

EVALUATION OF SYSTEMATIC BREEDING PROGRAMS IN LACTATING DAIRY COWS

by

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(ABSTRACT)

Observing cows in estrus and inseminating them at the optimal time are necessary steps for effective reproductive management of a dairy herd. However, increasing herd sizes can lead to reproductive inefficiency resulting in decreased profits on dairy herds. Synchronization of estrus, through pharmacological control, has been used to improve reproductive efficiency. Systematic breeding programs provide an organized approach for administering artificial insemination (AI) at first service. Moreover, reproductive management is based on a methodical approach for the entire herd rather than for the individual cow. Seven-hundred and thirty four Holstein cows from 16 commercial dairy herds were used to conduct this study evaluating three systematic breeding protocols; 14-d $\text{PGF}_{2\alpha}$, timed AI (TAI), and GnRH- $\text{PGF}_{2\alpha}$, in comparison with an untreated control group. Eight herds relied on visual observation as their primary method for detection of estrus, and 8 herds utilized the HeatWatch[®] (DDx, Inc., Denver, CO) electronic estrus detection system. The average days to first postpartum AI were longer for untreated control cows

when compared to the other breeding protocols. First AI conception rates did not differ among control, 14-d PGF_{2α}, or GnRH-PGF_{2α} protocols, but were higher than the TAI protocol. However, first AI pregnancy rates were higher for untreated controls versus hormonally treated cows. Estrus characteristics associated with each protocol were also evaluated and no difference was detected across treatments. An economic analysis determining cost per pregnancy for each protocol when considering drug costs, and pregnancy rates, resulted in the highest cost per pregnancy for TAI followed by GnRH-PGF_{2α} and 14-d PGF_{2α}. These programs should be considered as tools for convenience and efficiency of estrus detection; however, reduced labor costs from less time spent on estrus detection may be offset by the cost of the drug protocols. Cost effectiveness must be calculated on an individual herd basis when deciding whether a systematic breeding program is the appropriate choice.

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INTRODUCTION

The major factor limiting reproductive performance in many dairy herds is the failure to detect estrus in a timely and accurate manner. Efficient and accurate estrus detection is essential to optimize the economic management of individual cows to yield a profitable dairy operation. To maintain economic efficiency, dairy herds are increasing their herd size and maximizing milk production. For example, during the past 10 yr Virginia Dairy Herd Improvement herds have experienced average increases of 33% in lactating cows and 20% in milk yield without the same increase in the labor force. During this same period the average days open increased by 25 d, or approximately one estrous cycle. These increases in herd size, milk yield, and average days open have been implicated as contributors to the lower reproductive efficiency that has been experienced by many dairy herds because reproductive performance appears to be directly related to herd management (7). With increases in the average herd size, detection of estrus remains major problem, thus, leading to economic losses from failure to efficiently and accurately detect estrus, which can be excessive. For dairy farming to remain a profitable enterprise, more attention and skill need to be devoted to increasing reproductive efficiency.

To efficiently maximize lifetime milk yield, the goal for most dairy operations is to generate the highest possible number of offspring in the shortest interval without compromising the well being of the dam or neonate. To accomplish this the following must occur; establishment of a voluntary waiting period (**VWP**) and efficient and

accurate detection of estrus which would, thereby, reduce the variability of days to first insemination leading to a decreased calving interval. Calving intervals are increasing rather than decreasing because of reproductive inefficiency, leading to a decrease in profits. Studies (1, 10, 40) have indicated that the calving interval for optimal milk production and profit is between 12 and 13 mo. Calving intervals longer than optimum occur when cows spend more time in the later, less profitable stage of lactation (10). Estrus detection and days to first AI, including the VWP, are the key factors affecting duration of calving intervals (40). Increased rates of estrus detection and proper timing of insemination will, ideally, increase pregnancy rates within set time limits, thereby leading to shorter calving intervals. Lucy et al. (40) suggested that methods of estrus synchronization that optimize first service conception and reduce variability in days to first service may reduce the variability and length of calving intervals for all cows in the herd.

Estrus detection rates on dairy herds are often less than 50% (4). Detection of estrus is often difficult due to short periods of standing estrus, silent estrus, changing nutritional regimens, environmental temperatures, and estrus onset during the late night to early morning hours. Dransfield et al. (17) reported the average duration of estrus to be 7.1 ± 5.4 h with 8.5 ± 6.6 standing events per cow. Unpublished data from our laboratory concluded that the average standing event, or mount, exhibited by lactating dairy cows lasts for only 3.3 s, therefore, the expression of true estrus occurs for only 28 s during a 7.1 ± 5.4 h period, every 21 d. The combination of short estrus duration and

low frequency of standing activity is, perhaps, the major implication for low rates of estrus detection. While inadequate estrus detection may be a major limitation for dairy herds, management systems which emphasize observation of estrus and development of techniques for detection of estrus could help overcome the problem of missed estrus periods (76).

Systematic breeding programs are a proactive response to optimizing reproductive management of the dairy herd, rather than taking a reactive approach, by waiting for cows to cycle and be identified in estrus before administering AI. Moreover, reproductive management is based on a methodical approach for the entire herd rather than focusing on individual cows. A systematic breeding program induces estrus and may allow for appointment breeding without the need for detection of estrus. Two of the more common protocols include administration of a GnRH-PGF_{2α}-GnRH combination over a period of time, TAI, and the 14-d PGF_{2α} protocol, which consists of a series of PGF_{2α} administrations at 14-d intervals followed by detection of estrus. These systems facilitate the insemination of more cows and increase the number of inseminations within a given time period because the induction of estrus will enable more efficient detection of estrus since cows on the program will be expected to come into estrus, with the exception of those cows receiving TAI. Primary benefits of systematic breeding programs include day-to-day convenience in managing the breeding herd and efficient use of labor for detection of estrus and AI, in addition to decreased days to first AI. Systematic breeding programs have been used to some degree on many dairy herds;

however, there is a lack of controlled field data evaluating reproductive performance of cows and herds that implement such breeding schemes.

The objective of the research reported herein was to determine the effectiveness of three systematic breeding programs, 14-d PGF_{2α}, TAI, and GnRH-PGF_{2α} (Figure 1), on increasing the reproductive performance of a dairy herd under typical field conditions. Reproductive performance was measured by estrus detection rate, days to first AI, percentage of cows pregnant by 120 d postpartum, and first service conception rates for each protocol. The percentage of cows observed in estrus and distribution of days postpartum to first AI, in addition to conception rates and pregnancy rates by 120 d postpartum, were determined for each breeding protocol and herd. Sixteen herds were used; 8 herds relied on visual observation as the primary method for detecting estrus, whereas, the remaining 8 herds utilized the HeatWatch[®] electronic estrus detection system (DDx Inc., Denver CO). Therefore, a second objective was to evaluate the efficiency of estrus detection programs when combined with the various systematic breeding programs. Parameters evaluated include number of standing events per estrus period, duration of estrus, estrus intensity, and interval from estrus onset to AI. The final objective for this study was to provide an economic evaluation of the three systematic breeding protocols described herein.

REVIEW OF LITERATURE

Hormonal regulation of reproductive processes

Reproduction is regulated through communication between the endocrine system and the nervous system. Hormones are chemical messengers produced in numerous locations throughout the body, which control or regulate the function of cells, tissues, and organs. Hormones are released into the blood and exert their effect by binding to a specific receptor on or in target tissues. Receptors have an affinity for a specific hormone that causes binding to occur. Receptors, or receptor domains, for protein hormones (LH and FSH) are located on the plasma membrane of the target cell. When the domain becomes occupied, configurational changes occur and G-proteins are activated (25). Once GnRH, for example, is stimulated for release from the hypothalamus, it binds to its receptor on the gonadotroph cell (59) in the pituitary and activates a calcium influx and the phosphatidylinositol (PI) cascade (13, 45, 53). The binding of GnRH to its receptor stimulates G-proteins in the cellular membrane and is thought to involve GTP conversion to GDP and activation of phospholipase C. Hydrolysis of PI is initiated by activation of phosphodiesterase (PDE) and the main product is diacylglycerol (DAG). Arachidonic acid is then produced as a result of metabolism of DAG and eventually, protein kinase-C stimulates phosphorylation. Phosphorylation is an important step in protein synthesis and, therefore, gonadotropin synthesis, which then leads to gonadotropin secretion from the anterior pituitary (13).

Both pituitary and steroid hormones regulate the synthesis, storage, and release of hypothalamic hormones through three feedback mechanisms: a long, short, and ultra-short-loop (59). Long feedback involves interaction between the gonad, pituitary, and hypothalamus. Short feedback involves levels of pituitary gonadotropins, which can influence the secretory activity of releasing hormones without mediation of the gonads. Ultra-short feedback refers to a neuronal action of hypothalamic hormones to regulate their own secretion. Steroid hormones may exert stimulatory or inhibitory feedback depending on their concentration in the blood.

Sexual promoters (estradiol (E_2), progesterone (P_4), and testosterone) are produced by the gonads of the male and female to help regulate the function of the hypothalamus and the anterior pituitary (59). This provides evidence of a relationship between the hypothalamo-hypophyseal portal system and the gonad (28). The ovary is dependent on secretion of gonadotropins from the anterior pituitary. Steroid hormones secreted by the ovary feedback on the hypothalamus and the pituitary gland to regulate secretion of gonadotropins (28, 45). Synthesis of ovarian steroids requires coordinated activity of LH, FSH and several ovarian cell types. Steps of steroid hormone action include steroid transport, movement through the cellular membrane and cytoplasm, binding of the steroid to a nuclear receptor, and mRNA and protein synthesis. The newly synthesized mRNA leaves the nucleus and attaches to ribosomes where it directs the synthesis of specific proteins that will enhance the reproductive process (46).

