Evaluation of 72 h Cosynch and 5 or 7 d post-AI gonadotropin releasing hormone on first service pregnancy rate in lactating dairy cows

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ABSTRACT

Two studies were conducted to evaluate the effects of 5 or 7 d post-AI GnRH on first service PR, plasma P_4, and CL volume in lactating dairy cows synchronized using 72 h Cosynch. All cows were synchronized and randomly assigned to one of three treatment groups: Control – no additional GnRH; 5 d – GnRH 5 d after TAI; 7 d – GnRH 7 d after TAI. In the first study, P_4 concentrations were evaluated in samples collected at five separate times and CL volume and number were recorded at 30 d pregnancy examination for Holstein (n = 77) and Jersey (n = 33) cows. GnRH treatment did not affect PR (Control - 47.2%, 5 d GnRH - 40.5%, 7 d GnRH – 44.7%) or P_4, but increased TCLV compared to controls (Control – 7.33 cm^3, 5 and 7 d GnRH – 10.77 cm^3). Incidence of accessory CL increased PR (94.7 vs. 60.6%), P_4 (6.95 vs. 5.88 ng/mL), and TCLV (15.51 vs. 6.78 cm^3) compared to cows with a spontaneous CL. Cows classified as cycling based on P_4 evaluation had significantly higher PR than acyclic cows (54.4 vs. 16.1%). In the second study, Holstein cows (n = 1055) were submitted to the same experimental protocol and evaluated for first service PR. Post-AI GnRH treatment did not significantly affect PR. Primiparous cows (32.8%) tended to have higher PR than multiparous cows (27.6%), but GnRH treatment had no influence on this relationship. In conclusion, GnRH post-AI did not affect PR. Further evaluation of accessory CL incidence is warranted as it significantly affected PR.

(Abbreviations: AI – artificial insemination, CL – corpus luteum, PR – conception rate, P_4 – progesterone, TCLV – total corpus luteum volume**)**

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LIST OF ABBREVIATIONS

AI: artificial insemination

AIPL: Animal Improvement Programs Laboratory

CL: corpus luteum, *pl.* corpora lutea

FSH: follicle stimulating hormone

GnRH: gonadotropin releasing hormone

LBF: liver blood flow

LH: luteinizing hormone

MCR: metabolic clearance rate

$PGF_{2\alpha}$: prostaglandin F_2 alpha

PR: pregnancy rate

TAI: timed artificial insemination

TCLV: total CL volume

USDA: United States Department of Agriculture

INTRODUCTION

The dairy industry in the United States has undergone dramatic changes in the last 10 to 15 years and continues to change even today. Between 1993 and 2003, the number of dairy farms in the U.S. decreased by 41% (from 160,000 to 90,000) while annual milk production per cow increased by 16% (from 7500 kg to 8600 kg). This phenomenon is (at least partially) explained by a shift towards larger farms versus the smaller, "family" dairies of the past. In 1997, farms of more than 500 cows accounted 29% of all milk production, compared with 39% in 2001 (USDA, 2002). This trend has caused producers to change strategies in an attempt to maintain an acceptable level of efficiency (for larger farms) and to remain profitable in a competitive market (for smaller farms).

Managing reproduction is a time and labor-intensive process that is absolutely essential to the success and profitability of the modern dairy. Other aspects of dairy operations, including herd health, cow comfort (facilities), nutrition, general management, and skilled labor all interact with the reproductive performance of the dairy cow. Even with near-perfect conditions in these other areas, reproductive management constitutes a tremendous amount of work for producers. The two most important factors in a successful reproduction program are detecting cows in heat and deciding when those cows should be bred. Visual detection of estrus has always been the industry standard; unfortunately, visual detection must be done repeatedly throughout the course of the day, and the frequency of observation is directly linked to successfully detecting cows in estrus.

The concept of synchronization was first proposed in the 1970s, when researchers began manipulating the estrous cycle with reproductive hormones. Initially, the focus was synchronization of estrus alone, leading to easier (and less time-consuming) visual detection of estrus. Research later moved towards synchronization of both the period of estrus and ovulation, which gave rise to the idea of true "timed AI." While synchronizing estrus certainly gave producers an advantage, visual detection of estrus and timing of AI were still major concerns. Synchronizing ovulation produced protocols that successfully eliminated or concentrated the need for visual detection of estrus during the majority of each estrous cycle. Currently, there are many synchronization protocols using a variety of hormones. The most commonly used hormones are prostaglandin $F_{2\alpha}$ ($PFG_{2\alpha}$), gonadotropin releasing hormone (GnRH), estrogens (E_2), and progestins (P_4). Chorionic gonadotropins (equine and human) have also been used, although less frequently.

Synchronization as part of a sound reproduction program has the potential to increase farm efficiency, decrease labor needs, and produce PR equal to, or greater than visual detection of estrus. Additionally, synchronization ensures that all cows receive first service consistently, and at a pre-determined time following the voluntary waiting period. Researchers have taken synchronization programs in many different directions in the past 20 years; some are effective, but overly complex and/or too expensive to be practical, others are easy to implement but are of limited use. For example, protocols have been developed that effectively synchronize cows, but require many injections on an unusual schedule such as d 7, 14, 26, 33, and 35. Unusual schedules (those where injections are not given on the same day or days of the week) also become problematic when multiple groups are being synchronized that were started at different times. When injections from week one of a protocol and injections from week two of the same protocol fall in the same time period, different injections may be required three or four days a week. Other protocols are relatively simple, such as multiple injections of $PGF_{2\alpha}$, but require constant visual detection of estrus and therefore may or may not provide any real management benefits.

In 1995, Pursley et al. developed a short, easy to use protocol that utilized both $PGF_{2\alpha}$ and GnRH. This protocol was named Ovsynch because it accurately synchronized ovulation and allowed producers to virtually eliminate visual detection of estrus. This simple, 3-injection series quickly became the base for almost all other synchronization protocols.

In the early 1990's, sporadic research investigated the potential benefits of giving hormonal injections (typically GnRH) after artificial insemination. A great deal of research has been conducted on variations of a synchronization protocol with post-AI injections, but many of these protocols have deviated from the normal 7 or 14 d intervals commonly used to allow synchronization to be practical. This has led to some effective protocols that are not practical; producers must restrain cows multiple times in a single week as well as keep track of the synchronization schedule. These two problems prevent the otherwise successful protocols from ever being used.

The objectives of this study were to investigate the effect of GnRH injections given 5 or 7 d after insemination on pregnancy rate (PR) and to measure the resulting P_4 concentrations in plasma, formation of secondary corpora lutea (CL), and early embryonic losses in first service lactating dairy cows synchronized with a Presynch and 72 h Cosynch protocol.

LITERATURE REVIEW

DECLINING FERTILITY OF THE MODERN DAIRY COW

Dairy farmers who have been in the business for the last 20 or 30 yr have undoubtedly shared a common thought at some point during that time; "Why in the world is it so hard to get cows pregnant these days?" The issue of declining fertility used to be a hypothetical situation with supporters both for and against, but this attitude has seemingly changed in the last 10 yr. However, a consensus of researchers now agrees, regardless of cause, that the apparent level of fertility in U.S. dairy cows has decreased dramatically in the last 30 years.

During the 1950's, PR of dairy cows averaged approximately 55% when artificially inseminated at observed estrus. By comparison, cows bred at observed estrus in 2000 achieved PR of approximately 45%, and approximately 35% when bred by timed AI (Lucy, 2001). During this same time period, and not surprisingly, the average number of days open, the number of services per conception, and the average calving interval have all increased, reflecting a failure of new methods and ideas to stem the decline in fertility. An analysis of 73 Holstein herds in Kentucky from 1972 (4606 cows) to 1996 (7370 cows) showed an increase in days open from approximately 132 d to approximately 159 d, an increase in services per conception from 1.62 to 2.91, and an increase in calving interval from 13.5 to 14.4 months (Silvia, 1998).

Despite many years of investigation into the cause of this decline, no satisfactory answers have been found. Before examining some of the possible explanations, consider the "normal" sequence of events required for successful reproduction described by Silvia (2003). Cows must 1) develop healthy follicles containing fertile oocytes, 2) coordinate ovulation and estrus behavior to insure proper timing of insemination (natural or artificial), and 3) initially maintain a uterine environment conducive to sperm transport and fertilization, followed by an abrupt switch to an environment suitable to maintain a pregnancy. Even in summary, it is clear that chances for failure are abundant and inter-related with animal health, management, nutrition, environment, and numerous other factors.

Increased Milk Yield

The predominate theory to explain the decline in reproductive performance is the increase in milk yield during the period in question. In the last 10 yr (1995 to 2004), milk yield per cow has increased 16%, from 7500 kg to almost 8400 kg per year. During the same approximate time period (1993 to 2002), the number of dairy operations in the United States has decreased 41%, from 160,000 to less 90,000 (USDA, 2003). In short, fewer cows are now kept in increasingly concentrated areas but still manage to produce more milk than their predecessors. While the relationship between increased milk yield and decreased fertility is well documented and generally accepted, the specific mechanisms and causative agents are still not completely understood. The factors and events that lead to successful reproduction are complex and interconnected to such a degree that their genetic basis may never be fully elucidated, but selection for the desirable traits of today continues to have the potential to lower fertility.

Inbreeding is one genetic factor related to the intense selection for high milk yield. Percent inbreeding has been carefully tracked since the 1960's and has increased to the level of 5% in today's Holstein population and 7% in the Jersey population (AIPL, 2006). A study of Guernsey herds conducted in 1986 examined the effects of low-level inbreeding on reproductive performance over a span of 24 yr. The study concluded that each 1% increase in inbreeding resulted in a 0.17 increase in services per conception, a 2 d increase in days open, and a 3.3% decrease in PR (Hermas, et al., 1987). Although this study is somewhat dated, its results concur with other, similar research in other breeds and other parts of the world. Inbreeding in Holstein cows has increased by 4% since 1980 (AIPL, 2006). Applying the data from Hermas, this could account for a 0.68 increase in services per conception, an increase in 8 d open, and a 13% decline in PR for the last 25 yr. Even if the reality is not as dramatic as the theoretical, inbreeding is still a major cause for concern in the reproductive performance of dairy cattle.

