving, breeding, and production data were collected from Dairy Comp 305 (Valley Ag Software, Tulare, CA) for cows that were exposed to a breeding program at any time from June 1, 2007 to June 30, 2008 at Green Meadow Farms (Elsie, MI). During the data collection period, lactating Holstein cows were housed in free-stall barns with free access to water. Cows were fed a TMR composed mostly of corn silage balanced to meet or exceed nutrient recommendations for lactating dairy cows (NRC 2001). Cows were milked three times a day. During the 1st 6 months of data collection, cows received AI if detected in standing estrus following either a natural estrous cycle or following prostaglandin F_{2α}. During the final 6 months of data collection, cows received Presynch-11/Ovsynch and were timed inseminated for 1st AI. Cows that did not become pregnant received AI if detected in standing estrus following either a natural estrous or following prostaglandin F_{2α}. Pregnancy information was based on diagnoses that were performed via transrectal palpation of uterine contents at 39 days after AI or if returned to estrus and re-inseminated.

In Study 1, only cows that received a classification score with trait breakdowns and received at least 1 AI were included (n = 2299). Each type trait was divided into quartiles based on scores from 1 to 50. P/AI was calculated for 1st AI only for cows in each quartile. In Study 2, lactating dairy cows were assigned to 3 levels of fertility: 1) high fertility, cows that conceived on 1st service; 2) medium fertility, cows that conceived following 4 or more services and 3) low fertility, cows that never became pregnant following 4 or more services (n = 1656). In order to be

conservative, cows that conceived on the 2nd or 3rd service were not included in the high fertility group due to possible confounding factors, such as uterine infections, incorrect insemination technique, or negative energy balance in early lactation. Thus, only cows that conceived on the 1st service were considered to truly have high fertility and cows that conceived on 2nd or 3rd service were not included in this study. In each study, pregnancy status was considered only at the 39 d pregnancy diagnosis. Information acquired from Dairy Comp 305 included parity, number of inseminations, AI date, breeding outcome, breeding code (estrus or timed AI) for 1st service, and DIM. The groups were determined from generating a list based on number of inseminations and pregnancy outcome. Reproduction information was collected from the individual cow pages. Milk production data was acquired through creating a list of milk data of all cows in the herd from 2007-2008. Test day DIM closest to 90 DIM and corresponding ME milk values were utilized.

Linear breakdown scores provided by the Holstein Association USA, Brattleboro, VT to Green Meadow Farms were recorded in Microsoft Excel for cows that were classified from 1 year prior to 1st AI recorded to 1 year after the last AI recorded to ensure that classification scores were nearest as possible to 1st AI dates (Study 1) or AI periods (Study 2). The farm classified three times annually during the collection period. Twenty traits measured on a scale of 1-50 were recorded. Definitions of type traits and their corresponding scores are defined in Table 4.1. In 2008, the Holstein Association removed the trait “Thurl” from their scorecard; thus, not all cows have records for this trait (n = 1567). Composite scores (measured from 1-100) of front end/capacity, dairy strength, rump, feet and legs, udder, and a final classification score (measured from 50-100) were also included in the spreadsheet.

Table 4.1 Linear type traits scores and their respective meanings.			
Trait	Definition	Low Score	High Score
ST	Stature	Short	Tall
SR	Strength	Narrow and frail	Strong and wide
BD	Body Depth	Shallow body	Deep body
DF	Dairy Form	Tight	Open
RA	Rump Angle	High pins	Sloped rump
TH	Thurl Position	High	Low
RW	Rump Width	Narrow	Wide
LS	Rear Legs, Side View	Posty legs	Sickled legs
RL	Rear Legs, Rear View	Severe toe-out	No toe-out
FA	Foot Angle	Low angle	Steep angle
FU	Fore Udder Attachment	Loose	Strong
UH	Rear Udder Height	Low	High
UW	Rear Udder Width	Narrow	Wide
UC	Udder Cleft	Weak	Strong
UD	Udder Depth	Deep	Shallow
TP	Front Teat Placement	Outside quarter	Inside quarter
RT	Rear Teat Placement	Outside quarter	Inside quarter
TL	Teat Length	Short teats	Long teats
UT	Udder Tilt	Rear quarters deep	Front quarters deep

Analyses of Conception Rate to 1st service in Study 1

To examine the effect of each type trait on conception rate at 1st service, each type trait was separated into quartiles for both parity levels and analyzed using the Cochran-Mantel-Haenszel chi-square test. Parity had a significant effect on 1st service conception rate ($P < 0.01$) so data was stratified by parity (1st or 2nd +). Effect of milk production on conception rate at 1st AI based on estrus ($P = 0.45$) or timed AI ($P = 0.43$) was not significant so milk data was not used further in the analysis.