Specific hypothalamic neurons synthesize neuropeptides that stimulate or inhibit release of hormones from the anterior pituitary, which eventually influences ovarian

function (45). Positive feedback occurs when E_2 stimulates the release of a gonadotropin (LH). Estrogen can act as a positive or negative feedback agent (69). During proestrus, increasing levels of E_2 in the blood triggers the surge center of the hypothalamus, stimulating secretion of the preovulatory GnRH surge (28, 45). This release is caused by depolarization of the neurosecretory cell bodies. Estrogen has a negative feedback on the hypothalamus during the early stage of the estrous cycle (28). However, it is unlikely that E_2 has a direct effect on GnRH neurons, since no E_2 receptors have been discovered in the immediate proximity of the GnRH neuron (69). Perhaps, the E_2 receptors lie somewhere out away from the GnRH neuron near other neurons (i.e. noradrenergic and opioid neurons) that terminate on GnRH neurons. The noradrenergic and opioid neurons may serve as interneurons or mediators for the E_2 receptors to exert their effects on the GnRH receptors to stimulate release of LH (69).

FSH stimulates activity of the aromatase system and triggers the conversion of testosterone to E_2 through binding to its receptors on the granulosa cells (28). LH has several distinct actions on the ovary. Perhaps, the most important action of LH is to increase the synthesis of steroids by those cell types in the ovary that have LH receptors (theca interna, stroma, and granulosa cells). LH stimulates side-chain cleavage causing an increase in E_2 production in follicles. Under stimulation by LH, FSH influences E_2 production by causing the granulosa cells to convert androgens, produced in the theca cells, which are either secreted into the ovarian vein or traverse the basement membrane to the granulosa cells (28). Granulosa cells of mature follicles have high aromatase activity and convert most of the androgens produced by the theca cells to E_2 . Recruited

follicles begin producing E_2 (which enhances IGF_1 production) and low levels of inhibin (47, 53). During selection moderate levels of FSH and LH are being secreted and inhibin production is increasing (causing a negative feedback on the anterior pituitary, decreasing FSH release). Following the LH surge, the theca interna cells begin to produce P_4 , which exerts a negative feedback on the hypothalamus to reduce GnRH secretion by the tonic LH center (28, 53). High P_4 , therefore, prevents development of preovulatory follicles, E_2 production, and the preovulatory GnRH and LH surge (45, 66). Only E_2 active follicles are present at the time of the LH surge (28), and ovulatory follicles have a greater population of LH receptors on the theca and granulosa cells, which allow them to be more responsive to LH. The increased responsiveness to LH, in turn, increases the ability of the follicle to produce E_2 , while, at the same time, decreasing the FSH receptor population (53).

In summary, hormonal regulation of the reproductive processes is mainly confined to the hypothalamic-anterior pituitary-gonadal axis. Releasing hormones from the hypothalamus control the function of the anterior pituitary. For example, GnRH stimulates LH and FSH synthesis and secretion. Gonadotropins from the anterior pituitary, in turn, control the function of the ovary and influence steroid synthesis within the ovary. Through feedback mechanisms, both positive and negative, involving the hypothalamus, steroid hormones and proteins from the ovary regulate the release of LH and FSH.

Pattern of follicular development during the estrous cycle of the cow

The female reproductive system of the bovine exhibits a rhythmical change called the estrous cycle. A highlight of this cycle is the period of estrus, when the female is receptive to the male (55). It has been determined that the length of the estrous cycle in the cow normally lies between 18 to 25 d (42, 55). In cattle, behavioral changes signal the onset of estrus; however, hormone levels also play a role in both physical and physiological changes during the estrous cycle. Estrus may be characterized by sexual receptivity and a combination of peak E_2 secretion and disappearance of P_4 . In addition to standing to be mounted by a bull or herd mates, a display of behavioral characteristics of estrus may include redness and swelling of reproductive tissues, increased locomotion, vocal expression, nervousness, and attempts to mount other herd mates (58).

The estrous cycle of a cow can be divided into two phases: the follicular and luteal phases. The follicular phase of the estrous cycle, the period of regression of the corpus luteum (CL) to ovulation, can be further divided into three stages: recruitment, selection, and dominance (30, 39). There are numerous factors that influence the growth and development of oocytes, and at the same time, these factors influence the suppression of growth of other oocytes (28, 39, 53). The oocytes continuously begin to grow until the primordial reserve is exhausted. When any follicle is released from this reserve, it will continue to grow until it ovulates or becomes atretic, or E_2 inactive, and dies (28), which is the case for over 90% of all follicles (26, 60). The follicle is the environment for oocyte maturation and its two main functions are ovulation of a mature

oocyte for potential fertilization and supply of E₂ for the onset of estrus (58).

A general belief is that the initial stages of folliculogenesis occur without influence of gonadotropic hormones (21, 28, 53). During this time the oocyte increases in size and activity, which includes production of RNA and ribosomes. Follicular growth depicts a period of subcellular and molecular transformations of the granulosa and theca cells, as well as the oocyte. As the follicle grows the theca layer differentiates from surrounding connective tissue into the externa and interna tissue layers. The granulosa cells produce a glycoproteinaceous substance that forms the zona pellucida, which surrounds the oocyte (28). Granulosa cells maintain contact with each other and with oocytes by cytoplasmic processes that form gap junctions with the oocyte (27). These gap junctions are important for communication between the granulosa cells since they lack a direct blood supply, and also serve as a barrier limiting diffusion of materials into the oocyte (58).

FSH stimulates follicular development, but other factors can also mediate development (26, 53). In cows, elevated GnRH secretion is necessary for initiating the follicular phase of the estrous cycle (67). Removal of the negative feedback of P₄ (secretion from the ovary decreases) on the hypothalamus, allows an increase in GnRH pulse frequency and amplitude which, in turn, facilitates the release of LH and FSH from the anterior pituitary and initiates follicular development (28, 70). The increased secretion of gonadotropins from the pituitary, 1) causes an increase in inhibin secretion which blocks FSH release, and 2) stimulates the ovary to secrete E₂, which positively

feeds back on the hypothalamic nuclei in the preovulatory surge center. It is important to mention that specific GnRH receptors have not been detected on the bovine ovary (8). Therefore, it is commonly accepted that GnRH and its agonists act on the follicle indirectly through the induced secretion of LH and FSH from the anterior pituitary (13).

FSH influences E_2 production by causing the granulosa cells to convert androgens, produced in the theca cells under the influence of LH, to E_2 (28). FSH also induces its own receptors, which allows the follicle to become more responsive to the tonic release of FSH. Estrogens cause growth and cellular division of the granulosa and induce additional FSH receptors in the granulosa. As the granulosa cells begin to develop under FSH and E_2 influence, the antrum begins to form (28). Large antral follicles produce E_2 , which is responsible for accumulation of fluid. This follicular fluid comes from the peripheral plasma by transudation across the follicle basement lamina and accumulates in the antrum formed by the coalescence of small pockets of fluid. The fluid contains several compounds of major physiologic significance. In large follicles, the follicular fluid contains $E_2-17\beta$ during the follicular phase and minute quantities of P_4 as ovulation approaches.

It is important to remember that the follicular phase begins after luteolysis. Only E_2 active follicles are present at the time of the LH surge, and ovulatory follicles have a greater population of LH receptors on the theca and granulosa cells, which allow them to be more responsive to LH. The increased responsiveness to LH, in turn, increases the ability of the follicle to produce E_2 , while, at the same time, decreasing the FSH receptor

population. The largest, or dominant follicle, is responsible for most E_2 secretion by the ovary at estrus. However, this E_2 secretion rapidly decreases when the LH surge occurs prior to ovulation.

Both FSH and LH initiate growth and death of antral follicles (39). Antral follicles develop in response to tonic FSH and LH secretion and are always present on the ovary after puberty. The antral follicles may be classified by their size according to diameter. The dynamics of antral follicles consist of three major events: recruitment, selection, and dominance that are thought to be mediated by compounds in the follicular fluid, such as inhibin, activin, IGF₁ and their respective binding proteins (21). Small antral follicles are recruited, selected, and either become atretic or develop into dominant follicles that ovulate (28, 39, 53).

Recruitment is a gonadotropin dependent event during which a group of follicles from primordial pools begin to grow and produce E_2 (47, 53, 66). Some of the recruited follicles will become atretic and die if the proper endocrine conditions are not provided, but the remaining viable follicles will be selected (32, 39). These remaining viable follicles may then either become dominant or undergo atresia. It is important to remember that atresia occurs continuously throughout folliculogenesis. As the selected follicles move towards dominance, they continue to produce increasing amounts of E_2 and inhibin (47, 53). Inhibin is a protein hormone produced by the follicle that selectively blocks the release of FSH from the anterior pituitary. In the cow, several follicles are selected for dominance, however, only one will actually become the dominant follicle (39, 66). It is possible that larger follicles inhibit the growth and

development of their smaller recruited and selected cohorts through inhibin production and diversion of blood supply to the larger follicles (28, 53). Atresia occurs far more often than dominance and for a combination of reasons. Reduced blood supply and suppressed FSH concentrations contribute to the initiation of follicular atresia. Findlay (21), suggested that a feedback mechanism involving E_2 , inhibin, and FSH is involved with local regulators in determining the final number of selected antral follicles that become dominant and ovulate. In summary, hormonal control during the three stages of the follicular phase is as follows: during recruitment FSH is high to induce recruitment of follicles from the gonadotropin sensitive pool, LH is low, and no inhibin is being secreted. Recruited follicles begin producing E_2 (which enhances IGF_1 production) and low levels of inhibin (47, 53). During selection, moderate levels of FSH and LH are being secreted and inhibin production is increasing (causing a negative feedback on the anterior pituitary, decreasing FSH release). The large follicles have more FSH receptors, meaning they can still respond or grow with the small amount of FSH that is still present. Finally, during dominance, LH (for development of the dominant follicle) and inhibin are high while FSH is low, causing recruitment of other follicles to stop.

Cows experience anywhere from one to three follicular waves. For each of these waves follicles are recruited and selected (26, 39). However, only the follicle recruited after luteolysis will become eligible for ovulation due to a decline in P_4 . For example, in a cow with three follicular waves, dominant follicles produced during the first or second follicular wave will become atretic because of increasing or peak P_4 levels (26) since follicles cannot ovulate under P_4 influence. Differences exist between follicles that

become dominant or subordinate during the first wave of the estrous cycle in the cow (3, 53).