Recently the relationship between milk yield, multiple ovulation, and hormone profiles in lactating dairy cows has garnered increased attention. Lopez et al. (2005) published a paper that adds new data to this argument as well as summarizing the other available research. Milk yield, spontaneous ovulation rate (no hormonal treatments), serum P_4 and E_2 concentrations, preovulatory follicle volume, and luteal volume were measured in 267 lactating cows. Multiple ovulation rate was 1.6, 16.9, and 47.9% when cows were producing <35, 35 to <45, and >45 kg/d, respectively. Serum E_2 concentrations were lower (5.5 ± 0.3 vs. 7.8 ± 0.4 pg/mL) during periods of estrus with multiple ovulations. Serum P_4 concentrations were also lower 7 d after estrus in cows with multiple ovulations (2.5 ± 0.3 vs. 3.2 ± 0.1 ng/mL). Additionally, the duration of standing estrus was decreased in multiple ovulations compared to single ovulations (4.0 ± 1.0 vs. 9.8 ± 0.9 h) (Lopez, et al., 2005). Lower E_2 indicates a decrease in ovulatory follicle function, and possibly less fertile oocytes. Lower P_4 concentrations 7 d after estrus indicates decreased CL function, probably directly related to the poorly developed ovulatory follicles. These two hormonal deficiencies, along with the decreased duration of estrus, indicate that there may be some positive relationship between multiple ovulations and increased milk yield, and that multiple ovulations may be less viable than single ovulations, resulting in a further decrease in reproductive efficiency.

Reproductive Disorders

In addition to genetic factors related to selection for milk yield, other researchers have examined associations between milk yield and reproductive disorders. Grohn et al., (2000) gathered data from 61,124 Finnish Ayrshires to evaluate risk factors (parity, calving season, diseases) for the incidence of reproductive disorders (dystocia, abortion, retained placenta, early and late metritis, silent heats, prolapsed vagina, ovarian cysts, and other), relative to milk yields. Cows with relatively higher milk yields in previous lactations were more prone to retained placenta, early metritis, silent heats, and ovarian cysts, while cows with higher milk yields in the current lactation were more prone to ovarian cysts. Cows affected by these postpartum disorders are certain to suffer some decrease in reproductive efficiency, through longer periods of uterine involution (retained placenta, metritis), irregular cycling (silent heats, ovarian cysts), and normal metabolic impacts during any illness. Despite differences in breed, environment, and management styles between the U.S. and Finland, it is sensible to think that higher producing cows are under greater metabolic stress, and therefore more likely to be affected by reproductive disorders that ultimately lower fertility, regardless of their geographical location.

Metabolic Imbalances

The dairy industry has always dictated that gestation and lactation overlap prior to the dry period and the subsequent lactation. This creates several potential explanations for reduced reproductive efficiency. On average, a 600 kg Holstein cow requires about 10 Mcal of net energy daily for maintenance, but requires an additional 30 Mcal to produce 45 kg of milk per day. In simpler terms, a lactating cow is *ideally* consuming 4 times her maintenance needs each day, and most cows still lose weight during peak lactation (Silvia, 2003). These dramatic changes in metabolism can provide several possibilities for reduced reproductive efficiency.

The first explanation is the broadest and most difficult to test empirically, but is sensible given current knowledge of biological systems. Because cows lactate specifically to nourish their offspring, it stands to reason that lactation, not reproduction, would garner a larger percentage of the limited pool of nutrients and energy available, thus reducing reproductive function (Silvia, 2003). Again due to the multitude of factors present, it is difficult to measure these interactions, but some studies have linked nutritional factors to reproductive performance in dairy cattle. Most often, this relates to diminished function of the hypothalamic/pituitary axis or in hormone production by the ovaries (Butler, 2000). Additionally, substances such as growth hormone (GH), insulin-like growth factor-1 (IGF-1), insulin, and cortisol (Drackley, et al., 2001) have been implicated as factors of interest. In both cases, nutritional factors were directly linked to dramatic changes in the hormonal synthesis of periparturient cows.

Another possible explanation deals with abnormal metabolism of reproductive hormones due to increased feed intake and adaptations of the digestive viscera. Sangsritavong et al. (2002) examined changes in liver blood flow (LBF) and metabolic clearance rate (MCR) of E_2 and P_4 in lactating and non-lactating dairy cows fed different levels of (M) maintenance diet (ranging from no feed to 2.2 times M). Both LBF and

MCR of E_2 and P_4 increased when feed intake increased, and LBF and MCR increased even more substantially in lactating cows. It is therefore possible that the higher intakes required by lactating cows today are actually causing a reduction in reproductive hormones that is significant enough to decrease reproductive performance.

The combination of these many factors, which in no way comprise a complete list, offers some insight into the declining fertility of today's dairy cows. Add the management factor of larger numbers of cows per farm to the equation, and the reality of 15% PR does not seem as implausible. The question becomes, instead, how to compensate for this decline.

METHODS AND ORIGINS OF SYNCHRONIZATION

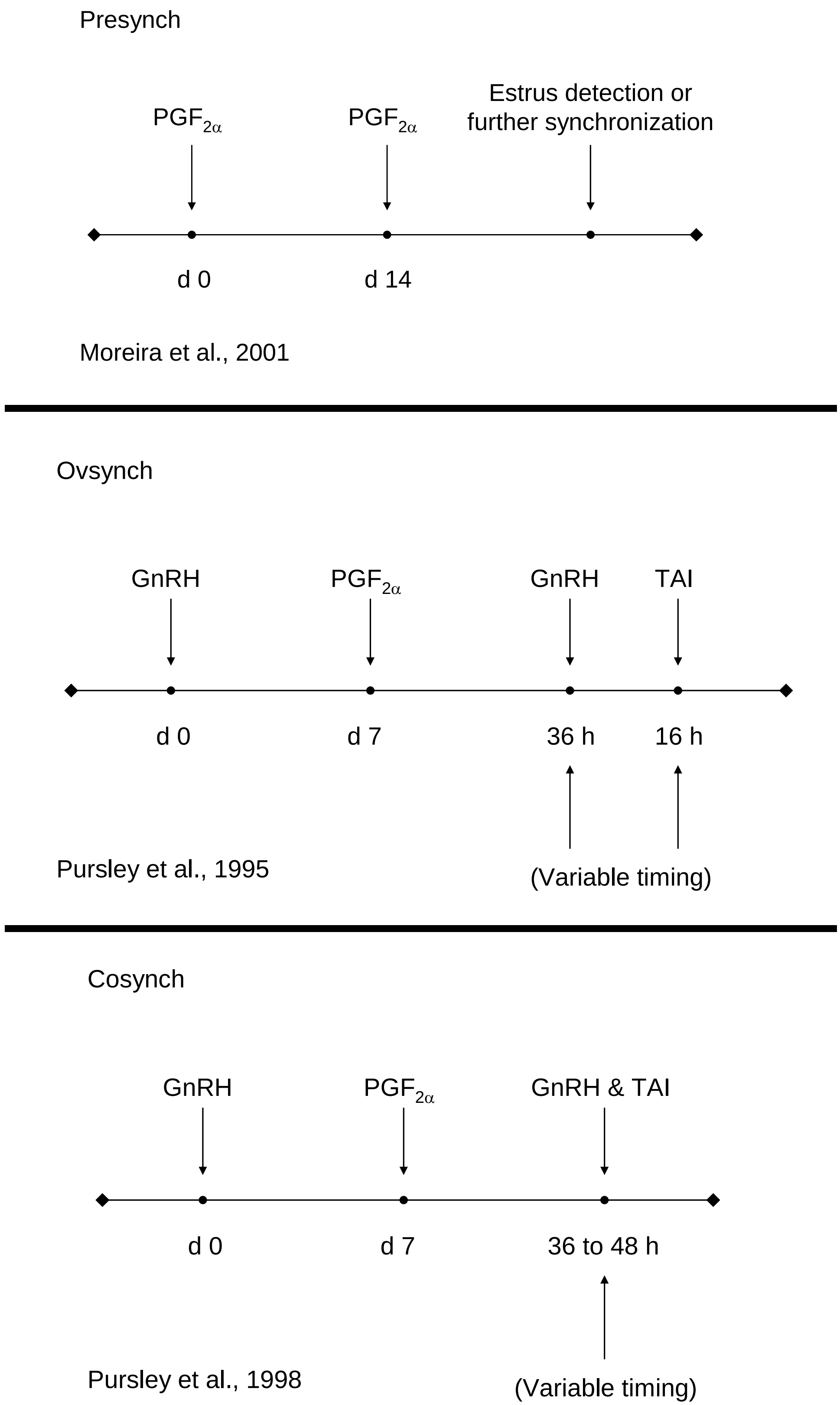
Even before declining fertility became a major issue, researchers and farmers sought ways to standardize their breeding programs, reduce or eliminate periods of visual detection of estrus, and ultimately improve first service PR. The first postpartum service has always been an important element in a successful breeding program as it heavily influences overall reproductive efficiency. Fricke (2003) outlined three strategies to be implemented early in the breeding period that will help establish an aggressive reproductive strategy: 1) all cows need to be submitted for first postpartum AI service at or near the end of the voluntary waiting period (VWP), 2) nonpregnant cows need to be identified as early post-AI as possible, and 3) cows that fail to conceive to first AI service need to be quickly returned to second AI service. Although this is a relatively recent summarization, it outlines the rationale that led to the development of protocols that synchronize estrus and/or ovulation.