Analyses of Fertility Groups in Study 2

Average score for each trait was analyzed between fertility groups using analysis of variance in SAS (Version 9.2, SAS Inst. Inc., Cary, NC). Cows were analyzed separately for parity 1 and parity 2+.

RESULTS

Tables 4.2, 4.3, 4.4 and 4.5 describe linear type traits that were analyzed in Study 1 and 2. Only the traits, Strength and Body Depth, were consistently different in Study 1 and Study 2 in both parities (1st or 2nd + parity). In Study 1, the only traits that were different in both 1st and 2nd + parity groups were Strength and Body Depth. In Study 2, the only traits that were different in both 1st and 2nd + parity groups were Strength, Body Depth, Foot Angle, and Udder Width. 1st parity cows had differences in Strength, Body Depth, Rump Width, Rear Leg Rearview, and Foot Angle in both Study 1 and 2. 2nd + parity cows had differences in Strength, Body Depth, Rump Angle, Thurl, and Rear Udder Width in both Study 1 and 2. In all cases, cows with lower Strength and Body Depth scores had greater fertility. Cows with lower Foot Angle scores and narrower Udder Width scores appeared to be more fertile. Cows with higher Rump Angle (more sloped) and Thurl (lower position) scores had greater fertility.

Parity had a significant effect on 1st service conception rate ($P < 0.01$). Cows that were inseminated based on detection of estrus had lower conception rates than those inseminated with timed AI in both parity 1 and parities 2+ (Figure 4.1). Effect of milk production on conception rate at 1st AI based on estrus or timed AI was not significant ($P = 0.45, 0.43$, respectively).

Table 4.2 Conception rates for 1st AI and quartile ranges for each trait by quartile for 1st parity lactating dairy cows in Study 1.

Trait	Quartile 1		Quartile 2		Quartile 3		Quartile 4		<i>P</i> - value
	Conception Rate (%)	Quartile Range	Conception Rate (%)	Quartile Range	Conception Rate (%)	Quartile Range	Conception Rate (%)	Quartile Range	
ST	41.67(n=228)	1-24	35.98(n=239)	25-29	32.83(n=265)	30-34	36.92(n=260)	35-50	0.24
SR	44.10(n=195)	2-20	34.82(n=247)	21-25	39.86(n=286)	26-31	29.55(n=264)	32-50	0.01
BD	44.12(n=204)	4-20	38.83(n=188)	21-25	30.84(n=308)	26-29	36.3 (n=292)	30-50	0.02
DF	36.84(n=209)	1-19	38.19(n=254)	20-24	32.78(n=180)	25-28	37.54(n=349)	29-47	0.67
RA	31.36(n=169)	1-14	38.69(n=305)	15-20	34.68(n=248)	21-28	39.63(n=270)	29-50	0.26
TH	36.49(n=285)	1-19	41.44(n=292)	20	33.76(n=157)	21-26	33.33(n=258)	27-50	0.2
RW	44.9(n=196)	1-20	36.82(n=220)	21-24	36.07(n=305)	25-31	31.37(n=271)	32-50	0.03
LS	34.09(n=132)	1-19	35.25(n=358)	20-26	37.00(n=200)	27-31	39.07(n=302)	32-46	0.72
RL	46.09(n=230)	1-17	38.17(n=241)	18-24	30.47(n=256)	25-29	33.21(n=265)	30-46	0.01
FA	43.5(n=246)	5-16	40.43(n=188)	17-20	35.47(n=172)	21-24	31.09(n=386)	25-49	0.01
FU	36.99(n=219)	2-15	41.36(n=220)	16-20	36.90(n=290)	21-27	32.32(n=263)	28-50	0.24
UH	36.32(n=201)	1-19	35.14(n=185)	20-25	37.21(n=344)	26-32	37.40(n=262)	33-50	0.96
UW	40.42(n=240)	1-18	36.76(n=204)	19-24	36.07(n=219)	25-31	34.35(n=329)	32-50	0.52
UC	43.67(n=229)	2-24	36.64(n=262)	25	32.24(n=183)	26-32	34.28(n=318)	33-50	0.07
UD	42.73(n=220)	5-19	36.20(n=221)	20-28	32.94(n=252)	29-33	35.79(n=299)	34-50	0.16
TP	41.67(n=216)	1-18	36.24(n=229)	19-24	34.56(n=298)	25-31	35.34(n=249)	32-50	0.38
RT	44.35(n=248)	1-22	34.23(n=222)	23-25	34.65(n=254)	26-32	33.58(n=268)	33-50	0.04
TL	38.74(n=191)	1-14	32.42(n=256)	15-20	41.03(n=290)	21-25	34.51(n=255)	26-50	0.16
UT	38.35(n=206)	1-28	38.19(n=199)	29-31	30.99(n=313)	32-34	40.88(n=274)	35-50	0.08

Table 4.3 Conception rates for 1st AI and quartile ranges for each trait by quartile for 2+ parity lactating dairy cows in Study 1.