Automated detection of estrus

Proper estrus detection and timing of insemination play vital roles in improving a cow's reproductive efficiency. Foote (23) stated that accurate estrus detection is the key to efficient reproduction and optimal milk production. While improper estrus detection may be a major limitation for dairy cattle, management systems which emphasize observation of estrus and development of techniques for detection of estrus could help overcome the problem of missed periods of estrus (76). Technology utilized by the HeatWatch[®] electronic estrus detection system is known as radio frequency data communication and provides the user with the ability to use mounting activity to determine estrus.

Incorporation of the HeatWatch[®] system into reproductive management programs offers the potential for increasing reproductive efficiency because the technology employs 24-h surveillance 7 d per wk, in addition to consistency, which is essential to any reproductive program. The continuous surveillance of estrus activity should increase the detection of estrus and, thereby, decrease days open. The substantial and precise amount of mounting activity data when coupled with the HeatWatch[®] software management system provides the user with a diagnostic tool to observe and interpret the estrous cycles of normally cycling and problem cows.

Studies utilizing the HeatWatch[®] system have evaluated timing of ovulation (73)

and optimal insemination (17) relative to the onset of estrus, as determined by standing activity. The average timing of ovulation relative to the first standing event associated with estrus was 27.6 ± 5.5 h and did not differ for spontaneously occurring estrus or estrus induced by $\text{PGF}_{2\alpha}$ (73). Optimal timing of AI was determined from a large field trial utilizing 2661 services in conjunction with HeatWatch[®]. Results indicated that the highest conception rates for AI occurred between 4 and 12 h after the onset of standing activity. Additionally, it was determined that the period of standing estrus in lactating cows lasts for 7.1 ± 5.4 h with an average of 8.5 ± 6.6 standing events (17). Research indicates that animals in close confinement show more signs of estrus during night hours than in the daytime hours, therefore, contributing to the high incidence of undetected estrus (18). It is not known whether this higher incidence of nighttime estrus expression is due to the absence of other distractions or to an inherent rhythm favoring night (75). However, A recent field study concluded that the onset of estrus distribution (n=2661 periods) was not different during a.m. versus p.m. hours (17).

Estrus synchronization with $\text{PGF}_{2\alpha}$

Synchronization of lactating dairy cows primarily has been limited to the use of $\text{PGF}_{2\alpha}$ or its analogs to regulate the luteal phase of the estrous cycle (14, 15, 22, 24, 35, 36, 37, 48). Prostaglandin $\text{F}_{2\alpha}$ generally causes regression or lysis of the CL, which is an irreversible degradation characterized by a decrease in P_4 concentration in the blood. It

is widely accepted, that $\text{PGF}_{2\alpha}$ (from the endometrium) and oxytocin (from the CL) are the two main hormones that control luteolysis. Communication between the CL and the uterus are necessary to induce a successful luteolysis. Subsequent expression of estrus occurs after lysis of the CL, followed by ovulation within 2 to 5 d after its administration (19, 22). Therefore, the success of estrus induction with $\text{PGF}_{2\alpha}$ depends upon the presence of a functional CL during the diestrus stage of the estrous cycle (35).

When $\text{PGF}_{2\alpha}$ is used as a luteolytic agent for a group of cows, subsequent detection of estrus is enhanced because many cows are in estrus simultaneously (31). Unfortunately, estrus is not precisely synchronized because the follicular population varies at the time of luteal regression; thus, some cows take longer to mature a dominant follicle for ovulation. If cows selected for the $\text{PGF}_{2\alpha}$ protocol were in diestrus, 90 to 95% of the cows should be in estrus by 7 d after injection, and 70 to 90% of these estruses should occur on d 3 to d 5 after $\text{PGF}_{2\alpha}$ administration (19).

Use of $\text{PGF}_{2\alpha}$ has benefits beyond the induction of estrus in individual cows. Scheduled use of $\text{PGF}_{2\alpha}$ can synchronize estrus among cows, resulting in less time spent on estrus detection and fewer veterinary visits for pregnancy examinations (19). Fourteen day $\text{PGF}_{2\alpha}$, an aggressive, proactive program, involves administration of $\text{PGF}_{2\alpha}$ before the end of the VWP, combined with subsequent $\text{PGF}_{2\alpha}$ administrations given at 14 d intervals until detection of estrus and insemination or AI timed at 80 h following the third $\text{PGF}_{2\alpha}$ administration.

In general, $\text{PGF}_{2\alpha}$ and its analogues are not luteolytic when administered in the

early stage of the estrous cycle (37). The 14-d $\text{PGF}_{2\alpha}$ protocol was designed to initiate a stage of the cycle that contains a mid-cycle CL, when cows are most responsive to the second administration of $\text{PGF}_{2\alpha}$ 14 d later (48). A study conducted in beef cows showed that cows administered $\text{PGF}_{2\alpha}$ during mid-cycle (d 10 to 15) had a higher estrous response than cow given $\text{PGF}_{2\alpha}$ between d 5 and 9 of the estrous cycle (34).

The VWP is designed to allow involution of the cervix and uterus after calving. The reproductive tract typically returns to normal size by 35 d after calving, while the uterus takes approximately 45 d to return to normal. The VWP may be intentionally extended in an attempt to alleviate the occurrence of lower conception rates in high producing, early postpartum cows. In addition, maintaining profitable levels of milk production for longer lactation cycles will allow for later, and possibly, more efficient postpartum AI.

Once a VWP has been established for a herd, cows are usually listed chronologically on dairy management lists according to calving dates. Cows within 14 d of the end of the VWP are given the setup $\text{PGF}_{2\alpha}$ administration, and, for convenience, injections are usually administered once a week for all cows that surpassed the specified VWP. Fourteen days later, cows receive the first breeding administration of $\text{PGF}_{2\alpha}$ and are observed for estrus and AI. Cows that are not observed in estrus are given additional $\text{PGF}_{2\alpha}$ 14 d later, and the cycle continues; $\text{PGF}_{2\alpha}$ is then administered at 14-d intervals until estrus is exhibited and detected. The 14-d $\text{PGF}_{2\alpha}$ protocol contains a fixed-time AI at 80 h after the third $\text{PGF}_{2\alpha}$ administration. It should be noted, however, that the

interval to estrus after $\text{PGF}_{2\alpha}$ is shorter for heifers than for cows (34), and timed AI at 80 h after the second $\text{PGF}_{2\alpha}$ may be too late, leading to a decrease in potential conception rates (6).

From controlled field studies researchers have questioned the dosage (5, 24) and interval (22) of the $\text{PGF}_{2\alpha}$ protocol. The most widely recommended dosage to synchronize estrus adequately has historically been 25 mg (Lutalyse[®], Pharmacia-Upjohn, Kalamazoo, MI). However, a reduction in the dose of $\text{PGF}_{2\alpha}$ from 25 to 17.5 mg does not affect estrus response or pregnancy rate in dairy cows (24). The stage of the estrous cycle, and presumably the stage of the functional CL, affects the efficacy of a reduced dosage of $\text{PGF}_{2\alpha}$ to induce luteolysis (5).

The 11 and 14-d $\text{PGF}_{2\alpha}$ programs are modifications of the original 7-d program used when $\text{PGF}_{2\alpha}$ was introduced in the 1970s for synchronization of estrus in cattle. A potential disadvantage of the 11-d program is that repeat administrations and subsequent estrus detection and AI do not occur on similar days of subsequent weeks. A study (22) on the length of the interval (11 or 14 d) between $\text{PGF}_{2\alpha}$ administration reported that more ($P < 0.05$) primiparous cows conceived within 30 d of first AI when they received $\text{PGF}_{2\alpha}$ at 14-d intervals than when they received $\text{PGF}_{2\alpha}$ at 11-d intervals (84% vs. 64%, respectively). It was suggested that the increase in conception rates might be attributed to higher plasma P_4 (54), and the positive relationship that exists between plasma P_4 and conception. Because cows must be in the luteal phase of the estrous cycle (between d 7 to 17) to respond to $\text{PGF}_{2\alpha}$, 11 or 14-d intervals have been recommended over 7-d

intervals (19, 22, 64).

A comparison of protocols for set interval PGF_{2α} when PGF_{2α} was administered to open cows after a CL was identified by rectal palpation suggested that cows receiving weekly doses of PGF_{2α} on the set interval protocol had a 30% higher conception rate than did cows that received PGF_{2α} based on rectal palpation (35). Based on results of this study, a set interval PGF_{2α} protocol decreased days open because of a decrease in days to first AI. Administration of PGF_{2α} on a weekly basis enhanced earlier AI because cows that lacked a functional CL at the time of the first PGF_{2α} were administered PGF_{2α} 7 d later (35). To further validate results from studies using PGF_{2α} protocols based on rectal palpation for identification of an existing CL, on-farm milk P₄ enzyme radioimmunoassay, or based on a fixed injection schedule, Heuwieser et al. (29) conducted a simulation study utilizing seven PGF_{2α} protocols evaluated against controls. Estrus detection efficiency levels were 35, 55 and 75%. The PGF_{2α} protocol based on the best case assumptions, fixed-time injections and superior estrus detection efficiency, had the greatest influence on reproductive performance versus PGF_{2α} protocols based on rectal palpation or enzyme radioimmunoassay. After 12 mo, the fixed-time PGF_{2α} schedule, at 75% estrus detection efficiency, reduced days open by 31 d and days to first AI by 15 d.

Dailey et al. (14, 15) conducted experiments similar to those using 14-d PGF₂; however, E₂ benzoate was added to the protocol 40 to 48 h after PGF_{2α} administration. Synchrony of estrus in cows with high P₄ concentrations was higher (90.3%) with E₂ treatment than with PGF_{2α} alone (60.0%). Similar results were obtained for dairy heifers

using the same protocol (15).