The goal of synchronization, at a basic level, is to control the dominant structures of the two phases of the estrus cycle, the corpus luteum (luteal phase) and follicular development (follicular phase). Synchronizing the estrus cycle is generally done by manipulating the CL in one of two ways: 1) administer $PGF_{2\alpha}$ to regress the CL, thereby shortening the cycle and initiating a new follicular wave, or 2) administer P_4 to maintain the CL, thereby lengthening the cycle. Regimens that regress the CL have been around for almost 40 yr (Lauderdale, et al., 1974, McCracken, et al., 1972, Stevenson, et al., 1987), but they were imprecise and required standard visual detection of estrus in order to be even moderately effective. These protocols successfully synchronized the cycles of a large percentage of cows, but problems with anestrous cows and cows that did not show luteal regression, along with the detection of estrus requirements served to limit their efficacy. In time, these $PGF_{2\alpha}$ based protocols expanded to include the use of GnRH to synchronize ovulation. The primary function of GnRH is to stimulate the release of luteinizing hormone (LH), which causes ovulation of the dominant follicle (if present) and of follicle stimulating hormone (FSH), which allows for the start of a new follicular wave. Predominate synchronization protocols of today use both $PGF_{2\alpha}$ to shorten the luteal phase and GnRH to stimulate follicular growth and cause ovulation at a given time. The three most common protocols, Presynch, Ovsynch, and Cosynch, are summarized in Figure 1.

Ovsynch

It was not until 1995 that a protocol was developed that effectively, and consistently, synchronized both estrus and ovulation, allowing for TAI with PR comparable to visual detection of estrus and AI following the AM-PM guidelines.

Figure 1. Frequently used synchronization protocols used to program first service in lactating dairy cows; Presynch, Ovsynch, and Cosynch.



Pursley et al. (1995) developed a protocol, now coined Ovsynch, that involved an injection of 100μg GnRH on a random day of the estrus cycle, 25mg of $PGF_{2\alpha}$ 7d later, a second 100μg GnRH 48h later and TAI 24h after the second GnRH. First service PR from this protocol was similar to non-synchronized results from the same herds, and thus showed that synchronization and TAI were viable options.

Several studies (Pursley, et al., 1997, Stevenson, et al., 1996) validated the Ovsynch protocol and soon researchers were altering the basic protocol in an attempt to improve PR and make it more practical for large commercial herds. Schmitt et al. (1996) compared the relative fertility of oocytes from persistent follicles versus that of newly induced dominant follicles. Oocytes from persistent follicles have diminished fertility compared to newly induced follicles. This partially explains the success of the Ovsynch protocol, and also set the stage for other protocols to induce new follicular waves after hormonally adjusting the length of the luteal cycle. Though Schmitt et al. (1996) conducted this work after Pursley et al. (1995) developed Ovsynch, it does help explain the efficacy of Ovsynch ; the first GnRH induces a new follicular wave and eliminates any potential persistent follicles, while $PGF_{2\alpha}$ and the second GnRH prepare and synchronize the ovulation of a second, newer dominant follicle.

Fricke et al. (1998), validated the use of half the conventional dose of GnRH for synchronization protocols similar to Ovsynch. GnRH is labeled as a treatment for cystic follicles, and despite a completely different physiologic purpose, the 100μg dose is extended for use in synchronization protocols as well. Fricke et al. hypothesized, correctly, that such a large dose would not be required to achieve acceptable results in synchronization programs and that reducing the dose could give substantial economic benefit to producers. Currently, the use of 50μg of GnRH versus 100μg reduces the cost of each GnRH injection by half and makes the use of synchronization protocols much more appealing to producers.

Peters and Pursley (2003) examined the timing for the final GnRH injection of the Ovsynch protocol (0, 12, 24, or 36 h after $PGF_{2\alpha}$) and its effects on ovulatory follicle size. Previous knowledge of $PGF_{2\alpha}$-induced synchrony limited the final GnRH injection to 36 h, as spontaneous LH surges typically occur at approximately 48 h and render GnRH useless if administered too late. Administration of GnRH 36 h after $PGF_{2\alpha}$ in the Ovsynch protocol provided the most benefits of the four times tested. Synchronization rate (87.8%), ovulatory follicle size (14.6 ± 0.44 mm), and PR (28%) were all increased by the 36 h GnRH treatment. The most commonly used times for administration of the final GnRH injection of Ovsynch are 36 or 48 h; however, 36 h consistently shows superior results.

Finally, given the physiologic requirements for synchronization (a CL to be affected by $PGF_{2\alpha}$ and/or a dominant follicle to be affected by GnRH), research has been conducted examining the efficacy of Ovsynch based on the day of the cycle on which it is initiated. Lactating Holsteins at known days of their estrous cycles were subjected to the Ovsynch protocol and categorized into four groups; d 1 to 4, d 5 to 9, d 10 to 16, or d 17 to 21, and ovulation status was evaluated by frequent per rectum ultrasonagraphy. Cows started on the Ovsynch protocol during the middle of the cycle (d 5 to 9) were synchronized substantially better than cows started either earlier or later in the cycle. The most important indication of successful synchronization was the percentage of cows that ovulated in response to the first GnRH, as non-responders to this injection are much less likely to respond to further injections in the protocol. In the 5 to 9 d group, 96% of cows ovulated to the GnRH injection, compared with 23% for d 1 to 4, 54% for d 10 to 16, and 77% for d 17 to 21 (Vasconcelos, et al., 1999). This demonstrated a clear advantage in initiating the Ovsynch protocol during d 5 to 9 of the cycle, but no indications of a practical way to implement the concept were given.

Presynch

Presynch is really a new name for an old protocol with a modified use. As mentioned previously, $PGF_{2\alpha}$ has been used for nearly 40 yr to alter the estrous cycle by regression of the CL (Lauderdale, et al., 1974, McCracken, et al., 1972, Milvae, et al., 1996, Stevenson, et al., 1987). A single injection of $PGF_{2\alpha}$ is given, followed by visual detection of estrus during the next 3 to 5 d, with a second injection later for those cows not detected in estrus. Moreira et al. (2001) reported that two injections of $PGF_{2\alpha}$ 14 d apart, followed by the initiation of Ovsynch 12 d later dramatically improved the PR of Ovsynch by correctly targeting the d 5 to 9 window of the cycle. Although the use of $PGF_{2\alpha}$ was not new, the authors labeled the protocol Presynch for its novel use in conjunction with Ovsynch. Pregnancy rate among the control groups in the study was 37%, compared with an average PR of 54% for Presynch groups (Moreira, et al., 2001).

As was the case with Ovsynch, further research attempted to refine, explain, or improve the Presynch protocol. Alnimer et al. (2002) reported several different variations of Presynch including a single injection of $PGF_{2\alpha}$ followed by Ovsynch as well as single or double injections of $PGF_{2\alpha}$ alone followed by visual detection of estrus. Pregnancy rates were below 20% for both stand-alone treatments and 37% for the reduced Presynch/Ovsynch. Although the intention of the study was not to compare various alterations of Presynch, it did show that both injections of $PGF_{2\alpha}$ were necessary in order to improve upon the original Ovsynch protocol. Adding Presynch to an Ovsynch protocol increases the number of cows successfully synchronized, thereby potentially improving the PR.

While Presynch was effective for synchronization, it presented a problem to producers implementing the protocol. By deviating from the otherwise constant 7 d routine, it required cows to be sorted an extra day each week and made compliance difficult. The efficacy of two synchronization protocols; one using Ovsynch alone and a second using a modified Presynch with a 14 d interval between Presynch and Ovsynch (versus the original 12 d interval) were also compared (Navanukraw, et al., 2004). Pregnancy rate using the modified Presynch (49.6%) gave a substantial advantage over Ovsynch alone (37.3%).

Cosynch

Once the Ovsynch protocol was reported in 1995 as a viable alternative to visual detection of estrus and Presynch had been reported to enhance Ovsynch, the last area available for improvement was the timing of the final injection of GnRH and the time of insemination. The name Cosynch arises from the modification of Ovsynch that allows AI to occur at the same time as the final injection of GnRH.

Timed artificial insemination 0, 8, 16, 24, and 32 h following the final GnRH injection of Ovsynch was conducted on a total of 732 lactating dairy cows (Pursley, et al., 1998). Calving rates ranged from 29 to 33% for groups inseminated at 0, 8, 16, and 24 h after the final GnRH injection. However, the calving rate was lower for cows inseminated at 32 h post GnRH (20%). It should be noted that this study was not designed specifically to evaluate the Cosynch protocol; it simply showed that 0 h TAI produced acceptable results (31%) compared to the standard timing, 16 h (33%), and allowed for one less handling of cows being synchronized. This relatively minor 2% discrepancy is often pointed out in direct comparisons between synchronization methods (DeJarnette, 2000, Fricke, 2003, Peters, 2005), usually as a "user-beware" statement against Cosynch. As the decision to implement a synchronization protocol usually comes down to cost and ease-of-use, the reduction in calving rate is another factor that producers must keep in mind when deciding on the protocol that is best for their situation.

All of the fine-tuning of the Ovsynch protocol, along with the introduction of Cosynch, caused researchers to go back and re-evaluate some elements of the protocols. The primary factor to be re-evaluated was the timing of the final injection of GnRH and the subsequent timing of TAI. Through the research discussed previously, the other injections of the Presynch and Ovsynch protocols have been well-established and there has been no interest in altering these "fundamental" injections. The final GnRH injection, however, controls the precise timing of ovulation and has the potential to increase the efficacy of the program. Portaluppi and Stevenson (2005), conducted the first study to examine the effect of altering the timing of the final GnRH injection across the broad range of synchronization procedures. The three treatments tested in this study were, essentially, 48 h Cosynch, 48 h Ovsynch with 72 h TAI (24 h after final GnRH), and 72 h Cosynch. All cows (n=665), received the Presynch protocol (2 injections of $PGF_{2\alpha}$ 14 d apart) before the initiation of one of the three Ovsynch protocols 14 d later. Pregnancy rates were 22.8, 23.5, and 31.4% for the 48 h Cosynch, 48 h Ovsynch with 72 h TAI, and 72 h Cosynch, respectively.