Trait	Quartile 1		Quartile 2		Quartile 3		Quartile 4		<i>P</i> - value
	Conception Rate (%)	Quartile Range	Conception Rate (%)	Quartile Range	Conception Rate (%)	Quartile Range	Conception Rate (%)	Quartile Range	
ST	27.19(n=320)	2-26	23.92(n=301)	27-33	20.12(n=343)	34-38	23.91(n=343)	39-50	0.20
SR	27.16(n=243)	2-24	27.81(n=356)	25-29	19.22(n=437)	30-34	22.51(n=271)	35-50	0.02
BD	27.04(n=196)	8-24	27.48(n=433)	25-28	18.93(n=280)	29-35	21.36(n=398)	36-50	0.03
DF	24.49(n=245)	1-21	27.04(n=392)	22-27	21.97(n=314)	28-34	21.07(n=356)	35-46	0.22
RA	17.00(n=253)	1-14	28.32(n=392)	15-23	18.26(n=334)	24-26	28.96(n=328)	27-50	0.01
TH	17.98(n=366)	1-17	16.03(n=131)	18-19	26.22(n=492)	20-25	29.27(n=328)	26-50	0.01
RW	23.79(n=248)	2-24	24.54(n=383)	25-28	28.05(n=246)	29-34	20.47(n=430)	35-50	0.16
LS	20.92(n=325)	1-21	24.58(n=297)	22-25	23.36(n=351)	26-31	26.05(n=334)	32-50	0.47
RL	24.14(n=319)	2-19	25.53(n=141)	20-24	26.15(n=478)	25-32	19.51(n=356)	33-50	0.14
FA	22.15(n=325)	2-18	25.96(n=312)	19-24	26.20(n=332)	25-27	20.71(n=338)	28-50	0.25
FU	27.38(n=263)	1-16	28.16(n=368)	17-24	22.19(n=338)	25-31	20.71(n=338)	32-50	0.21
UH	27.33(n=311)	3-23	26.48(n=321)	24-27	21.39(n=346)	28-34	20.06(n=329)	35-50	0.07
UW	30.68(n=251)	1-24	25.27(n=376)	25-28	20.83(n=336)	29-34	19.77(n=344)	35-50	0.01
UC	25.27(n=273)	2-24	27.56(n=352)	25-27	22.30(n=269)	28-32	20.34(n=413)	33-50	0.11
UD	23.97(n=267)	1-18	25.00(n=356)	19-24	22.51(n=351)	25-31	23.42(n=333)	32-50	0.89
TP	24.00(n=300)	1-19	25.12(n=203)	20-24	24.44(n=401)	25-31	22.08(n=403)	32-50	0.81
RT	25.16(n=318)	1-24	21.70(n=318)	25-26	26.90(n=316)	27-33	21.41(n=355)	34-50	0.27
TL	29.07(n=313)	1-14	22.83(n=311)	15-23	21.30(n=336)	24-26	22.03(n=345)	27-50	0.08
UT	28.87(n=284)	1-27	23.49(n=166)	28-29	23.30(n=526)	30-33	20.06(n=329)	34-50	0.08

Table 4.4 Average type trait scores and standard errors for each fertility group for 1st parity lactating dairy cows in Study 2.