Some estrus synchronization studies utilizing three PGF_{2α} injections given at 11 or 14-d intervals also measured P₄ concentrations in milk (14) or plasma (22, 40, 62) of cows receiving PGF_{2α}. A positive relationship exists between concentrations of P₄ and conception rates, indicating that conception rates of cows given PGF_{2α} depend on the plasma P₄ concentrations that are reached during the days preceding luteolysis (22). Therefore, in a PGF_{2α} protocol for estrus synchronization, the conception rate of any given group of cows depends on the proportion within the group of cows with high P₄ concentrations and the proportion of cows with low concentrations. Cows with plasma P₄ concentrations <5 ng/ml 2 d prior to the second PGF_{2α} administration had lower conception rates (36%) than did those cows with P₄ concentrations >5 ng/ml (75%) (22). Using a PGF_{2α} protocol, cows with milk P₄ concentrations ≥8 ng/ml had a higher rate of synchronization than did those with concentrations <8 ng/ml (14). Stevenson et al. (64) experimented with exogenous P₄ in conjunction with PGF_{2α} prior to AI. Prebreeding treatment with exogenous P₄ did not improve conception rates, but the efficiency of estrus expression increased from 54% for those cows receiving only PGF_{2α} to 71% for the cows receiving the combination of PGF_{2α} and P₄ (64). The type or dose of progestin given prior to breeding may influence conception for cows in which no functional CL is present. Smith and Stevenson (61) reported a difference in pregnancy rates ($P < 0.01$) for heifers and cows given PGF_{2α} and prebreeding (d 40 to 54) exogenous progestins, either natural (41.5%) or synthetic (26.7%).

Results of estrus synchronization using a $\text{PGF}_{2\alpha}$ protocol have varied over numerous studies. Stevenson et al. (64) reported no reduction in the length or variation of calving intervals using $\text{PGF}_{2\alpha}$; however, that study suggested that $\text{PGF}_{2\alpha}$ offered an option of prolonging the VWP without extending calving intervals. In contrast, Lucy et al. (40) found that treatments utilizing $\text{PGF}_{2\alpha}$ reduced intervals to first service, which effectively shortened calving intervals.

Xu et al. (78) conducted a $\text{PGF}_{2\alpha}$ study on five New Zealand herds using 518 control cows and 521 $\text{PGF}_{2\alpha}$ synchronized cows. Synchronized cows were given $\text{PGF}_{2\alpha}$ on a 13-d interval. Breeding did not begin until after the second $\text{PGF}_{2\alpha}$ administration. Compared with control cows, synchronization of estrus in the $\text{PGF}_{2\alpha}$ treated cows significantly reduced conception rates to first AI (61.1% versus 70.5%). However, Xu et al. (78) reported that the stage of the estrous cycle at the time of the second $\text{PGF}_{2\alpha}$ injection affected fertility because cows in the later stage of their cycle (d 14 to 19) had a higher response rate to $\text{PGF}_{2\alpha}$ than did those in the middle or early stages of their cycle.

Recently, LeBlanc et al. (38) reported that administration of GnRH 7 d prior to the second of two $\text{PGF}_{2\alpha}$ administrations 14 d apart, did not alter the expression of estrus or fertility in lactating dairy cows. Based on previous studies, GnRH was hypothesized to synchronize the follicular status at the time of luteolysis and to increase the size and E_2 secretion from the ovulatory follicle. However, the first administration of $\text{PGF}_{2\alpha}$ may have caused those cows that responded to the $\text{PGF}_{2\alpha}$ to be too early in their subsequent follicular development to respond optimally to GnRH.

Synchronization of ovulation

Use of GnRH has generally been as a therapeutic agent in reproductive management; however, recent studies have incorporated GnRH into a synchronization protocol (2, 9, 38, 40, 49, 50, 51, 52, 57, 63, 67, 70). Studies have indicated that GnRH or its agonist alters ovarian follicular development and luteal function (41, 68). In the past, GnRH has been effective for treating ovarian follicular cysts (33) and for initiating the LH surge to stimulate ovulation (71, 79). Incorporation of known uses of GnRH has led researchers to a new synchronization program (51, 77) by which GnRH administration 6 to 7 d prior to PGF_{2α} increased the percentage of cows that were synchronized and reduced the time and variability of estrus for beef cows (67) and heifers (71). It has been suggested that treatment with a combination of GnRH and PGF_{2α} is a practical method for controlling ovarian follicular and luteal functions and increases estrus synchrony in both cyclic and acyclic postpartum cows and heifers (70). A recently developed protocol, TAI, synchronizes ovulation, allowing for potentially higher conception rates to TAI than with PGF_{2α} (50, 51). The program involves administration of 100 µg of GnRH, followed by 25 mg of PGF_{2α} 7 d later and a second administration of GnRH 36 to 48 h after PGF_{2α}. The first administration of GnRH is given at a random stage of the estrous cycle, causing either luteinization or ovulation of the largest follicle (52, 68) in approximately 85% of all cows. Administration of PGF_{2α} regresses the CL or the luteinized follicle induced by GnRH. A new dominant follicle

forms and is available for ovulation by the time of the second GnRH administration, given 36 to 48 h after PGF_{2α}. The second administration of GnRH has been shown to synchronize ovulation rather than estrus.

An important difference between TAI and 14-d PGF_{2α} is the elimination of estrus detection. With TAI, cows are not observed for estrus but are inseminated at a specified time (16 to 20 h) following the second GnRH administration. A small percentage of cows may express signs of estrus prior to the PGF_{2α} injection because the first administration of GnRH failed to ovulate the dominant follicle or the follicle is not sufficiently mature to ovulate. Cows expressing estrus just prior to or just after PGF_{2α} administration tend to be mid-cycle (d 13 to 15), or they may be late-cycling cows that are returning to estrus for the first time after parturition.

A recent study compared 14-d PGF_{2α} with TAI (50). One group of nulliparous and multiparous dairy cows received the TAI protocol (Figure 1), and the other group received 25 mg of PGF_{2α} at 14-d intervals until detection of estrus and AI according to the a.m.-p.m. guidelines. Response to the TAI protocol was dramatically different between heifers and lactating cows (50). Pregnancy rates for multiparous cows were similar for both treatments: 38.9% and 37.8% for 14-d PGF_{2α} and TAI, respectively. However, heifers treated with 14-d PGF_{2α} had a much higher pregnancy rate (74.4%) than did heifers on the TAI protocol (35.1%). Serum P₄ concentrations were evaluated at each administration of GnRH and PGF_{2α} for both nulliparous and multiparous cows, and results indicated that heifers did not respond to synchronization of luteal function by

exogenous GnRH, possibly because of inconsistent follicular wave patterns (50). Therefore, TAI was not effective for heifers because of lack of synchrony, but lactating cows that were synchronized with the GnRH-PGF_{2α}-GnRH combination had more precise estrus synchronization than did cows treated with a single PGF_{2α} administration (77).

To determine the optimal time of AI relative to ovulation, 732 lactating dairy cows were randomly assigned to five treatment groups by stage of lactation and parity (49). Cows were synchronized using the TAI protocol and inseminated at 0, 8, 16, 24, or 32 h after the second administration of GnRH. Pregnancy rates were similar for cows inseminated at 0, 8, 16, and 24 h, 37%, 41%, 45%, and 41%, respectively, and lower for cows inseminated at 32 h (32%, $P < 0.05$). Similarly, calving rates were similar among the groups inseminated at 0, 8, 16, and 24 h and lower ($P < 0.05$) for cows in the 32 h group, 31% at 0 and 8 h, 21% at 16 and 24 h, and 20% at 32 h. Highest pregnancy rates per AI were obtained when AI occurred at 8, 16, and 24 h after the second GnRH administration, however, pregnancy rates and calving rates were low across all groups.

It has also been suggested that stage of lactation of multiparous cows affects pregnancy rates. Pregnancy rates per AI were evaluated for different parities and various stages of lactation (49). Second lactation cows bred between 76 and 100 d postpartum had higher pregnancy rates, 70%, in comparison with cows in lactations 1 or ≥ 3 . It should be noted, however, that only a small group of cows (n=23) were represented in the second lactation group for 76 to 100 d postpartum. Additionally, during the same

period, the overall sample consisted of only 96 cows, compared to 428 cows in the 50 to 75 d group and 208 cows in the >100 d group. Pregnancy rates for cows submitted for AI in early lactation (50 to 75 d) were less ($P < 0.025$) than that of cows in later lactation (>75 d). Similarly, (50) cows >75 d postpartum had higher pregnancy rates per AI (43.3%, $P < 0.01$) than did cows that were between 60 to 75 d postpartum, 26.0%. Based on these data it can be suggested that the VWP for TAI be extended to at least 75 d postpartum to optimize pregnancy rates.

Burke et al. (9) compared the effectiveness of TAI versus AI at detection of estrus in multiparous cows using the combination of GnRH and PGF_{2α}. Conception rates were noticeably higher for cows when AI occurred after detection of estrus (41.5%) than when cows received timed AI (26.5%). However, because a lower proportion of cows were detected in estrus, pregnancy rates were similar, 30.5% after AI at detection of estrus and 29.0% for TAI. Days open were fewer ($P < 0.07$) for cows that received TAI and conceived by 120 d postpartum than for cows receiving AI at detected estrus (79.2 d and 83.6 d, respectively). The number of days to first AI, with a VWP of 60 d, was 9.7 d fewer for multiparous cow receiving TAI than for cows receiving AI after estrus detection (9). A similar study (52) also showed a mean reduction of 27 d to first AI using the combination of GnRH and PGF_{2α} and a 50-d VWP. Overall, conception rates were more consistent for cows in the TAI group from month to month; dramatic fluctuations in these rates occurred for cows receiving AI at detected estrus (9). The use of GnRH-PGF_{2α} protocols has increased pregnancy rates by increasing the number of

cows receiving AI; however, at the same time, conception rates have not shown the same increase in efficiency (9, 40, 50, 51).