There is a body of research that supports the increased performance of the 72 h Cosynch treatment. Several studies in the last 10 yr in lactating beef cattle (Dejarnette, et al., 2001a, Stevenson, et al., 2000) and in lactating dairy cattle (Badinga, et al., 1994) have reported that the time to estrus after various GnRH + $PGF_{2\alpha}$ treatments was approximately 60 h after $PGF_{2\alpha}$. Another study in 2003 (DeJarnette and Marshall) examined lactating dairy cows subjected to Presynch, then submitted for Ovsynch. In that study, 65% of the Presynchronized cows showed estrus 3 d after the $PGF_{2\alpha}$ injection of Ovsynch. The most recent study by Portaluppi and Stevenson (2005) showed similar results, with 62% of cows in the 72 h Cosynch group being detected in estrus 72 h (3 d) after the Ovsynch $PGF_{2\alpha}$. These studies indicate that estrus is frequently (~60% of the time) occurring 2 to 3 d, or 48 to 72 h, after the PGF_{2a} injection of Ovsynch. It therefore makes sense that protocols placing GnRH and TAI closer to the end of that period would be more effective than protocols initiating GnRH and TAI before the onset of estrus around 60 h.

Another possible explanation of the increased success of the 72 h Cosynch is that the additional 24 h period from 48 h to 72 h allows for a more mature ovulatory follicle and oocyte at the time of GnRH administration and TAI. This is a physiologically sensible explanation that also could account for the lower rate of pregnancy loss in the 72 h Cosynch treatment (1.6%) versus 5.9% and 13.3% in the 48 Cosynch and 48 h Ovsynch with 72 h TAI, respectively (Portaluppi and Stevenson, 2005). A larger, more mature ovulatory follicle should reasonably be expected to develop into a larger CL capable of increased synthesis of P_4, necessary for recognition and maintenance of pregnancy.

POST AI HORMONE TREATMENT

As early as 1990, work was being conducted to examine the effects of GnRH injection following AI. Most of these studies examined the period 12 to 14 d after insemination, based on the idea that maternal recognition of pregnancy occurs around this time. Exogenous GnRH supplementation will stimulate the growth of existing CL and/or stimulate developing follicles to lutenize, increasing P_4 secretion. High concentrations of P_4 during this phase of gestation are essential to the continuation of the pregnancy. Low concentrations of P_4 initiate the luteolytic cascade, where endogenous production of $PGF_{2\alpha}$ causes regression of the CL of pregnancy and resumption of normal cycling.

Despite continuing research, post AI hormone treatments in dairy cows continue to have mixed results. For almost every study that reports positive results, a similar study can be found that showed no advantages, regardless of presynchronization, chosen hormones, timing of administration, etc. There have been enough positive results to suggest that post AI treatment has potential, but is difficult to standardize and replicate results across the extremely varied pool of environments, climates, breeds, management styles and experimental designs that exist in today's dairy industry. Discussed below are five studies representative of post-insemination hormone protocols, each with differing approaches to the same concept and differing results.

Lajili (1991) conducted a study examining the effect of administration of 10μg of a GnRH analogue, buserelin, 12 to 14 d after AI in two groups of cows (n = 210); one group was bred on visual detection of estrus, the other group was partially synchronized using a single $PGF_{2\alpha}$ injection. Cows used as controls were injected with saline instead of GnRH between d 12 and 14. Post AI GnRH treatment resulted in an increase in PR compared to control animals (60 vs. 44%). Cows from the group treated with $PGF_{2\alpha}$ prior to AI showed an increased response to the GnRH as well; PR 62 vs. 40% in treated vs. control animals. In cows treated with GnRH that were diagnosed open, an increased rate of estrus detection following AI (91 vs. 74%) and a higher fertility rate on the following AI (59 vs. 44%) were observed.

Willard et al. (2003) examined the effects of post AI GnRH treatment at 5 or 11 d after AI using lactating Holstein cows (n = 106). Specifically, the 11 d treatment was intended to create accessory CL in cows with a 2 wave cycle, while the 5 d treatment was intended to create accessory CL in cows with a 3 wave cycle. All cows were submitted to the Ovsynch protocol using the standard 0, 7, 9 d plus 16 h (GnRH, $PGF_{2\alpha}$, GnRH, TAI) schedule. These cows then received one of three treatments; no GnRH or control (n = 37), 100µg GnRH 5 d after TAI (n = 34) or 100µg GnRH 11 d after TAI (n = 34). Serum P_4 was evaluated, number and size of CL were evaluated by transrectal ultrasound, and pregnancy diagnosis was performed 30 d after TAI. Serum P_4 was significantly higher in both GnRH-treated groups compared to the control group (5 d = 6.1 ± 0.4 ng/mL; 11 d = 6.2 ± 0.3 ng/mL; control = 3.8 ± 0.3ng/mL), as was the PR (5 d = 32%; 11 d = 38%; control = 19%). While statistical significance is not clear between the 5 d and 11 d treatments, both produced better results when compared to non-treated controls.

Bartolome et al. (2005) reported the effects of GnRH injections d 5 and 15 after AI. Lactating dairy cows (n = 831) were synchronized using standard Presynch-Ovsynch protocols for first service. All cows were assigned to one of four treatment groups on the day of TAI; G1 (n = 214) received GnRH on d 5; G2 (n = 209) received GnRH on d 15; G3 (n = 212) received GnRH on both d 5 and d 15; and G4 (n = 196) received no GnRH post-AI and served as controls. Pregnancy diagnosis was performed on d 27 and again on d 45 after TAI, but blood hormone concentrations were not monitored. No significant positive results were reported from any treatment group, but a decline in PR was observed in G3, which received GnRH on both treatment days; G1 = 47.7%, G2 = 43.5%, G3 = 36.8%, G4 = 44.4%. This study is in agreement with previous research (Macmillan, et al., 1985, Macmillan, et al., 1986, Milvae, et al., 1984) that has indicated that multiple injections of GnRH or GnRH analogues (such as buserelin) after AI are routinely detrimental to reproductive efficiency, not to mention the impracticality of their use.

Ryan et al. (1991) examined the use of post-AI GnRH on various dairies in Saudi Arabia. Lactating Holstein cows (n = 1535) were used, but no synchronization methods were employed. Trained observers conducted visual detection of estrus twice daily with the aid of tail chalking, and AI was performed according to the standard A.M. /P.M. guideline during the cooler hours of the early morning or late evening. Cows were assigned randomly to one of three treatment groups; G1 (n = 514) received 10µg buserelin at the time of AI; G2 (n = 503) received 10µg buserelin at the time of AI and again 12 d after AI; G3 (n = 516) received no injections and served as controls. Pregnancy rates were significantly lower for G3 (42.4%) than for G1 (48.8%) and G2 (51.1%), but there was no difference in PR between G1 and G2.

Lopez-Gatius et al. (2006) examined three treatment groups as in the previous study; G1 (n = 429) received no injections and served as controls; G2 (n = 431) received 100µg GnRH at the time of AI; G3 (n = 429) received 100µg GnRH at the time of AI and again 12 d after AI. In this study, cows were bred on visual heats until 60 DIM, at which time weekly exams per rectal palpations were performed to evaluate reproductive status.

Cows with palpable CL larger than 15mm were treated with cloprostenol (a $PGF_{2\alpha}$ analogue) and then bred on detected estrus. Cows considered to be anestrous were treated with a P_4-releasing intravaginal device and cloprostenol and were then bred on detected estrus if they returned to normal cyclicity. Results were substantially different compared to the 1991 study by Ryan et al.; G1 (control) again had the lowest PR (20.6%), but G3 (AI and 12 d) had a significant increase in PR compared to G2 (AI), 35.4 and 30.8% respectively.

These five studies are representative of the extreme variation that exists in study protocols for post-AI hormone treatment. Even a partial listing of these variations shows an inherent problem with directly comparing data from one study to another; environment/climate, level and method of synchronization, hormone used and dosage of that hormone, and timing of administration are all key factors that are rarely, if ever, consistent across any number of studies. Additionally, this is only a partial list and makes no consideration for differing management practices, general cow health and comfort, nutrition, etc. Frustration due to these complicating factors led researchers to a method of comparison for synchronization protocols and all of their many variations that would allow more meaningful evaluation of the large pool of heterogeneous data currently available.

Peters et al. (2000) performed a meta-analysis of a large set of data from studies examining the effects of GnRH administration 11 to 14 d after AI. The analysis was conducted on 19 studies from 14 published papers representing 10,945 cows. According to the authors, normal meta-analysis procedure (the Mantel-Haenszel method) was not appropriate for this data set, and logistic regression was used instead. Due to the large number of explanatory variables in the data set (trial, treatment, cow type, age, synchronization, method and time of pregnancy diagnosis), a saturated model best fit the data, but limited the pool to 2,541 observations out of the total 10,945. Further analysis of the limited data set allowed for the non-significant treatment by trial interaction to be removed and the data set was then re-evaluated. Once the interaction term was removed, the odds ratio of pregnancy in GnRH treated animals increased and became significant (odds ratio = 1.33; $P < 0.01$). While the authors were careful to point out that this value has limited use in extrapolation to other situations, the study reported that post-AI GnRH treatment produced significant benefits in some situations and merits further study to understand and standardize those benefits. It is also important to note that, to the best of the author's knowledge, no meta-analyses have been conducted more recently that would compare some of the newer modifications to the Ovsynch protocol. The analysis conducted by Peters in 2000 examined post AI GnRH administration from d 11 to 14 in which cows were subjected to Ovsynch protocols existent at that time. As protocols have changed in the intervening years, this study provides a good historical look at procedures of the time, but has limited use in extrapolation to protocols used currently.