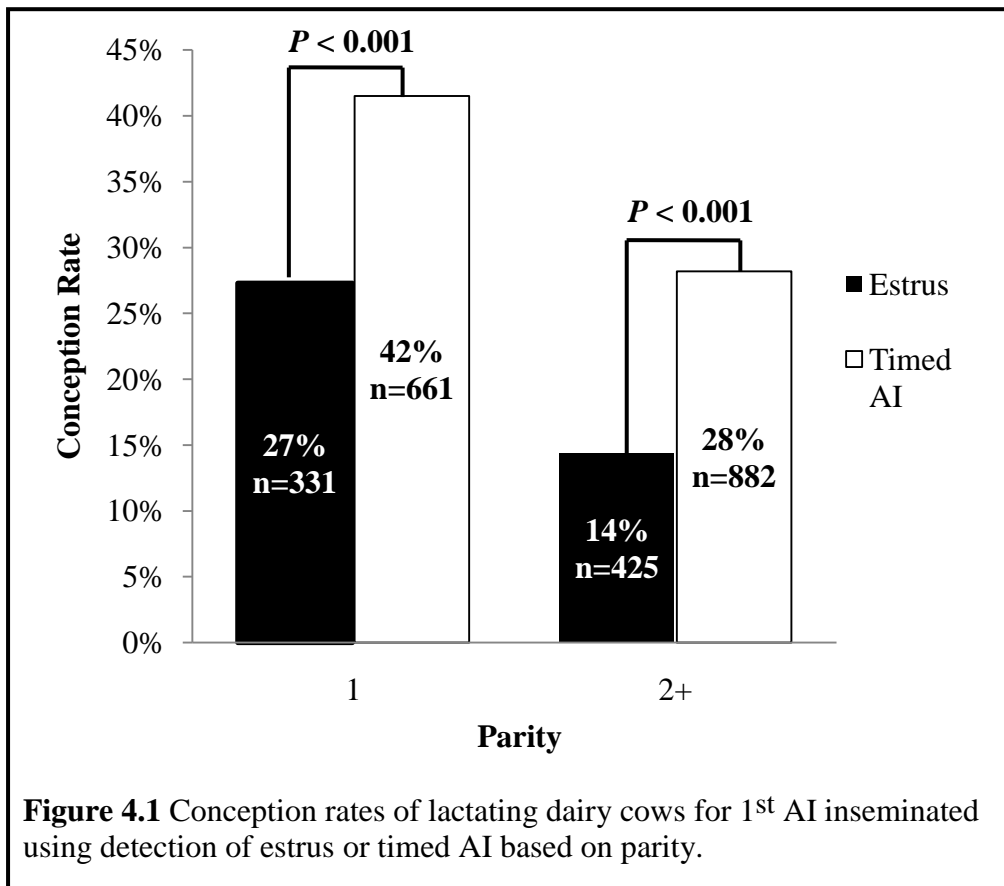
Trait	High Fertility	Medium Fertility	Low Fertility	Overall <i>P</i> -value
ST	28.14 ± 0.41	28.96 ± 0.40	28.80 ± 0.98	0.36
SR	24.37 ± 0.36	25.49 ± 0.39	27.11 ± 0.94	0.01
BD	24.11 ± 0.33	24.83 ± 0.44	25.97 ± 0.89	0.05
DF	22.58 ± 0.36	23.05 ± 0.39	22.61 ± 0.70	0.66
RA	22.67 ± 0.44	21.42 ± 0.53	22.84 ± 1.22	0.15
TH ¹	22.27 ± 0.40	22.20 ± 0.50	22.61 ± 1.19	0.93
RW	23.38 ± 0.36	24.94 ± 0.36	25.09 ± 0.86	0.01
LS	26.99 ± 0.35	26.35 ± 0.44	25.33 ± 0.80	0.18
RL	23.03 ± 0.42	24.33 ± 0.43	25.53 ± 0.89	0.02
FA	21.32 ± 0.35	22.28 ± 0.40	23.55 ± 0.76	0.02
FU	21.92 ± 0.43	23.24 ± 0.47	21.59 ± 1.03	0.08
UH	25.75 ± 0.40	26.14 ± 0.43	24.13 ± 0.89	0.15
UW	23.49 ± 0.38	24.89 ± 0.44	23.33 ± 1.00	0.04
UC	26.24 ± 0.37	27.60 ± 0.41	27.50 ± 0.95	0.04
UD	29.36 ± 0.41	30.25 ± 0.42	30.98 ± 0.98	0.16
TP	24.51 ± 0.48	25.61 ± 0.49	27.38 ± 1.09	0.04
RT	27.20 ± 0.48	28.23 ± 0.48	29.78 ± 1.09	0.06
TL	20.75 ± 0.41	21.43 ± 0.46	20.06 ± 1.22	0.35
UT	32.76 ± 0.35	31.66 ± 0.35	32.38 ± 0.98	0.10
n	365	300	64	729

¹ Due to removal of TH from Holstein Association's classification, not all cows have data for TH
(n = 321, 287, 62, 670, respectively)

Table 4.5 Average type trait scores and standard errors for each fertility group for 2+ parity lactating dairy cows in Study 2.