The TAI protocol had never been tested in the summer months when estrus detection and pregnancy rates are often low due to heat stress. Therefore, de la Sota et al. (16) evaluated a TAI protocol against a control group of lactating dairy cows during summer heat stress. Control cows were administered $\text{PGF}_{2\alpha}$ on day 60 postpartum and AI at detection of estrus. Conception rates were lower ($P < 0.05$) for the TAI group, 13.2%, than for the control estrus detection group, 22.9%. However, pregnancy rates were higher ($P < 0.05$) for the TAI group, 13.9%, versus 4.8% for the estrus detection group. It was suggested that although TAI did not protect the embryo from heat stress induced embryonic death, limitations on detection of estrus induced by heat stress were eliminated. The TAI protocol could have exerted a stimulatory effect on the subsequent fertility of treated cows because the advantage of a higher pregnancy rate at the initial TAI was maintained throughout the 365-d postpartum period. Furthermore, Aréchiga et al. (2) evaluated a TAI program during periods of heat stress on two commercial dairy herds in northern Florida. Cows were inseminated at each observed estrus after 50 or 70 d or were included in a TAI program consisting of 8 μg of a GnRH agonist (Receptal[®], Hoechst-Roussel Agri-Vet Co., Somerville, NJ) followed by 25 mg of $\text{PGF}_{2\alpha}$ (Lutalyse[®], Pharmacia-Upjohn Kalamazoo, MI) 7 d later and 8 μg of a GnRH agonist 2 d later. Insemination occurred 17 to 25 h after the last GnRH agonist administration. Pregnancy rates for first AI were similar for cows inseminated after visual observation of estrus and

cows that received TAI. However, the percentage of cows pregnant by 90 d postpartum was greater for cows in the TAI group, 11% versus 20%, respectively. Thus, an enhancement of fertility with the TAI protocol was evident from data obtained from subsequent inseminations in the studies conducted by de la Sota et al. (16) and Aréchiga et al. (2).

Vasconcelos et al. (72) conducted a study utilizing 354 lactating dairy cows to determine if conception rates with TAI were influenced by day of the estrous cycle at initiation of hormone treatment. Cows in mid-cycle (d 5 to 12) had higher conception rates (41%, $P < 0.05$) than cows in either early or late stages of the cycle (32%). Cows beginning the TAI protocol in the early stage of the cycle had a high rate of synchronization after the second GnRH administration (94%), but, in contrast, had the lowest conception rate (28%). It was postulated that the presence of a large persistent follicle may be the cause for the low conception response.

MATERIALS AND METHODS

The study population consisted of 734 primiparous and multiparous lactating Holstein cows from 16 commercial dairy herds in Virginia. Herds were selected from Holstein dairy herds participating in the Virginia Dairy Herd Improvement program, with records processed by the Dairy Records Management System (Raleigh, North Carolina). Herds were divided into 8 visual observation herds using visual observation to identify signs of estrus behavior and 8 herds relying on the HeatWatch[®] electronic estrus detection system for detection of estrus. Selection of herds utilizing visual observation for detection of estrus was based on the following: 1) above state average for milk yield; 2) above state average for estrus detection efficiency; and 3) geographically located near HeatWatch[®] herds. In addition, all selected herds agreed to follow instructions on assignment of cows to various protocols and to consult with researchers on monthly visits concerning assignment of cows, hormone treatments, and other study-related areas. The 8 HeatWatch[®] herds were selected from a pool of 32 herds in Virginia which utilize the HeatWatch[®] system. All HeatWatch[®] herds were loaned 15 transmitters for the duration of the study because most herds did not have the 60 transmitters necessary to conduct this study.

Prior to beginning the study, rolling herd averages for the 16 herds ranged from 8096 to 12264 kg per year (Table 1). Virginia DHI estrus detection rate in August 1997, was 40% and the rolling herd average for milk yield prior to initiation of this study was 8532 kg. Estrus detection rates for the 16 herds were above the state average for all

TABLE 1. Profile of herds prior to initiation of research trial.

Herd	Estrus detection ^a	Cows	Days open	Days to first service	EDR ^b (%)	Milk yield ^c (kg)
1	HW	150	166	151	100	9101
2	HW	205	159	110	94	9789
3	HW	164	132	105	78	9570
4	HW	101	151	104	27	8455
5	HW	122	139	92	40	12264
6	HW	150	129	92	68	9302
7	HW	203	188	142	64	10680
8	HW	135	162	125	74	8096
\bar{x}	<i>HW</i>	<i>154</i>	<i>153</i>	<i>115</i>	<i>68</i>	<i>8600</i>
9	VO	201	137	92	51	9892
10	VO	83	151	126	84	10116
11	VO	77	128	89	43	12572
12	VO	116	116	89	70	9519
13	VO	130	155	86	43	10207
14	VO	152	136	101	90	9890
15	VO	85	132	105	47	10729
16	VO	177	115	72	54	9435
\bar{x}	<i>VO</i>	<i>128</i>	<i>134</i>	<i>95</i>	<i>60</i>	<i>10295</i>
\bar{x}	<i>All herds</i>	<i>141</i>	<i>144</i>	<i>105</i>	<i>64</i>	<i>9448</i>
VA State Average ^d		127	149	98	40	8532

^a Method of estrus detection was either visual observation (VO) or was determined by the HeatWatch[®] (HW) electronic estrus detection system (DDx, Inc., Denver, CO).

^b EDR = estrus detection rate, (%), calculated using the equation [(services per conception - 1) / {(average days open - days to first service) / 21} (100)] from Fetrow et al. (20).

^c Dairy Herd Improvement Association rolling herd averages for test day closest to initiation of the study.

^d Virginia Dairy Herd Improvement Association rolling herd averages for August 1997.

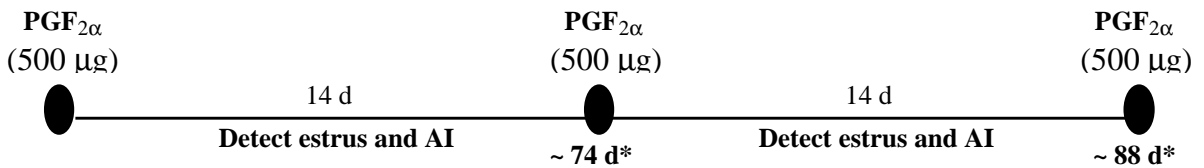
herds, 28% above for HeatWatch[®] herds and 20% above state average for visual observation herds. In addition, HeatWatch[®] herds were 68 kg above the state average for milk yield, while visual observation herds were 1766 kg above the Virginia State average.

Individual information obtained for all treated and untreated control cows in each herd included calving date, test day milk yield closest to AI, and lactation number. Cow data were obtained through the Personal Computer Direct Access to Records by Telephone (PCDART) software program. Herd information for the 16 participating herds was downloaded monthly, and a custom report was constructed with PCDART software to select only cows less than 60 d postpartum that had not yet been inseminated during the current lactation. At parturition, 60 cows (15 cows per protocol) were assigned randomly within each herd to one of the four reproductive management protocols, 14-d PGF_{2α}, TAI, GnRH-PGF_{2α}, or an untreated control group, (Figure 1) utilizing the routine method for detection of estrus used by that herd (visual or HeatWatch[®]). All cows calving during the study period in participating herds had equal opportunity for inclusion in the study; however, cows identified by the herd manager as ineligible for breeding were excluded. Criteria for ineligibility was herd specific and generally based on difficulties during the previous lactation.

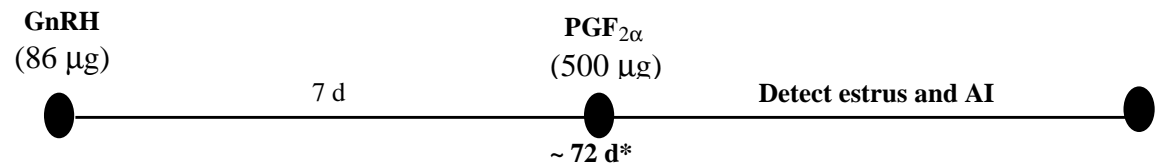
The 14-d PGF_{2α} protocol was initiated on the first Monday after 60 d postpartum, and consisted of a maximum of three, 500 µg, i. m. administrations of PGF_{2α} (Estrumate, Bayer, Shawnee Mission, KS), 14 d apart. Cows were observed for estrus and

Control (Detection of estrus and AI by normally practiced methods for each herd, beginning the first Monday past 60 d postpartum)

14-d PGF_{2α} Protocol (First Monday past 60 d postpartum)



GnRH-PGF_{2α} Protocol (First Monday past 65 postpartum)



TAI Protocol (First Monday past 65 postpartum)

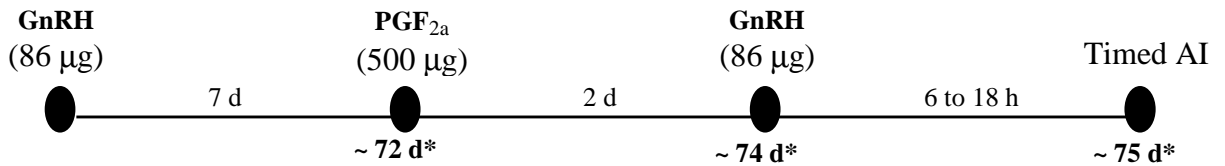


Figure 1. Description of protocols, identifying timing of injections, used to conduct the systematic breeding study comparing 14-d PGF_{2α}, GnRH-PGF_{2α}, and TAI against an untreated control group.

*Depict approximate number of days postpartum.

inseminated according to the a.m.-p.m. guideline for visual observation herds, or within 14 h after the onset of estrus for herds utilizing the HeatWatch[®] system. Only cows that were not detected in estrus after PGF_{2α} administration were given subsequent administrations of PGF_{2α} but no cow received more than 3 doses. Estrus detection for the visual observation herds was conducted according to the existing program for each individual herd.

The TAI protocol was initiated the first Monday after 65 d postpartum. The initial hormone administration consisted of 86 µg of GnRH i. m. (Fertagyl, Intervet, Millsboro, DE). Seven days later, 500 µg of PGF_{2α} was administered, followed by a second 86 µg GnRH administration 2 d later. All cows in the TAI group were submitted for AI 6 to 18 h after the second administration of GnRH and were not inseminated according to observation of estrus (Figure 1).

The GnRH-PGF_{2α} protocol was designed in the same manner as TAI; however, the second administration of GnRH was eliminated and cows were observed for estrus after PGF_{2α} administration and inseminated accordingly. Only TAI cows inseminated between 73 and 85 d postpartum were included in the analysis because anything outside this time period were not inseminated according to protocol. Additionally, cows in the 14-d PGF_{2α}, and GnRH-PGF_{2α} groups were eliminated if AI did not occur within 10 d following PGF_{2α} administration.