PRACTICAL USE OF SYNCHRONIZATION TODAY

Breeding cows by way of synchronization has become a complicated and confusing practice, at least when viewed from the outside with little understanding of the underlying principles. One of the most important things to remember is that synchronization alone cannot currently, and will probably never, be able to completely solve declining reproductive efficiency issues. Farm operations as a whole must be evaluated and adjusted to find the best balance of cow health, production, reproduction, and economics, and it is very likely that no two farms will ever be exactly alike.

W. W. Thatcher (2006) wrote a review of ways to improve fertility in today's dairy cows. Many options were discussed, but most fall into broad categories including; careful nutritional management to reduce reproductive disorders around parturition and to maintain a suitable energy balance that allows for normal resumption of cyclicity; programming both first inseminations and repeat inseminations as a means of better management and reduction of labor devoted to visual detection of estrus; and finally, use of other supplements, such as bovine somatotrophin (bST) and dietary supplements like bypass fats and rumen-protected choline, all of which have been shown to increase reproductive efficiency.

Benefits of Synchronization vs. Breeding on Detected Estrus

Fricke et al. (2005) presented a very good comparison of three common methods used to submit cows to first AI service; the first method used visual detection of estrus only; the second method used what the author called "back-door" Ovsynch, where cows are monitored for estrus from the VWP (45 DIM) until 70 DIM, at which point all cows not bred as a result of visual detection of estrus are submitted to Ovsynch and bred by TAI; the third method submitted all cows to Presynch-Ovsynch between 25 and 32 DIM, TAI occurred between 65 and 73 DIM, and no cows were bred as a result of visual detection unless they displayed standing estrus between the VWP and the day of the first Presynch injection.

When the first approach, visual detection of estrus only, is considered, it is immediately apparent that this method is very inefficient. Approximately 10% of the cows scheduled for breeding by this approach were submitted to first AI before the VWP, and almost 35% were not submitted to first AI until after 100 DIM (range = 100 to 190 DIM). Obviously, the latter 35% had no chance of becoming pregnant in the ideal 70 to 80 DIM range, because they were not inseminated for the first time until greater than 100 DIM. This method also creates a management nightmare in the form of rebreeding cows bred on random days over a 5 month period (<40 to 190 DIM). Of course, it is entirely possible to implement a successful first service program based on visual detection of estrus, but this example shows how difficult such a program can be when managed incorrectly.

The second approach, the so-called "back-door" Ovsynch is a blend of visual detection of estrus and synchronization where Ovsynch is used to account for cows that have not displayed estrus within a set amount of time following the VWP. Visual detection of estrus on all cows started at 45 DIM (the set VWP) and occurred daily until 70 DIM. Cows not bred by this point are submitted to stand-alone Ovsynch and bred by TAI 10 d later. This method effectively eliminates all of the late first service AI, an effect seen in the visual detection of estrus scenario. However, this method still leaves a substantial number of cows (40% to 50%) bred randomly between 45 and 70 DIM that must be closely observed for non-pregnancy and submission to second service.

The third method involves a full synchronization protocol of Presynch and Cosynch with no visual detection of estrus during most of the synchronization procedure. Using this method, all cows are submitted to Presynch-Cosynch once a week between 25 and 32 DIM, and bred by TAI between 65 and 73 DIM. The only visual detection of estrus occurs during the 4 to 5 d immediately preceding TAI, and this detection is limited to the much smaller group of cows synchronized to be bred that week. Using this method, 98% of the cows in this scenario received timed AI for their first service between 65 and 73 DIM, and less than 5% of the cows received AI after a detected estrus (either in the days preceding TAI or in cows that displayed standing estrus several weeks after TAI).

One very clearly illustrated point in these scenarios is that compliance to the chosen protocol, regardless of which method is chosen, is essential for success. A properly managed system using visual detection of estrus will be much more effective than a poorly managed system using synchronization. When using synchronization protocols, "poorly managed" might be a misleading term; "anything less than perfect" might be more appropriate. Consider the Presynch-Ovsynch method discussed previously; this protocol involves 5 injections for each individual cow, each to be given on a specific day for a specific physiological reason. Assume that a farm achieves 95% injection accuracy on any given injection day, i.e. 95 out of 100 cows scheduled to receive an injection actually receive the correct injection on that day. Examining the figures for a five injection protocol (0.95 x 0.95 x 0.95 x 0.95 x 0.95 = 0.77) shows that 95% accuracy will result in 1 of every 4 cows not successfully completing the protocol, making the TAI of that cow ineffective. Compliance of 98% results in 1 of 10 cows failing the protocol, while 99% compliance results in only 1 of 100 cows failing the protocol. As a result, farms that cannot maintain compliance of 98% or higher should not attempt to use synchronization as their primary method of submitting cows to first service AI (Fricke, et al., 2005). In addition, there are two other very important facts to keep in mind when choosing a synchronization protocol over a visual detection system.

First, it is generally accepted that Ovsynch and its many variations do not provide substantial increases in CR compared to properly managed visual detection of estrus (the improvement is seen in PR). Most of the benefits of synchronization come from improved management and the fact all cows can be submitted to service in a timely fashion rather than from physiological benefits from the actual synchronization. In addition to the many individual studies discussed here, this trend has recently been confirmed by meta-analysis of Ovsynch and its variations compared to visually detected controls (Rabiee, et al., 2005).

Second, the use of a synchronization program does not allow for complete elimination of visual detection of estrus. DeJarnette et al. (2001b) specifically examined the incidence of premature estrus in dairy cows (n = 345) submitted to several variations of the Ovsynch protocol. All cows were treated with GnRH (100μg) followed 7 d later by $PGF_{2\alpha}$ (25mg) as per normal Ovsynch procedure. All cows were then observed twice daily from d 7 until d 9, and any cows visually detected in estrus were bred by AI 8 – 12 h later. Cows not detected in premature estrus were then submitted to either the continuation of normal Ovsynch (GnRH on d 9 and TAI 16 – 18 h later) or 72 h Cosynch (GnRH and TAI 72 h after $PGF_{2\alpha}$, on d 10). Premature estrus occurred in 20% (68/345) of the tested cows within 48 h after the $PGF_{2\alpha}$ injection of Ovsynch. If these cows are submitted to TAI 48 or 72 h after $PGF_{2\alpha}$, the timing between ovulation and insemination is incorrect and will usually result in very low PR. Even when a suitable synchronization program is chosen and managed efficiently, there is good reason to continue visual detection of estrus the week prior to TAI in order to catch and appropriately breed cows who display early or premature estrus relative to the timing of the protocol.

SELECTING THE BEST SYNCHRONIZATION PROGRAM

Looking at all of the possible options, pitfalls, and requirements for synchronization programs makes it fairly easy to see why farmers sometimes throw their hands up in frustration when deciding on the best reproductive strategy for their herd. The issue is further complicated by the fact that there truly is no “best” protocol that can be widely and successfully applied to two farms right across the street from one another, much less in different parts of the country or the world. Reproductive strategies must be individually formulated for each farm (sometimes even for specific groups of cows on a particular farm) and must take into account management styles, environment, nutrition, labor, level of training and understanding, and any number of other factors that have significant impact on cow health, comfort, and reproductive efficiency. Once a strategy is formulated, it must be closely adhered to in order to be effective, but the management must also keep track of the overall effectiveness and be ready to identify problems, or problem cows, and adjust strategies to accommodate those problems. Failure to choose an appropriate synchronization method or to successfully implement that protocol will ultimately result in lowered reproductive efficiency as well as a great deal of frustration. Consulting with reproductive specialists or knowledgeable veterinarians, understanding the physiologic “how and why” of the protocol being used, and developing the proper level of management can result in effective synchronization protocols that are easy to use and beneficial.

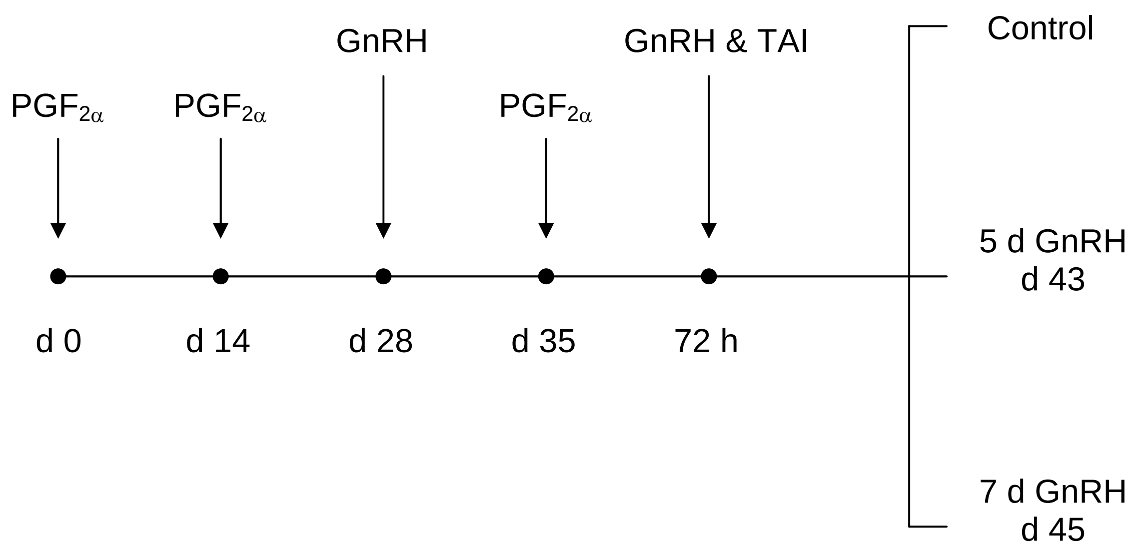
Effects of 5 and 7 d post-AI GnRH on first service PR, plasma P_4, and CL volume in lactating dairy cows submitted to 72 h Cosynch at the Virginia Tech Dairy Center

MATERIALS AND METHODS

This trial was conducted at the Virginia Tech Dairy Center from December 2004 to December 2005 using primiparous and multiparous lactating dairy cows (n = 116). Both Jersey (n = 36) and Holstein (n = 80) cows received a TMR balanced to meet or exceed the nutritional requirements for lactating dairy cows. Cows were housed in free-stall barns and bedded using mattresses and saw dust.