Trait	High Fertility	Medium Fertility	Low Fertility	Overall <i>P</i> -value
ST	34.05 ± 0.53	35.06 ± 0.41	35.37 ± 0.64	0.19
SR	29.53 ± 0.43	30.78 ± 0.37	31.45 ± 0.53	0.02
BD	29.97 ± 0.43	31.68 ± 0.36	32.51 ± 0.53	0.01
DF	29.59 ± 0.43	30.87 ± 0.36	30.50 ± 0.62	0.08
RA	23.25 ± 0.51	21.96 ± 0.42	21.28 ± 0.63	0.04
TH ¹	24.05 ± 0.50	22.29 ± 0.41	20.50 ± 0.70	0.01
RW	29.83 ± 0.48	30.62 ± 0.38	30.20 ± 0.63	0.42
LS	27.10 ± 0.40	26.93 ± 0.34	25.62 ± 0.51	0.06
RL	25.64 ± 0.46	26.89 ± 0.40	26.28 ± 0.67	0.13
FA	23.35 ± 0.38	23.94 ± 0.35	25.24 ± 0.56	0.02
FU	22.83 ± 0.47	24.42 ± 0.42	23.94 ± 0.63	0.04
UH	28.00 ± 0.46	29.44 ± 0.40	29.71 ± 0.58	0.03
UW	28.57 ± 0.47	30.89 ± 0.39	30.73 ± 0.63	0.01
UC	28.18 ± 0.43	28.81 ± 0.39	29.01 ± 0.66	0.46
UD	24.08 ± 0.46	23.41 ± 0.41	24.41 ± 0.66	0.34
TP	25.59 ± 0.52	26.22 ± 0.45	24.64 ± 0.68	0.16
RT	27.86 ± 0.52	27.99 ± 0.46	27.98 ± 0.72	0.98
TL	22.45 ± 0.49	23.78 ± 0.41	23.75 ± 0.69	0.10
UT	29.02 ± 0.38	30.00 ± 0.30	29.36 ± 0.56	0.13
n	311	441	176	927

¹ Due to removal of TH from Holstein Association's classification, not all cows have data for TH (n = 294,428,175, 897, respectively).



DISCUSSION

The principal finding of this study was that there was a negative relationship between fertility and the body volume traits of Strength and Body Depth in both studies and both 1st and 2nd + parities, indicating that smaller cows have a greater likelihood of pregnancy. Similar results were reported by Berry et al., 2004 indicating that taller, wider, deeper cows had lower genetic merit for pregnancy rate and required more inseminations. The authors found negative genetic correlations between body-related type traits (Stature, Chest Width, Body Depth, Rump Angle, and Rump Width) and interval to 1st service (-0.33, -0.51, -0.17, -0.83, -0.53, respectively). Different results were reported by Pryce et al., 1998 in which cows with narrower

chesters had lower conception rates to 1st AI. Royal et al., 2002a also found that frailer, narrower cows had poorer fertility based on commencement of luteal activity.

Since the majority of the cows in this trial were inseminated by timed AI, we speculate that dosage of GnRH and PGF_{2α} may be hindering fertility in larger cows. Since all cows, regardless of body size, are assigned to receive the same dose of GnRH and PGF_{2α}, fertility may be lower in larger cows as seen in this study due to ineffective dosage. Lower than recommended doses of PGF_{2α} have been seen to have undesirable rates of estrus synchronization in beef heifers (Berardinelli and Adair, 1989). This could also be the case for cows with larger body sizes. However, reducing GnRH dosage did not affect efficacy of the synchronization protocol in lactating dairy cows (Fricke et al., 1998). Still, further research is warranted to determine if a higher dose is required for larger cows and if so, to discover the ideal dose based on body size.

Lack of complete CL regression in a percentage of cows is still a limiting factor in Ovsynch (Brusveen et al., 2009). Based on a study by Brusveen et al., 2009, a second dose of PGF_{2α} one day later increased luteal regression and numerically increased P/AI. However, due to too few cows on their trial, they did not see significant differences in P/AI. We speculate that larger cows are not receiving either a sufficient dose or number of approved doses of PGF_{2α} to fully regress their CL and this is hindering their conception rates.

Another possible explanation for improved fertility in smaller cows is that smaller cows may have a tendency to produce less milk (Tsuruta et al., 2004). Gong et al., 2002 found that cows selected for higher genetic merit for milk yield had a longer interval to 1st service, required more services, and had lower conception rates than cows with lower genetic merit for milk yield. The high genetic merit cows weighed significantly more than the low genetic merit cows,

although body condition score was similar for both groups; however, the magnitude of weight loss and body condition loss during the 1st 5-6 weeks postpartum was significantly higher for the high genetic merit cows. Although it is not fully understood why there is a difference in reproduction in cows with high versus low genetic merit for milk yield, the researchers suggest that high genetic merit cows have a higher incidence of negative energy balance early in lactation which then inhibits reproductive capacity (Gong et al., 2002). In order to obtain the high milk production, larger cows potentially consume more feed. The increased feed consumption leads to increased blood flow to the liver and increased steroid metabolism, which then decreased reproduction (Wiltbank et al., 2006). This model may help explain the decline in fertility, particularly in larger cows, producing more milk. As a result, larger cows may have higher milk production, which may be involved in their decreased reproduction.