Cows selected for the control group were either visually observed or monitored for estrus with HeatWatch[®]. HeatWatch[®] transmitters were applied the first Monday

after 60 d postpartum. The HeatWatch[®] software default was set to recognize a "standing" estrus as three standing events, lasting ≥ 2 s each, within a 4-h period. However, the inseminator made the final determination of estrus and decision to AI. Service sires were selected at the discretion of the herd manager, and AI was performed according to individual herd procedures. Additionally, all hormones were administered by the herd manager or herd personnel.

Pregnancy diagnosis was usually determined between 35 and 55 d of gestation by transrectal uterine palpation, during monthly or bi-monthly herd practitioner visits. Cows that returned to estrus following AI did not receive subsequent treatment. However, second and third services up to 120 d postpartum were recorded for all cows diagnosed open following the first insemination. Additionally, cows that expressed estrus prior to examination for pregnancy following the first AI, were considered open and inseminated at the discretion of the herd manager.

HeatWatch[®] System

Components of the HeatWatch[®] system included a miniaturized pressure sensitive radio transmitter, powered by a replaceable 3 V battery and secured in a water-resistant pouch attached to a 35 x 20 cm nylon mesh patch. The patch was glued directly to the hair and hide of the sacral region of the cow using a contact-type adhesive. Activation of the pressure sensor, emitted a radio transmission to a signal receiver (0.4-km range), which was hard-wired to a data buffer unit. The buffer received and stored

standing activity data and formatted it for acceptance by the on-farm microcomputer when downloaded using HeatWatch[®] software. The HeatWatch[®] system software integrated data which was received from standing activity into management lists which included standing heat, suspect heat, brief cycles, inactivity, and non-return to estrus. In addition to these lists, a mount data log compiled information on the date, time of day, duration of individual standing events, and kept a running log of standing events for the entire herd based on data obtained from individual cows.

Data Management and Statistical Analysis

Cow identification number, date and time of estrus onset, and time of AI were recorded by inseminator in a logbook provided by the researchers and later entered into a computer spreadsheet. All HeatWatch[®] information was downloaded at each farm by the researchers. This allowed for verification of estrus activity for each individual cow and further characterization of onset and termination of estrus, as well as number of standing events and estrus duration. Estrus duration was defined as the interval (h) from first to last standing event as recorded by HeatWatch[®] software. In addition HeatWatch[®] information was used to analyze estrus intensity, defined as number of standing events per hour. Estrus intensity and duration were further classified into four groups: low intensity (< 1.5 mounts per hour) and short duration (< 7 h), low intensity and long duration (> 7 h), high intensity (> 1.5 mounts per hour) and short duration, and high intensity and long duration. Onset of estrus was classified into four 6-h increments:

0001 h to 0600 h, 0601 h to 1200 h, 1201 h to 1800 h, and 1801 to 0000 h.

Preliminary models characterizing conception rates included effects of herd, method of estrus detection, synchronization protocol, parity, and production variables. Test day milk yield was recorded at the test day closest to insemination in order to observe the effect of milk production on fertility. Statistical analyses were conducted using the general linear model procedures in SAS[®] (56).

Preliminary analyses were conducted to evaluate relationships and associations between independent variables and conception rates, days to first AI, and estrus characteristics including estrus duration, estrus intensity, and number of standing events for each protocol. The final model used for characterizing conception rates included effects of herd, method of estrus detection, synchronization protocol, and interactions between protocol and method and protocol and herd within method. The error term for testing the effects of method of estrus detection on conception rate, days to first AI, and estrus characteristics was herd within method. In addition the error term, protocol by herd within method, was used to test the effects of protocol and protocol by method interaction. The final statistical model used for characterizing conception rates and days to first insemination was:

$$Y_{ijkl} = \mu + P_i + M_j + H_{(j)k} + (PM)_{ij} + (PH)_{(j)ik} + e_{ijkl}$$

where:

$$Y = \begin{array}{l} \text{pregnancy outcome for an individual cow diagnosed 35 to 55 d post} \\ \text{AI (0,1)} \end{array}$$

- μ = overall mean
- P_i = the effect of the i^{th} protocol ($i = 1, 2, 3, 4$)
- M_j = the effect of the j^{th} method of estrus detection ($j = 0, 1$)
- $H_{(j)k}$ = the effect of the k^{th} herd within the j^{th} method of estrus detection ($k = 1, 2, 3, \dots, 16$)
- $(PM)_{ij}$ = the effect of the i^{th} protocol and j^{th} method of estrus detection
- $(PH)_{(j)ik}$ = the effect of the i^{th} protocol and k^{th} herd within j^{th} method
- e_{ijkl} = random error

The final model used to evaluate pregnancy rates for first, second and third inseminations was:

$$Y_{ijkl} = \mu + P_i + M_j + H_{(j)k} + (PM)_{ij} + e_{ijk}$$

The preliminary model for analysis of the 14-d PGF_{2 α} protocol included effects of number of PGF_{2 α} administrations, method of estrus detection, herd within method and number of PGF_{2 α} administrations by method of estrus detection interaction. Nonorthogonal contrasts were conducted to test for differences between the number of PGF_{2 α} administrations, and the error term used in this analysis was herd within method. The final statistical model used to characterize conception rates by the number of PGF_{2 α} administrations was as follows:

$$Y_{ijkl} = \mu + A_i + M_j + H_{(j)k} + (AM)_{ij} + (AH)_{(j)ik} + e_{ijklm}$$

where:

Y = pregnancy outcome for an individual cow diagnosed 35 to 55 d post AI (0,1)

μ = overall mean

A_i = the effect of the i^{th} PGF_{2 α} administration ($i = 1, 2, 3$)

M_j = the effect of the j^{th} method of estrus detection ($j = 0, 1$)

$H_{(j)k}$ = the effect of the k^{th} herd within the j^{th} method of estrus detection ($k = 1, 2, 3, \dots, 16$)

$(AM)_{ij}$ = the effect of the i^{th} PGF_{2 α} administration and the j^{th} method of estrus detection

$(AH)_{(j)ik}$ = the effect of the i^{th} PGF_{2 α} administration and k^{th} herd within j^{th} method

e_{ijklm} = random error

Economic analysis

The 16 commercial dairy herds participating in the present study were surveyed to establish average drug costs for PGF_{2 α} and GnRH and their respective analogues. Cost for labor associated with estrus detection was also calculated. Herd managers were each asked four questions ([Appendix, Figure 4](#)) which were used to establish how much time per day was devoted to estrus detection, average wage per hour paid for detection of estrus, and drug costs associated with PGF_{2 α} and GnRH.

RESULTS AND DISCUSSION

To the knowledge of the researchers, no large, multi-herd field study utilizing commercial dairy operations has compared 14-d PGF_{2α}, GnRH-PGF_{2α}, and TAI protocols to an untreated control group. Therefore, the objective of this study was to evaluate three dairy synchronization protocols versus the standard breeding program adopted by most herds, which consisted of visual or electronic detection of estrus followed by AI. In addition, this was the first study to evaluate estrus characteristics in conjunction with systematic breeding programs. The idea to conduct such a study was brought about by recent literature which attempted to validate a protocol that is believed to synchronize the time of ovulation (3, 49, 50, 51, 52, 57) and involves AI without detection of estrus.

Inseminating cows without observing them in estrus is a new experience for producers because it contradicts what they have read, learned, and practiced. Therefore, the researchers conducting the present study believed it was necessary to evaluate a well-established protocol, 14-d PGF_{2α}, against a relatively new protocol, TAI, and a modification of the TAI protocol, GnRH-PGF_{2α}, as well as an untreated control group. A combined evaluation of these protocols enabled observation of the effects on first AI conception rates, interval from parturition to first AI, cumulative pregnancy rates, and estrus characteristics associated with hormonally induced estrus periods

Sixteen commercial dairy herds were used to collect data from 734 lactating dairy cows. Herds were grouped according to estrus detection method; 8 visual observation herds and 8 herds relying on the HeatWatch[®] electronic estrus detection system

(individual farm results are shown in [Appendix, Table 13](#)). All analyses were conducted comparing each protocol within the two methods of estrus detection. However, when no difference ($P > 0.05$) was detected between methods of estrus detection, all herds were grouped regardless of estrus detection method and results were reported accordingly. All cows with first insemination occurring prior to 60 d postpartum or beyond 120 d postpartum were removed from analyses.

Effects of synchronization on reproductive performance

Preliminary analyses were conducted to evaluate the effects of parity and milk yield on days to first AI and pregnancy results for all protocols. It was determined that neither parity nor milk yield had a significant influence on conception rates or the interval from parturition to first postpartum AI. Therefore, these variables were removed from the final models.

The VWP for all protocols was set to target 75 d postpartum as the average days to first AI. Mean intervals from parturition to first postpartum AI are summarized in Table 2. The untreated control protocol had the longest interval to first AI, 80.9 d, probably because no hormonal intervention was present, whereas, the 14-d PGF_{2α} protocol resulted in the shortest postpartum interval to AI, 74.9 d. Days to first AI for the control and GnRH-PGF_{2α}, 78.0 d, protocols were higher ($P < 0.05$) than the TAI, 77.1 d, and 14-d PGF_{2α} protocols.

The average interval to first postpartum AI was 77.7 d across all protocols, and

TABLE 2. Interval from parturition to first AI characterized by protocol across all herds (n=16).

Protocol	Cows (no.)	Days to first AI ($\bar{x} \pm SD$)
Control ^a	209	80.9 ± 16.0 ^f
14-d PGF _{2α} ^{b,d}	187	74.9 ± 10.6 ^h
GnRH-PGF _{2α} ^{c,d}	129	78.0 ± 3.0 ^{f,g}
TAI ^e	209	77.1 ± 2.5 ^{g,h}

^a Control cows were inseminated based upon the first spontaneously occurring estrus period following 60 d postpartum.

^b PGF_{2α} administered at 14-d intervals.