Once a week, all cows between 25 and 35 DIM were randomly assigned to one of three treatments, without regard to current stage of the estrus cycle, parity, or breed. All treatment groups received Presynch and 72 h Cosynch injections for synchronization of estrus and ovulation. Each cow received two 25 mg i.m. injections of $PGF_{2\alpha}$ (Lutalyse®; Pfizer Animal Health Inc., New York, NY) 14 d apart starting on d 0. On d 28, Cosynch injections began with a 62.5 μg i.m. injection of GnRH (Cystorelin®; Merial Ltd., Iselin, NJ), followed on d 35 with 25 mg of $PGF_{2\alpha}$. The final 62.5 μg injection of GnRH was given on d 38 concurrent with TAI. Cows then received either no further treatment (Control), GnRH 5 d after AI (5 d GnRH), or GnRH 7 d after AI (7 d GnRH). This protocol is shown in Figure 2. GnRH dosage in this protocol is based on research conducted by Fricke et al. (1998). Fricke tested the efficacy of a 50 μg dose compared to the usual 100 μg dose and found no differences when used in synchronization protocols. The 62.5 μg dose is a 25% increase above a half dose, providing a safety buffer for injection errors. All cows were synchronized such that TAI occurred on Friday mornings, but those cows observed to be in standing estrus during that week were bred according to

Figure 2. Timing of hormone injections for the Presynch + 72 h Cosynch protocol used to program first service AI.



the AM-PM guideline. The results of pregnancy exams were recorded for these cows, but no further injections were given.

Blood samples were collected via the tail vein on d 0, 14, 28, 38, and 52 (14 d post TAI), placed immediately on ice, centrifuged (3,000 x *g* for 20 min), and the resulting plasma was frozen at -20°C until analysis. Plasma P_4 was evaluated by radioimmunoassay (Coat-A-Count®; DPC, Los Angeles, CA). Pregnancy diagnosis was performed by ultrasonagraphy using a portable SonoSite 180+ (SonoSite Inc., Bothell, WA) 32 to 39 d following TAI. Pregnancy status was re-evaluated 60 to 67 d post TAI to confirm pregnancy and determine embryonic loss. Embryonic death was calculated as the number of cows diagnosed open at recheck divided by the number of cows diagnosed pregnant at the initial exam. Additionally, during the first pregnancy diagnosis (32 to 39 d), ovaries were scanned for occurrence of accessory CL, and all CL found were measured by ultrasound. In this discussion, the CL formed from the ovulatory follicle is referred to as the spontaneous CL; therefore, any additional CL that developed are referred to as accessory CL. The original 2-dimensional measurements were converted into 3-dimensional estimates of volume using the following equations: radius (cm) = (length/2 + width/2)/2; volume (cm^3) = $4/3 * \pi * R^3$. Concentration of plasma P_4 was used to evaluate cyclicity and luteal function related to the experimental treatments.

Data and Statistical Analysis

First service PR was analyzed using the logistic procedure of SAS (SAS® Institute, Cary, NC). The analysis model included terms for treatment, season, parity, breed, AI technician, and cyclicity. Months were grouped into seasons for the analysis as follows: Summer = June, July, August; Fall = September, October, November; Winter =

December, January, February; Spring = March, April, May. No differences were found between cows for lactations greater than 1, so in the final analysis parity was grouped as primiparous (lactation = 1) or multiparous (lactation >1). Breed was categorized as Holstein (n = 77) or Jersey (n=33). Cyclicity was determined using plasma P_4 concentrations taken at 4 times during the synchronization protocol. Plasma P_4 was determined on both days of the Presynch injections and on both days of GnRH administration of the Ovsynch protocol. Cows were classified as cycling if their plasma P_4: was > 1 ng/mL in the first three samples and < 1 ng/mL in the fourth sample. Results from the logistic regression were presented as odds ratios and 95% confidence limits. Odds ratios are interpreted as the relative chance of a pregnancy occurring for a particular level of an explanatory variable relative to another level when the other explanatory variables of the model are controlled. As a result of this interpretation, odds ratios indicate the following: 1 = no effect on the chance of pregnancy; <1 = decreased chance of pregnancy; >1 = increased chance of pregnancy. The 95% confidence limits demonstrate the precision of the odds ratio estimates. A confidence limit that contains the value "1" indicates no significant differences between the levels of the variables being tested.

Total CL volume (TCLV) was analyzed using the GLM procedure of SAS. The analysis model included treatment, season, parity, breed, pregnancy status at 30 d, and the interaction of treatment and pregnancy status. Season, parity, and breed were organized exactly as in the logistic regression model discussed previously. Total CL volume between treatment groups was further evaluated using estimate statements to compare control versus treatment group averages and treatment 1 versus treatment 2.

Progesterone data was analyzed using the GLM procedure of SAS as repeated measures. The analysis model included treatment and plasma P_4 concentrations from the final sample (d 52).

Correlations between P_4 and TCLV were calculated using the correlation procedure of SAS. The analysis model included TCLV and plasma P_4 concentrations from the final sample (d 52). Significance was declared at $P \leq 0.05$ and tendencies at $P \leq 0.10$ for all analyses.

RESULTS AND DISCUSSION

First Service Conception Rate

Treatment (*P* = 0.89), season (*P* = 0.75), parity (*P* = 0.12), breed (*P* = 0.78), and AI technician (*P* = 0.30) did not significantly influence first service PR in this study (Table 1). A breakdown of the data by parity and treatment is shown in Table 2. First service PR was 30.9, 43.0 and 34.8% for control, 5 d, and 7 d treatments respectively. Early embryonic loss between initial and repeat pregnancy exams was calculated as 12.5% (6/48); PR at 60 d was 33.5, 33.7, and 20.9% for control, 5 d, and 7 d treatments respectively. Cows that were classified as acyclic differed significantly from those cows that did respond to the protocol (odds ratio = 0.151, *P* = 0.0011). Cyclicity was determined based on plasma P_4 concentrations collected throughout the trial; 72% of the cows were cyclic while 28% were acyclic (Table 3). Only 5 acyclic cows were diagnosed pregnant at the initial exam, therefore 90% of the cows diagnosed pregnant were from the cycling group.

Numerous studies have been conducted to determine the effects of administration of GnRH following AI on PR. Results of these studies have indicated no

Table 1. Logistic regression of first service pregnancy rate on GnRH treatment, season of AI, parity, breed, cyclicity status, and AI technician for cows bred either without a GnRH injection post-AI or with GnRH administration 5 or 7 d post-AI at the Virginia Tech Dairy Center.

Category	AI (no.)	Pregnant LS Mean (%)	SE (%)	Odds ratio [1]	95% CL [2]	*P* value [3]
Treatment						0.89
Control	36	38.6	9.7	1.00	…	…
5 d GnRH	37	39.3	10.0	1.06	(0.352, 3.176)	…
7 d GnRH	38	34.5	9.6	0.83	(0.290, 2.367)	…
Season						0.75
Winter	34	46.5	13.3	1.00	…	…
Spring	26	33.9	8.2	0.58	(0.156, 2.147)	…
Summer	46	39.7	10.1	0.78	(0.182, 3.148)	…
Fall	4	29.7	14.6	0.45	(0.087, 2.371)	…
Parity						0.12
2+	76	30.0	7.5	1.00	…	…
1	34	44.9	9.7	2.10	(0.833, 5.278)	…
Breed						0.78
Jersey	33	38.8	10.2	1.00	…	…
Holstein	77	36.1	6.9	0.88	(0.349, 2.212)	…
Cyclicity						0.0011
Cyclic	79	60.0	6.8	1.00	…	…
Acyclic	31	18.9	10.4	0.15	(0.048, 0.469)	…
AI Technician						0.30
7	47	46.8	8.3	1.00	…	…
5	48	33.5	9.1	0.52	(0.204, 1.327)	…
1	15	32.1	13.7	0.50	(0.131, 1.880)	…

[1] Odds ratio is the estimated chance of pregnancy at AI in a single category considering the other variables in the model. Baseline represented as 1.00. Ratios > 1 indicate increased chance of pregnancy. Ratios < 1 indicated decreased chance of pregnancy

[2] 95% CL that do not include 1.00 are different from 1.00 at $P < 0.05$

[3] Significance declared at $P < 0.05$, trends at $P < 0.10$

Table 2. First service pregnancy rate by parity and treatment for cows bred either without a GnRH injection post-AI or with GnRH administration 5 or 7 d post-AI at the Virginia Tech Dairy.

		AI (no.)	Pregnant LS Mean (%)
Parity 1			
	Control	12	31.6
	5 d GnRH	9	53.0
	7 d GnRH	10	44.5
Parity 2+			
	Control	24	30.1
	5 d GnRH	38	32.9
	7 d GnRH	26	25.0
Totals		111	36.2

Table 3. First service pregnancy rate by cyclicity status, parity, and treatment for cows bred either without a GnRH injection post-AI or with GnRH administration 5 or 7 d post-AI at the Virginia Tech Dairy.

		Cyclic [1]		Acyclic	
		AI (no.)	PR (%) [2]	AI (no.)	PR (%) [2]
Parity 1					
	Control	9	71.2	3	0.0
	5 d GnRH	5	80.6	4	25.3
	7 d GnRH	10	54.7	3	34.2
Parity 2+					
	Control	19	54.7	5	5.5
	5 d GnRH	18	36.6	10	29.1
	7 d GnRH	18	48.9	6	1.0
Totals		79	57.8	31	15.9

[1] Cyclicity determined using P_4; $P_4 > 1$ ng/mL on d 0, 14, and 28 and < 1 ng/mL on d 38 indicated normal cyclicity.