In this trial, we found cows that had straighter rear legs and steeper foot angle had lower conception rates for 1st AI. This was confirmed by Berry et al., 2004, who indicated that cows with more posty rear legs and steeper foot angles had an increased interval to first service and a lower pregnancy rate to 1st service.

Some of our results suggest there may be a relationship between fertility and Rear Teat Placement, indicating that cows with teats on the inside of the rear quarters had lower fertility. The opposite was seen by Berry et al., 2004 who found that cows that had teats wider in the rear view and closer together from the side view had decreased pregnancy rates to 1st AI and required an increased number of inseminations. Our study also indicates there is a tendency for cows with longer teats to have decreased fertility, while Berry et al., 2004 found that cows with shorter teats had a decreased pregnancy rate to 1st AI and required more services.

Berry et al., 2004 sometimes had conflicting results than what our trial uncovered. Their research was conducted on spring-calving dairy herds in southern Ireland. One reason they may have seen conflicting results is that genetics of cattle and management practices are different in Ireland. The fertility patterns that have emerged during this trial may not be similar in farms with different management practices.

Increased selection for larger cows in the recent past may have a detrimental impact on fertility. Based on this trial, cows with lower body volume had increased fertility. In addition, larger cows with similar milk production as smaller cows required more health care and thus increased costs (Mahoney et al., 1986). Selection programs aimed at increasing body size may not be reasonable or economically justifiable.

CONCLUSION

Consistent patterns from this study illustrate that increased body volume may be contributing to decreased fertility. This is potentially an artifact of incorrect dosage amount of GnRH and/or PGF_{2α} during timed AI protocols. Further research needs to be conducted to determine if this is the case and subsequently discover the most effective dose of GnRH and PGF_{2α} in synchronization programs. The decrease in fertility in larger cows may also be partially explained by the potential increased level of milk production and feed intake in larger cows over smaller cows. Cows with straighter rear legs and steeper foot angles also consistently showed decreased fertility in this trial. More research is warranted to determine the impact of this relationship. Other type traits did not consistently have an impact on fertility.

CHAPTER 5

CONCLUSION:

ENHANCING CONCEPTION RATES OF LACTATING DAIRY COWS FOLLOWING A NOT-PREGNANT DIAGNOSIS WITH ULTRASOUND

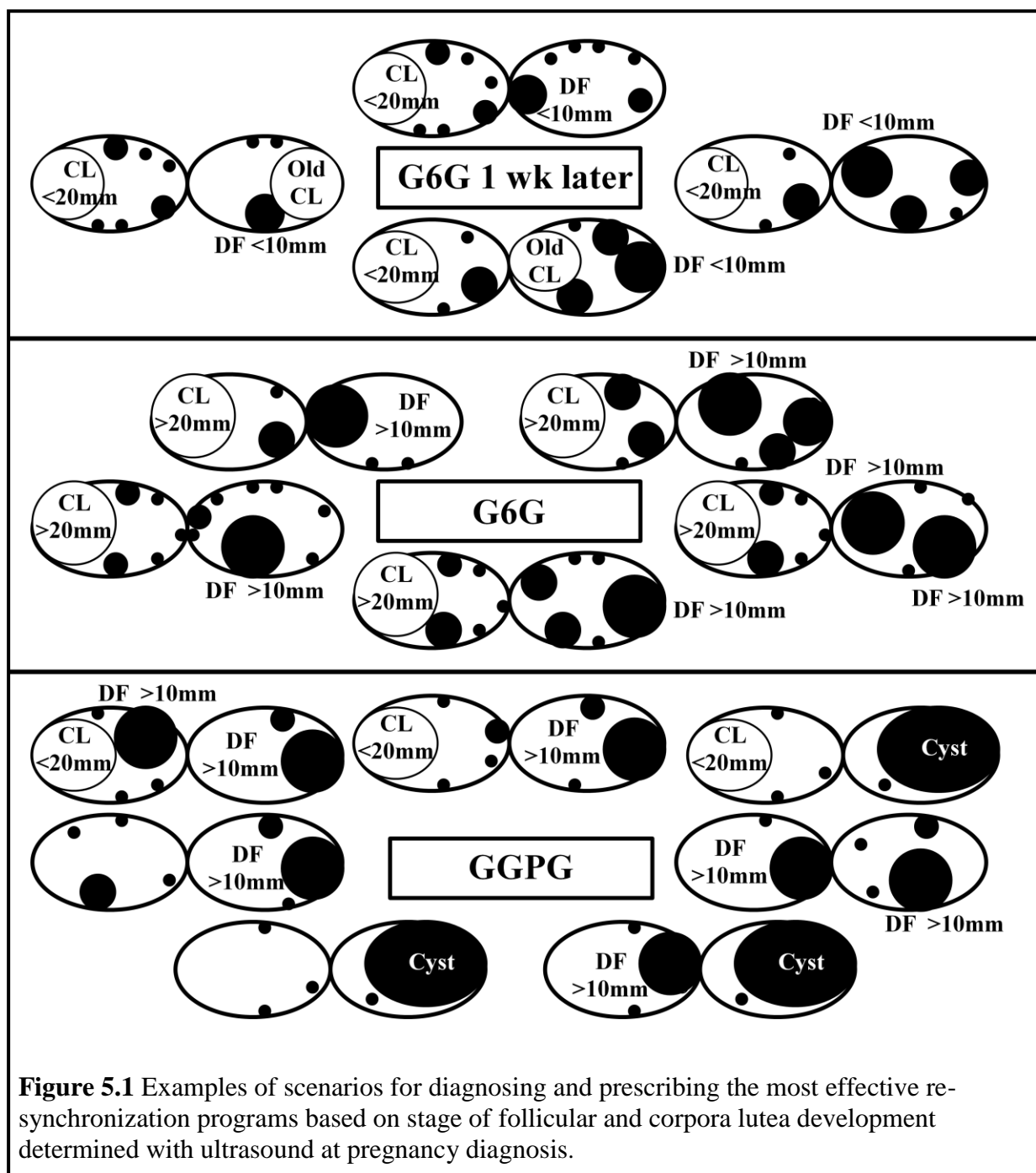
Article will be submitted for publication in a lay veterinary journal

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It is evident that reproduction is a major factor affecting dairy farm profitability. Thus, improving reproduction is an important goal for many producers. You, as the veterinarian, play a critical role in helping producers boost profit. Another way to enhance this relationship is through an additional use of ultrasonography at time of pregnancy diagnosis. This article will provide insights on how to utilize ultrasound at pregnancy diagnosis to determine the most successful re-synchronization program for non-pregnant cows based on specific profiles of follicular and corpora lutea development. This will allow you to enhance your role as veterinarian in improving fertility on dairy farms.

There are 3 specific ovarian structural profiles in not-pregnant cattle that can be easily identified with ultrasound (Figure 5.1) that can be utilized for selecting a re-synchronization program. Each profile has a specific re-synchronization program that can be assigned to it. The 1st profile describes cows with small CL (< 20 mm) and small follicles (< 10 mm). This profile is generally found in cows in the first few days of the estrous cycle. GnRH will likely not cause ovulation since there is no dominant follicle and PGF_{2α} will likely not cause luteal regression. For this reason, the most effective thing to do is wait 7 days and then initiate a G6G program to re-synchronize. The 2nd profile describes cows with CL (> 20 mm) regardless of size of follicles. This CL should be responsive to PGF_{2α} induced luteolysis. There is a very high likelihood that there will be at least 1 follicle >10 mm in size that will eventually be responsive to GnRH. In these cows, initiation of a G6G program is recommended. The 3rd ovarian profile describes cows with at least one large dominant follicle (> 10 mm) and a regressing CL (< 20 mm) or no CL. Cows with follicular cysts or other anovular conditions (Wiltbank et al., 2002) would also fall into the 3rd profile. In these cows, a GGPG treatment is recommended.



There are specific re-synchronization actions for each of these 3 ovarian phenotypes. But before we outline these actions, it is important to understand how we developed the rationale for reducing the estrous cycle down to these three characteristics.