^c GnRH, 86 μg, - 7 d, PGF_{2α}, 500 μg.

^d Cows inseminated > 10 d post PGF_{2α} were removed from analysis.

^e TAI consisted of GnRH, 86 μg, - 7 d, PGF_{2α}, 500 μg, - 2 d, GnRH, 86 μg, followed by AI 6 to 18 h later.

^{f,g,h} Means with different superscripts differ ($P < 0.05$).

there was no difference in the days to first service between methods of estrus detection. The TAI protocol was designed for AI to occur on d 75 postpartum. Minimal variation around this time period occurred due to hormone administration schedule changes by herd managers. It should be noted that any cows inseminated prior to 73 d or after 85 d postpartum for the TAI protocol were removed from all analyses to ensure proper AI according to the specified protocol. In addition, any cows in the 14-d PGF_{2α} and GnRH-PGF_{2α} groups inseminated more than 10 d after PGF_{2α} administration were also removed.

Cows in the 14-d PGF_{2α} group had a shorter postpartum interval to AI than cows in the control and GnRH-PGF_{2α} groups ($P < 0.05$). Therefore, administering PGF_{2α} every other week enhanced earlier insemination in the 14-d PGF_{2α} group. Cows lacking a CL at the time of the first administration or not inseminated following PGF_{2α} administration were treated again in 2 wk, thereby allowing for earlier detection of estrus versus waiting for a spontaneous estrus to occur. Barr (4) established that a decrease of 1 d to first postpartum insemination corresponds to a decrease of 0.8 d open. Comparison between the 14-d PGF_{2α} and untreated control groups yielded a decrease of 6 d to first service, which according to Barr, would result in a savings of 4.8 d open for the 14-d PGF_{2α} group. These findings are similar to those of Pankowski et al. (48) which resulted in a savings of 6 d open when using a set-interval PGF_{2α} program versus insemination based upon rectal palpation. Comparison between the 14-d PGF_{2α} and GnRH-PGF_{2α} groups yielded a 3 d difference in days to first AI, potentially resulting in a

savings of 2.4 d open for the 14-d PGF_{2α} group.

Table 3 provides a comparison of the interval from parturition to first postpartum AI prior to the initiation of this study with data obtained from all cows that were inseminated according to one of the four protocols. Table 3 enables an overall evaluation of the effect that the systematic breeding protocols had on the interval to first AI. The HeatWatch[®] herds experienced the greatest decrease in days to first AI, 38 d, versus 17 d for visual observation herds. There was a greater time allowance for when cows were inseminated for the HeatWatch[®] herds possibly because herds with HeatWatch[®] believe they have the option of waiting until the next cycle to AI. Therefore, first service is delayed and herd managers rely on the HeatWatch[®] system to identify estrus, providing for a higher first AI conception rate. This fact can be validated by observing the difference between HeatWatch[®] and visual observation herds prior to the study, where the difference in the interval to first AI was 20 d greater for HeatWatch[®] herds, approximately one estrous cycle. Some HeatWatch[®] herds were not fully utilizing the HeatWatch[®] system prior to the study due to an inadequate supply of transmitters. The shorter interval to first AI for visual observation herds can also account for the smaller difference before the study began versus after. Reduction in days to first AI may also be attributed to having someone review each herd's breeding program and visit the herd on a monthly basis. Overall difference in the interval to first postpartum AI decreased by 28 d, suggesting that incorporation of systematic breeding programs, including the untreated control group, forced the participating herds to closely monitor the facilitation of the first

TABLE 3. Profile of interval from parturition to first AI across all herds (n=16) prior to initiation of systematic breeding programs versus after the incorporation of breeding programs in to reproductive management practices.

Herd	Estrus detection ^a	Days to first AI			
		Before ^b \bar{x}	Cows (no.)	After ^c \bar{x}	Difference ^d
1	HW	151	43	80	-71
2	HW	110	49	79	-31
3	HW	105	55	78	-27
4	HW	104	27	77	-27
5	HW	92	32	77	-15
6	HW	92	51	77	-15
7	HW	142	43	76	-66
8	HW	125	42	76	-49
\bar{x}	<i>HW</i>	<i>115</i>	<i>342</i>	<i>77</i>	<i>-38</i>
9	VO	92	53	76	-16
10	VO	126	43	79	-47
11	VO	89	48	80	-9
12	VO	89	50	76	-13
13	VO	86	50	76	-10
14	VO	101	52	77	-24
15	VO	105	49	83	-23
16	VO	72	47	80	8
\bar{x}	<i>VO</i>	<i>95</i>	<i>392</i>	<i>78</i>	<i>-17</i>
\bar{x}	<i>All herds</i>	<i>105</i>	<i>734</i>	<i>78</i>	<i>-28</i>

^a Method of estrus detection was either visual observation (VO) or was determined by the HeatWatch[®] (HW) electronic estrus detection system (DDx, Inc., Denver, CO).

^b Rolling herd average for days to first AI prior to the study were obtained from Dairy Herd Improvement Association herd summary sheets

^c Days to first AI obtained as a result of the study were calculated utilizing only cows inseminated according to one of the four systematic breeding protocols.

^d Difference was calculated as (the interval from parturition to first AI prior to the study) – (the interval from parturition to first AI as a result of the study); a "-" sign indicates a decrease in days to first AI.

TABLE 4. Conception and pregnancy rates for first AI characterized by protocol across all herds (n=16).

Protocol	Conception rate ^a		Pregnancy rate ^b	
	(no.)	(%)	(no.)	(%)
Control ^c	209	46.9 ^h	241	40.7 ^h
14-d PGF _{2α} ^{d,e}	187	42.2 ^{h,i}	238	33.2 ^{h,i}
GnRH-PGF _{2α} ^{e,f}	129	45.7 ^h	231	25.4 ⁱ
TAI ^g	209	30.1 ⁱ	209	30.1 ^{h,i}

^a Conception rate = number of cows diagnosed pregnant / total number of cows inseminated.

^b Pregnancy rate = number of cows diagnosed pregnant (inseminated according to each specified protocol) / total number of cows per submitted for each protocol.

^c Control cows were inseminated based upon the first spontaneously occurring estrus period following 60 d postpartum

^d PGF_{2α} administered at 14-d intervals.

^e Cows inseminated > 10 d post PGF_{2α} were removed from analysis.

^f GnRH, 86 µg, - 7 d, PGF_{2α}, 500 µg.

^g TAI consisted of GnRH, 86 µg, - 7 d, PGF_{2α}, 500 µg, - 2 d, GnRH, 86 µg, followed by AI 6 to 18 h later.

^{h,i,j} Protocol means within a column with different superscripts differ ($P < 0.05$).

postpartum AI.

Conception rates and pregnancy rates resulting from first postpartum AI were characterized by protocol across all herds (n=16) in Table 4. No differences in conception rates by protocol were found between visual observation herds and HeatWatch[®] herds. As indicated in Table 4, cows in the 14-d PGF_{2α} and GnRH-PGF_{2α} groups inseminated more than 10 d after PGF_{2α} administration were not included in the analysis of conception rates to ensure that AI was conducted according to treatment. Using Tukey's Studentized Range Test, no difference in conception rates were detected between the control, 46.9%, GnRH-PGF_{2α}, 45.7%, and 14-d PGF_{2α}, 42.2% groups. However, the first AI conception rate for the TAI group, 30.1%, was lower than the control and GnRH-PGF_{2α} groups ($P < 0.05$).

First AI pregnancy rates across all protocols (n=4) were different ($P < 0.05$) between methods of estrus detection, 36.6% for visual observation herds and 28.2% for HeatWatch[®] herds. First AI pregnancy rates were calculated as the number of cows pregnant divided by the total number of cows per protocol. For the control protocol, 241 cows were inseminated, however, only 209 were inseminated within the 120-d postpartum time period. Therefore, the 32 cows inseminated beyond 120 d were included in the model used to analyze first AI pregnancy rate, but not conception rate. In the GnRH-PGF_{2α} and 14-d PGF_{2α} groups, cows inseminated more than 10 d after PGF_{2α} administration were used in the model to calculate first AI pregnancy rates because all cows were treated with specified hormones and had equal opportunity to be inseminated.

In the GnRH-PGF_{2α} protocol, 44% (n=102) of the cows did not respond to treatment, while 21% (n=51) of the cows in the 14-d PGF_{2α} protocol did not respond to treatment with PGF_{2α} after 1, 2 or 3 administrations. First AI pregnancy rates were higher ($P < 0.01$) for the control group, 40.7%, versus the GnRH-PGF_{2α} protocol, 25.4%. However, no differences were detected between the GnRH-PGF_{2α}, 14-d PGF_{2α}, 33.2%, or TAI, 30.1%, protocols. Pregnancy rate and conception rate for the TAI protocol did not change because there was a 100% submission rate for insemination.

Overall first AI conception rates across all herds were 45.1% for visual observation herds (n=392) and 37.2% for HeatWatch[®] herds (n=342). However, individual protocol conception rates by herd varied significantly, as should be expected with this type of field data. First AI conception rates for untreated controls ranged from 21 to 85%, 15 to 86% for 14-d PGF_{2α}, 0 to 88% for GnRH-PGF_{2α}, and 0 to 60% for TAI. The tremendous variation among conception rates within each protocol may be explained by the small sample size taken from each herd. Because there were only fifteen cows per protocol from each herd, each cow could account for as much as a 7% difference in conception rates. A larger sample size per herd would be ideal, eliminating the significant effects of an individual cow without the more obvious effect on conception rates. Furthermore, management factors may have also been involved in the variation obtained from the 16 herds. For example, the expectation that synchronized cows would be in estrus shortly after hormone administration may have influenced herd managers to over-estimate subtle or secondary indications of estrus behavior. Therefore, cows may

have been inseminated before they were actually in estrus. Because individual herd reproductive management factors could not be excluded from the present study, the variation in first service conception rates provide a representation of how these protocols may perform on a variety of commercial dairy herds. The differences detected among herds enabled an objective evaluation of systematic breeding programs under normal field conditions.