[2] LS means

benefits in some cases (Bartolome, et al., 2005, Ryan, et al., 1991) and significant improvements in PR in others (Lajili, et al., 1991, Lopez-Gatius, et al., 2006, Peters, et al., 2000, Sianangama and Rajamahendran, 1992, Thatcher, et al., 2001, Willard, et al., 2003). The day of administration varied between studies, but was most often d 5, 7, 11, 12, or 14. In studies that showed benefits, PR for treated groups ranged from 5 to 20% higher than non-treated controls.

Lopez-Gatius et al. (2006) and Willard et al. (2003) conducted studies similar to the current study using GnRH administration at 0 and 12 d post-AI and at 0, 5, and 11 d post-AI, respectively. Control groups had lower PR (20.6 and 19%) compared to cows receiving GnRH (32, 38, and 35.4%) at 5, 11, and 12 d respectively. Peters et al. (2000) conducted a meta-analysis of GnRH administered to 11,000 cows 11 to 14 d after AI and concluded that there was a significant improvement in PR for GnRH treated cows (odds ratio = 1.33, $P < 0.01$). At this time, no meta-analysis has been conducted on GnRH administered 0 to 10 d after AI.

Progesterone and CL Volume

Administration of GnRH at either 5 or 7 d post AI did not affect plasma P_4 concentrations ($P = 0.30$). There was a moderate positive correlation (r = 0.40, $P = 0.0035$) between the final P_4 and TCLV. As CL tissue is responsible for biosynthesis of P_4, the correlation between the P_4 concentration on d 38 and TCLV was not as strong as might be expected. There was also a moderate negative correlation (r = -0.42, $P < 0.0001$) between the fourth and final P_4 samples. The fourth P_4 sample was taken at the time of AI; consequently this negative relationship would be expected in cyclic cows. Plasma P_4 should be low at the time of AI indicating a dominant, estrogenic follicle at that time. Having a dominant follicle and the resulting low P_4 at the time of AI increases the chance of a pregnancy and therefore of having a CL of pregnancy 14 d later at the time of the final P_4 sample collection. Treatment ($P = 0.034$) and pregnancy status at 30 d ($P < 0.0001$) had a significant effect on TCLV. The TCLV for the average of 5 and 7 d treatments was significantly higher than non-treated controls ($P = 0.011$). However, there was no difference between 5 and 7 d treatments ($P = 0.93$).

Multiparous cows tended to have greater TCLV compared to primiparous cows ($P = 0.077$). However, season ($P = 0.27$), breed ($P = 0.90$), and treatment by pregnancy status interaction ($P = 0.70$) did not significantly effect TCLV. As shown in Table 4, cows with an accessory CL had significantly higher TCLV (15.54 cm^3) than cows with a spontaneous CL (8.27 cm^3) ($P < 0.0001$). First service PR for cows with an accessory CL was significantly higher (95%) compared to 61% in cows with only a spontaneous CL ($P = 0.0069$). Plasma P_4 was also significantly increased (6.95 vs. 5.88 ng/mL) in cows with accessory CL ($P = 0.025$). Cows with no CL at the initial pregnancy exam (n = 39) were considered anovulatory; PR for cows in this group was 7.7%. Although no differences were observed in first service PR due to GnRH treatment in this study, the incidence of accessory CL seems to have a significant effect on PR (Table 5). Both 5 and 7 d GnRH induced accessory CL (25 and 30%) and the resultant PR was correspondingly increased for both (100 and 90%, respectively).

Numerous studies have examined relationships between plasma P_4 and CL size and function in conjunction with synchronization protocols; all such studies have reported increases in either P_4 biosynthesis, amount of CL tissue, or both in response to post-AI treatment with GnRH (Bartolome, et al., 2005, Lajili, et al., 1991, Lopez-Gatius,

Table 4. Characteristics of cows determined to have either a spontaneous or accessory corpus luteum at ultrasound examination 32 to 39 d post AI.

Category	AI (no.)	Pregnant LS Mean (%)	TCLV LS Mean (cm^3) [1]	P_4 LS Mean (ng/mL)	SE (%)	*P* value [2]
CL Incidence						
PR (%)						0.0069
Spontaneous	33	60.6	…	…	7.3	…
Accessory	19	94.7	…	…	9.6	…
TCLV (cm^3)						<0.0001
Spontaneous	33	…	8.27	…	71.4	…
Accessory	19	…	15.54	…	94.1	…
P_4 (ng/mL)						0.0248
Spontaneous	33	…	…	5.88	28.2	…
Accessory	19	…	…	6.95	36.6	…

[1] Total CL volume – Measurements taken as L x W (cm). Radius (R) calculated by R = (L/2 + W/2)/2. Volume (V) calculated by $V = 4/3 \times \pi \times R^3$

[2] Significance declared at $P < 0.05$, trends at $P < 0.10$

Table 5. First service pregnancy rate by incidence of corpora lutea and treatment for cows bred either without a GnRH injection post-AI or with GnRH administration 5 or 7 d post-AI at the Virginia Tech Dairy.

	No CL		Spontaneous CL		Accessory CL	
	AI (no.)	PR (%) [1]	AI (no.)	PR (%) [1]	AI (no.)	PR (%) [1]
Treatment						
Control	15	6.7	17	76.5	0	0.0
5 d GnRH	21	9.5	6	50.0	9	100
7 d GnRH	13	0.0	10	40.0	10	90.0
Totals	39	7.7	33	60.6	19	94.7

[1] LS means

et al., 2006, Peters and Pursley, 2003, Ryan, et al., 1991, Sianangama and Rajamahendran, 1992, Thatcher, et al., 2001, Willard, et al., 2003). However, these studies did not report direct correlations between the amount of CL tissue present and P_4 concentrations. Differences in the hormone used (or the amount administered), the synchronization protocol used to initiate TAI, and the day of post-AI hormone injections also limits the ability to directly compare one study to another, but all have shown variable benefits over no treatment.

More recent studies by Howard et al. (2005) and Chagas e Silva and Lopes da Costa (2005) have examined similar treatments and their effects on P_4, CL size and number, and PR as in the current study. Howard et al. (2005) used 100 μg of GnRH 5 d after AI and Chagas e Silva used 1500 IU hCG 7 d after AI; cows in the study by Howard et al. (2005) were synchronized, cows in the Chagas e Silva study (2005) were not. One hundred percent of the GnRH-treated cows (n = 12) in the study by Howard et al. developed an accessory CL; 78% of the hCG-treated cows (n = 64) in the Chagas e Silva study developed an accessory CL. In both studies, cows with accessory CL had higher PR than non-treated controls. Plasma P_4 concentrations in both studies were not significantly different between treated and non-treated animals until d 13 to 15 after AI, at which point a difference of 2.00 to 5.00 ng/mL became apparent and significant in the treated cows. Multiple blood samples were taken in both studies before and after the 13 to 15 d post-AI period, allowing the investigators to examine the P_4 relationship over time. Time, labor, and financial constraints limited post-AI blood sampling in the current study and may have resulted in potentially significant results not being observed. Although the PR in cows with accessory CL in these two studies was not as dramatic as in the current study, similar trends in PR and P_4 were observed, indicating the potential benefits of inducing accessory CL. Further study will be needed to clarify the causative agents of this phenomenon.

Summary

Treatment, season, parity, breed, and AI technician did not significantly influence first service PR in this study. Control, 5 d, and 7 d groups had PR of 38.6, 39.3, and 34.5% respectively. Early embryonic death rate of 12.5% reduced PR at 60 d to 33.5, 33.7, and 20.9% for control, 5 d, and 7 d treatments respectively. Cows considered to be cycling based on plasma P_4 had a dramatically increased chance of pregnancy in this study. Although similar studies have shown post-AI GnRH to increase plasma P_4, differences were not significant in the current study. Total CL volume was shown to be significantly increased in multiparous cows. Treatment with GnRH following AI resulted in significant increases in TCLV, but there were no differences between post-AI treatments. Cows with accessory CL had increased TCLV and higher PR than cows with a spontaneous CL. No relationship was found between the increased TCLV and the P_4 concentrations for GnRH-treated groups. This study presented no evidence that post-AI GnRH treatment increased first service PR or plasma P_4.

Effects of 5 and 7 d post-AI GnRH on first service PR in lactating dairy cows submitted to 72 h Cosynch at Myers Dairy

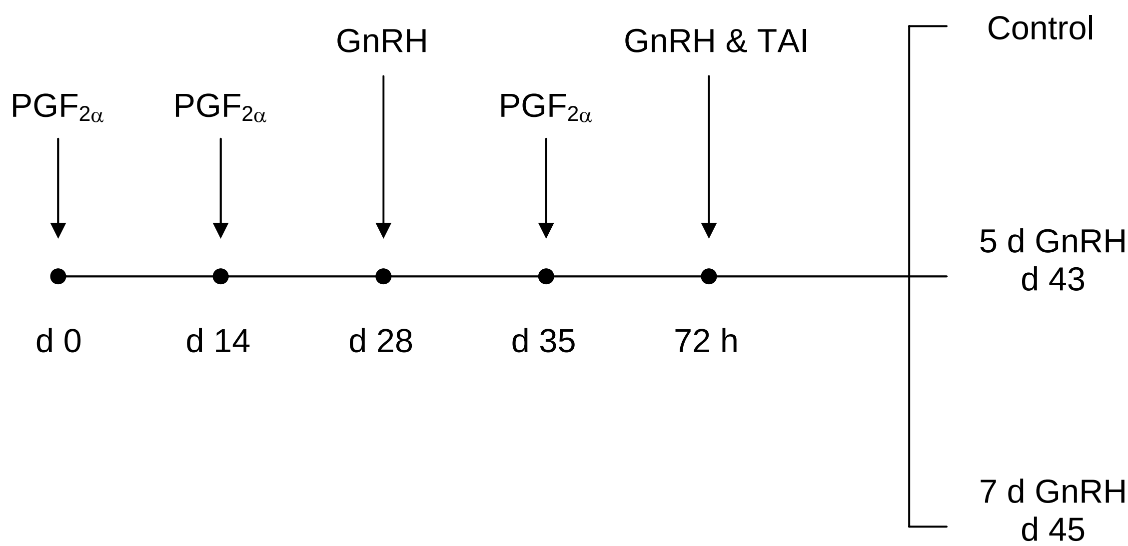
MATERIALS AND METHODS

This trial was conducted at Myers Dairy, Jonesville, NC from December 2004 to March 2006 using primiparous and multiparous lactating dairy cows (n = 1055). All cows were housed in free-stall barns and received a TMR balanced to meet or exceed the nutritional requirements for lactating dairy cows.