In a descriptive study just completed, we assigned cows to 3 synchronization programs based on 4 stages of follicular and corpora lutea development identified at a not-pregnant diagnosis 35 to 41 days following previous AI. Treatments were: 1) If small follicles (< 10 mm) and CL (< 20 mm), recheck in 1 week and place on treatment 2 or 3 based on new exam; 2) If 1st wave dominant follicle (> 10 mm) and CL (> 20 mm), cows were treated with Ovsynch (GnRH - 7d PGF_{2α} - 2d GnRH - 16h AI); 3) If cows had at least n = 1 follicle > 10 mm and growing follicles (2nd wave) or 2 follicles > 10 mm and CL > 20 mm, they were treated with G6G (PGF_{2α} - 2d GnRH - 6d Ovsynch); and 4) If cows had at least n = 1 follicle > 10 mm and regressing (< 20 mm) or no CL they were treated with GGPG (GnRH - 6d Ovsynch). We were conservative with our decisions to place cows in the Ovsynch group. Cows with 2 dominant follicles were generally placed in Group 3, thus the percentage of cows that fell in the Ovsynch group was less than expected based on percentage of the estrous cycle. We found that 6% of cows were in the 1st stage, 7% in stage 2, 63% in stage 3, and 24% in stage 4 (n = 514). Conception rates from our study were 27.8% for Ovsynch, 36.6% for G6G, and 45.3% for GGPG. The ovulation rate for the 1st GnRH of Ovsynch was low for Ovsynch (54 %) and was the primary reason for low conception rates in this group. As a result of fewer than expected cows and lower than expected ovulation rate to the 1st GnRH of Ovsynch, in the Ovsynch group, we do not recommend using only Ovsynch as a re-synchronization program.

DECISIONS, DECISIONS, DECISIONS

So, as a veterinarian, what actions should you take based on diagnosis of follicular and corpora lutea development? Since Ovsynch was not successful, we recommend utilizing only the 3 stages lined out in Fig. 5.1: 1) If cows are in the early part of their estrous cycle and have CL < 20 mm and follicles < 10 mm, there is no treatment that will yield any progress since GnRH will

not ovulate follicles < 10 mm and PGF_{2α} will generally not regress CL < 20 mm. In our study, the majority of cows in this stage of follicular and corpora lutea development fell into the G6G category 1 week later as they had already started a 2nd follicular wave. ACTION: Postpone the start of G6G for 1 week. 2) If cows have a CL > 20 mm in diameter, start on G6G; 3) If cows have one or more large follicles (> 10 mm in diameter) and either no CL or a regressing CL (< 20 mm in diameter), then start on GGPG.

There is significant variability in follicular and corpora lutea development between cows and it can be challenging to determine the stage of the cycle, so we are developing a reference guide to help understand caveats and their solutions. For instance, if unsure if the CL is regressing or new, there are several clues to look at. Firstly, pattern of follicular growth can be a deciding factor. For the majority of cows in early cycle, there are no follicles larger than 10 mm and there are many small follicles whereas in late cycle, there is at least 1 follicle larger than 10 mm. Based on our data, cows with a CL ≤ 20 mm and at least 1 follicle > 10 mm, 86.6% (n = 67) of the CL were in fact regressing. In addition, new CL are darker in color due to increasing blood flow in the CL while regressing CL are lighter in color since blood flow is decreasing. The reference guide will focus on steering you in the right direction in decision-making based on caveats we have found.

WHY DO WE HAVE PROBLEM COWS?

Another aspect of our recent trials led us to the discovery that larger cows have inferior fertility. In a study looking at the effects of type traits on measures of fertility, body volume traits, particularly strength and body depth, were seen to consistently impact conception rates. As part of a subsequent study, we examined the effects of heart girth measurements on various measures of fertility, including their effect on reproductive hormones. We found that cows with

less heart girth had increased conception rates and luteolysis rates to the final PGF_{2α} of Ovsynch. Since complete CL regression is a critical factor in the cow becoming pregnant, conception rates are hindered as a result. These findings potentially indicate that current dosage recommendations of hormones utilized in synchronization protocols may not be effective. Since all cows are recommended to receive the same dose of GnRH and PGF_{2α}, perhaps larger cows are not receiving either a large enough dose or number doses of PGF_{2α} to cause complete CL regression. Future research is warranted to determine the most effective dose or series of doses of PGF_{2α}, particularly for larger cows, in order to cause complete CL regression and thus boost conception rates.

SUMMARY

This research brings the industry another step closer to improving conception rates in lactating dairy cows. Bovine practitioners now have the opportunity to diagnose ovarian status at not-pregnant diagnosis and subsequently place cows on the most effective synchronization protocol to enhance fertility. In addition, we have determined that a negative relationship exists between size and fertility so producers can change their breeding goals accordingly. We speculate that larger cows having lower fertility may be an artifact of ineffective dosage of reproductive hormones in synchronization programs. Thus, future research is warranted to determine the most effective dosage or series of doses to improve luteolysis rates and thus, conception rates. But most importantly, you as a veterinarian, have another opportunity to help producers improve profitability by increasing levels of reproduction on farms.

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