Myers Dairy uses synchronization as a normal part of its management procedures, therefore the timing of the synchronization protocols for this study were altered slightly compared to the study conducted at the VT Dairy. Once a week, all cows between 35 and 41 DIM were randomly assigned to one of three treatments, without regard to current stage of the estrus cycle or parity. All three treatment groups received Presynch and 72 h Cosynch injections for synchronization of estrus and ovulation. Cows then received either no further treatment (Control), GnRH 5 d after AI (5 d GnRH), or GnRH 7 d after AI (7 d GnRH). This protocol is illustrated in Figure 3. All cows were synchronized so that TAI occurred on Thursday mornings, but those cows observed to be in standing estrus during that week were bred according to the AM-PM guideline. The results of pregnancy exams were recorded for these cows, but no further injections were given.

Pregnancy diagnosis was conducted according to the normal schedule for the dairy. First pregnancy exams were done 35 to 42 d after TAI, and the follow-up exams were done 50 to 57 d after TAI. Embryonic death was calculated as the number of cows diagnosed open at recheck divided by the number of cows diagnosed pregnant at the initial exam.

Figure 3. Timing of hormone injections for the Presynch + 72 h Cosynch protocol used to program first service AI.



Data and Statistical Analysis

First service PR was analyzed using the logistic procedure of SAS. The analysis model included treatment, season, parity, and AI technician. Months were grouped into seasons for the analysis as follows: Summer = June, July, August; Fall = September, October, November; Winter = December, January, February; Spring = March, April, May. No differences were found between cows for lactation greater than 1, so in the final analysis parity was grouped as primiparous (lactation = 1) or multiparous (lactation >1). Twenty three cows were bred following visual detection of estrus in the 5 d prior to scheduled TAI. All of these cows were diagnosed pregnant, but were removed from the analysis since they were not bred according to the synchronization protocol.

Results from the logistic regressions were presented as odds ratios and 95% confidence limits. Odds ratios were interpreted as the relative chance of a pregnancy occurring for a particular level of an explanatory variable relative to another level when the other explanatory variables of the model are controlled. As a result of this interpretation, odds ratios indicate the following: 1 = no effect on the chance of pregnancy; <1 = decreased chance of pregnancy; >1 = increased chance of pregnancy. The 95% confidence limits demonstrate the precision of the odds ratio estimates. A confidence limit that contains the value “1” indicates no significant differences between the levels of the variables being tested. Significance was declared at $P \leq 0.05$ and tendencies at $P \leq 0.10$.

RESULTS AND DISCUSSION

First Service Conception Rate

Treatment (*P* = 0.60) and season (*P* = 0.91) did not influence first service PR in this study (Table 6). First service PR was 31.0, 30.3, and 33.8% for control, 5 d, and 7 d treatments respectively. A breakdown of the data by parity and treatment is shown in Table 7. Early embryonic loss between initial and repeat pregnancy exams was calculated as 1.8% (5/275); these losses did not significantly affect mean PR. There was a trend (*P* = 0.06) for the PR of primiparous cows (34.2%) to be higher than multiparous cows (28.8%) but there were no significant differences between treatments by parity. Cows bred by AI technician 1 (n = 332) versus 7 (n = 586) were 1.7 times more likely to become pregnant (*P* = 0.0003). Although the 23 cows that were bred on visual detection of estrus were not included in the analysis, they represent the importance of maintaining a visual detection program. When those cows were included in the data, the overall PR increased from 29.8 to 31.6%.

Summary

Treatment and season had no effect on first service PR at the Myers Dairy. Control, 5 d, and 7 d groups had PR of 31.0, 30.3, and 33.8% respectively. Cows bred by technician 1 were 1.7 times more likely to become pregnant than if they were bred by technician 7. Primiparous cows had a tendency towards higher PR than multiparous cows (34.2 vs. 28.8%). These analyses presented no evidence that post-AI GnRH increases PR, but suggests that small alterations in the timing of post-AI GnRH to accommodate existing synchronization schedules may be feasible in situations where this treatment does increase PR.

Table 6. Logistic regression of first service pregnancy rate for effects of treatment, season, parity, breed, synchronization status, and AI technician for cows bred either without a GnRH injection post-AI or with GnRH administration 5 or 7 d post-AI at Myers Dairy.

Category	AI (no.)	Pregnant LS Mean (%)	SE (%)	Odds ratio [1]	95% CL [2]	*P* value [3]
Treatment						0.60
Control	287	31.0	2.7	1.00	…	…
5 d GnRH	289	30.3	2.5	0.96	(0.666, 1.382)	…
7 d GnRH	346	33.8	2.7	1.137	(0.805, 1.605)	…
Season						0.91
Winter	297	33.0	2.7	1.00	…	…
Spring	200	32.3	3.3	0.97	(0.652, 1.438)	…
Summer	189	30.1	3.4	0.87	(0.576, 1.302)	…
Fall	235	31.3	3.0	0.92	(0.631, 1.342)	…
Parity						0.06
2+	522	28.8	2.1	1.00	…	…
1	399	34.5	2.3	1.318	(0.988, 1.757)	…
AI Technician						0.0003
7	586	25.9	1.9	1.00	…	…
1	332	37.4	2.5	1.72	(1.284, 2.295)	…

[1] Odds ratio is the estimated chance of pregnancy at AI in a single category considering the other variables in the model. Baseline represented as 1.00. Ratios > 1 indicate increased chance of pregnancy. Ratios < 1 indicated decreased chance of pregnancy

[2] 95% CL that do not include 1.00 are different from 1.00 at $P < 0.05$

[3] Significance declared at $P < 0.05$, trends at $P < 0.10$

Table 7. First service pregnancy rate by parity and treatment for cows bred either without a GnRH injection post-AI or with GnRH administration 5 or 7 d post-AI at Myers Dairy.

		AI (no.)	Pregnant LS mean (%)
Parity 1			
	Control	132	35.2
	5 d GnRH	121	30.1
	7 d GnRH	147	37.3
Parity 2+			
	Control	155	27.1
	5 d GnRH	168	29.0
	7 d GnRH	199	30.3
Totals		922	31.5

CONCLUSIONS

Treatment had no significant effect on first service PR at the Virginia Tech Dairy or the Myers Dairy. Small sample size at the Virginia Tech Dairy lowered the probability that significant differences would be found, along with numerous other factors relating to the dairy's status as a research herd; dramatic changes in management, nutrition, and cow location along with more frequent handling and the resulting cow stress are all potential factors that could have limited or altered the performance of the treatments in this study. Myers Dairy had an above-average reproductive program based on first service PR data in prior years. First service PR at Myers Dairy was approximately 28% in the year immediately before this study, and farm personnel were already implementing synchronization with a high degree of success. Such a well-managed operation makes conducting research studies there an enjoyable experience, but may limit the size and scope of the treatment differences observed in comparison to results reported by other researchers.

No significant relationships were observed between treatments, plasma P_4 concentrations, and CL volume at the Virginia Tech Dairy. Given the normally large variability in plasma P_4 necessary for a cow to maintain a pregnancy, small sample size in this trial may have resulted in the non-significant results. Larger sample size would allow for more variation and still result in an accurate median value necessary to show significance. Corpus luteum volume is also highly variable from cow to cow and any potential significance may have been lost in small sample size. The dramatic increase in PR in cows with accessory CL indicates that successful induction of accessory CL may have a more important role in pregnancy than the resultant P_4 profile. A study that examined similar treatments and collected P_4 and CL volume data in larger quantities might elucidate the cause of this increase in PR. Evaluating FSH and LH concentrations in addition to P_4 would provide a more complete endocrine profile, and more frequent CL measurement by ultrasound would give a clearer picture of ovarian dynamics during the period immediately following AI. Further research should be conducted to evaluate the effect of accessory CL on PR; if this proved successful, methods could be developed specifically to induce accessory CL and thereby increase PR.

Synchronization protocols that include post-AI treatments of GnRH have been proven effective in the past. Although this study did not show significant positive results, there is certainly benefit to be gained through continued research. Synchronization protocols can be effective and helpful to dairy producers with the knowledge and drive to correctly implement them, and research should continue to support this very important element of the dairy industry.

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College:

Virginia Polytechnic Institute and State University 1999-2003
Blacksburg, Virginia
Bachelor of Science in Agricultural and Applied Economics

Virginia Polytechnic Institute and State University 2004-2006
Blacksburg, Virginia
Master of Science in Dairy Science (in progress)

Virginia-Maryland Regional College of Veterinary Medicine 2006-2010
Blacksburg, Virginia
Doctor of Veterinary Medicine (starting August 2006)

Professional Positions:

Graduate Teaching Assistant 2005
ALS 4304 Physiology of Reproduction
Department of Dairy Science
Virginia Polytechnic Institute and State University

Graduate Teaching Assistant 2006
ALS 2304 Animal Anatomy and Physiology
Department of Dairy Science
Virginia Polytechnic Institute and State University

Honors Organizations:

Alpha Zeta Agricultural Honor Fraternity 2001-current