Variations in individual herd conception rates may further be explained by response to hormonal intervention. For example, $\text{PGF}_{2\alpha}$ and its analogues are not luteolytic when administered in the early stage of the estrous cycle (37). The 14-d $\text{PGF}_{2\alpha}$ protocol was designed to initiate a stage of the cycle that contains a mid-cycle CL, when cows are most responsive to the second administration of $\text{PGF}_{2\alpha}$ 14 d later (48). A study conducted in beef cows showed that cows administered $\text{PGF}_{2\alpha}$ during mid-cycle (d 10 to 15) had a higher estrous response than cow given $\text{PGF}_{2\alpha}$ between d 5 and 9 of the estrous cycle (34).

The present study, along with others (38, 63) found no distinct advantage in the addition of GnRH in conjunction with $\text{PGF}_{2\alpha}$ to an estrus synchronization program. Untreated controls and cows in the 14-d $\text{PGF}_{2\alpha}$ group had nearly identical conception rates to cows in the GnRH- $\text{PGF}_{2\alpha}$ group at less expense to the producer. Furthermore, first AI pregnancy rates for the GnRH- $\text{PGF}_{2\alpha}$ protocol were lower ($P < 0.05$) than the untreated control protocol, but did not differ from the 14-d $\text{PGF}_{2\alpha}$, and TAI protocols. These results should be an important consideration when producers are deciding which

breeding program, if any, to implement into a reproductive management program.

Stevenson et al. (63) found that the addition of GnRH into a 14-d PGF_{2α} program (GnRH administered 7 d prior to the second of two administrations of PGF_{2α}, 14 d apart) decreased conception rates when compared to animals receiving 14-d PGF_{2α} alone, 48.1% versus 63.5%, respectively. LeBlanc et al. (38) conducted a similar study, but reported no difference in conception rates for cows in the 14-d PGF_{2α} or PGF_{2α}-GnRH-PGF_{2α} groups, 38.1% versus 37.3 %, respectively. The different results obtained by the two studies could be explained by parity of the experimental cows used in Stevenson's study. Lower conception rates were associated with nulliparous animals, which comprised half of the study population. If nulliparous animals were removed from the analysis, LeBlanc and Stevenson obtained similar conception rates.

Based on previous studies, GnRH administration was meant to synchronize follicular status at the time of luteolysis and increase the size and secretion of E₂ from the ovulatory follicle. However, the CL resulting from ovulation induced by exogenous GnRH has been shown to have a lower capability for P₄ secretion (57). In addition, LH release following administration of a GnRH agonist (Buserelin[®], 8 µg, Hoechst-Roussel Agri-vet, Somerville, NJ), lasted for only 5 h in heifers (11), which is approximately half the duration of a naturally occurring or PGF_{2α} associated preovulatory LH surge in cattle. Further lack of response to the GnRH-PGF_{2α} protocol may have been attributed to a deficiency of maturing follicles or low serum E₂ concentrations (33).

Xu et al. (78) provided evidence that the stage of the estrous cycle at the time of

PGF_{2α} treatment affected both the percentage of cows that expressed estrus in response to PGF_{2α} and the conception rate to AI at synchronization of estrus. Cows in the later stage of the luteal phase (d 14 to 19) had higher conception rates than cows in the early or middle stages of their cycle. Kristula et al. (35) reported that routine weekly use of PGF_{2α} beginning on d 50 postpartum resulted in a shorter interval in days to first AI (72.5 d) over cows inseminated according to PGF_{2α} administered upon rectal palpation (78.3 d). However, resulting conception rates from first AI did not differ (46.9% for PGF_{2α} treated cows and 42.0% for rectal palpation cows). These data support the interval from parturition to first AI and conception rates obtained in the present study. Pankowski et al. (48) also obtained a similar conception rate, 47%, for cows given PGF_{2α} at 14-d intervals and 43% for cows inseminated after PGF_{2α} given according to rectal palpation.

Utilization of the 14-d PGF_{2α} protocol showed no advantage over the untreated control or GnRH-PGF_{2α} treatment group, in terms of increasing first AI conception and pregnancy rates, but the interval to first AI was lowest ($P < 0.05$) for the 14-d PGF_{2α} protocol. Scheduling PGF_{2α} administration to initiate estrus allows for centralization of AI within the days following PGF_{2α} treatment. Additionally, treatment with PGF_{2α} offers an option of grouping cows together for administration of PGF_{2α} which, thereby, increased the chances for detection of estrus because more cows will be expected to be in estrus simultaneously (31). Fourteen-day PGF_{2α} also allows for extending the postpartum VWP without extending calving intervals (64); however, the VWP was not

extended for 14-d PGF_{2α} cows in the present study.

Comparison between control, 14-d PGF_{2α}, GnRH-PGF_{2α}, and the TAI protocols examined whether TAI without estrus detection could be an effective method to manage reproduction in dairy cows when compared with daily detection of estrus and insemination according to the a.m.-p.m. guideline. Pursley et al. (50) reported similar pregnancy rates per AI after TAI compared with cows inseminated at detection of estrus. In contrast, different conception rates were obtained from the TAI protocol versus the other protocols in the present study and may be explained by many factors. Pursley et al. (50, 52) reported conception rates of 38% and 37%, for the TAI protocols and suggested that 85% of lactating dairy cows ovulated within 32 h after administration of GnRH at a random stage of the estrous cycle. To achieve successful synchronization with the TAI protocol, it is critical to ovulate a follicle when the first GnRH is administered during the late luteal phase. Vasconcelos et al. (72) found that cows in mid-cycle (d 5 to 12) had higher conception rates (41%, $P < 0.05$) than cows in either the early stage (28% conception rate) or the later stages (d 13 to 21), 35%. Variations in the time of initiation of the TAI protocol may contribute to the lower conception rate obtained from the TAI protocol in the present study because stage of the cycle was not taken into consideration. Future studies involving the TAI protocol may consider incorporation of PGF_{2α} given 14 d prior to the first GnRH administration. Induced luteolysis would help synchronize the stage of the estrous cycle, resulting in a recruitment and selection from the preantral follicle population for ovulation after GnRH-PGF_{2α} administration. Therefore,

subsequent GnRH administration for the TAI protocol would occur mid-cycle, between d 9 to 12, at the time for optimum fertility.

Lack of ovulation in the TAI protocol may be another factor contributing to decreased fertility. If a follicle does not ovulate or luteinize after the initial GnRH administration, the cow may cycle normally and would most likely be in estrus before the second administration of GnRH (50). A small percentage of cows may also express signs of estrus following the first GnRH administration prior to administration of PGF_{2α}.

Stage of lactation during the TAI protocol has also been shown to affect fertility. Cows inseminated > 76 d postpartum on the TAI protocol had higher pregnancy rates, 43% ($P < 0.01$), than cows inseminated between 60 to 75 d postpartum, 26% (9). Based on these results, the TAI protocol in the present study began on d 65 postpartum, allowing insemination to occur approximately 75 d postpartum.

An important difference between the TAI protocol and the 14-d PGF_{2α} and GnRH-PGF_{2α} protocols is the elimination of estrus detection for the first postpartum insemination. Timed AI could benefit producers because they can select a day for first AI. Insemination on a specified day would allow for control in AI programs and management of the calving interval. Previously reported data (50) indicated that the day of first AI should be after 75 d postpartum to achieve maximum fertility. Timed AI can be a profitable alternative for managing large commercial dairy herds where estrus detection rates are usually less than optimal during summer (16). In addition, there is a reduction in labor costs associated with TAI because there is no labor involving detection

of estrus prior to first AI.

Researchers investigating the TAI protocol repeatedly discuss the elimination of estrus detection from a reproductive management program when using TAI. However, estrus detection is only eliminated for the first AI, and with a 30% conception rate, cows will need to be inseminated a second, third, or fourth time for them to conceive. Subsequent inseminations cannot occur without detection of estrus unless a second TAI protocol is utilized following pregnancy diagnosis. Pursley et al. (49) suggested that multiple TAI protocols can be used with 28 d pregnancy diagnosis by ultrasound evaluation, thus completely eliminating the need for estrus detection. Because fertility is not improved with the TAI protocol, the producer must make decisions about if or when this protocol should be implemented on the herd. For example, if estrus was not detected from an individual cow by 100 d postpartum, TAI could be used as an alternative to estrus detection. However, if it is a routine breeding program that the producer desires, 14-d PGF_{2α} or GnRH-PGF_{2α} may be a better choice, depending on convenience, economic evaluation, and reproductive performance.

It is important to understand that with all systematic breeding programs, as well as untreated cows, first AI conception rates will not be 100%. Cows that do not respond to hormone administrations should be identified and closely observed for expression of estrus. Additionally, all cows inseminated following hormonal administration should be observed 18 to 25 d after the first AI for return to estrus. There has been speculation

among researchers that hormonal intervention in reproductive management programs has a positive effect on conception resulting from subsequent inseminations. De la Sota et al. (16) found that higher pregnancy rates were obtained with the TAI protocol during periods of heat stress and were maintained throughout the subsequent 365-d postpartum period following hormonal treatment. Careful observation for detection of estrus should provide the opportunity for a second insemination as soon as possible, while maximizing the positive effects of the exogenous hormones administered more than 40 d earlier.

Reproductive performance of experimental cows was followed for 120 d postpartum, and cumulative pregnancy rates are represented in Figure 2. No differences were detected between untreated control and treated cows. The TAI group, however, had a noticeably lower pregnancy rate by 120 d postpartum. This was surprising because TAI at 75 d allowed the opportunity for more repeat services in comparison with the other protocols. The low first AI conception rate may account for the inability of the cows in the TAI group to achieve the same pregnancy rate as cows in the other groups. Different results were obtained by de la Sota et al. (16) who found that the TAI protocol, during periods of heat stress, improved pregnancy rates associated with TAI versus 14-d $\text{PGF}_{2\alpha}$. The advantage of a higher pregnancy rate at the initial TAI protocol was maintained throughout the postpartum period. However, a lower number of cows presented for AI in the 14-d $\text{PGF}_{2\alpha}$ group may account for the lower pregnancy rate resulting from 14-d $\text{PGF}_{2\alpha}$.

Slight increases in the percentage of cows diagnosed pregnant, with the exception

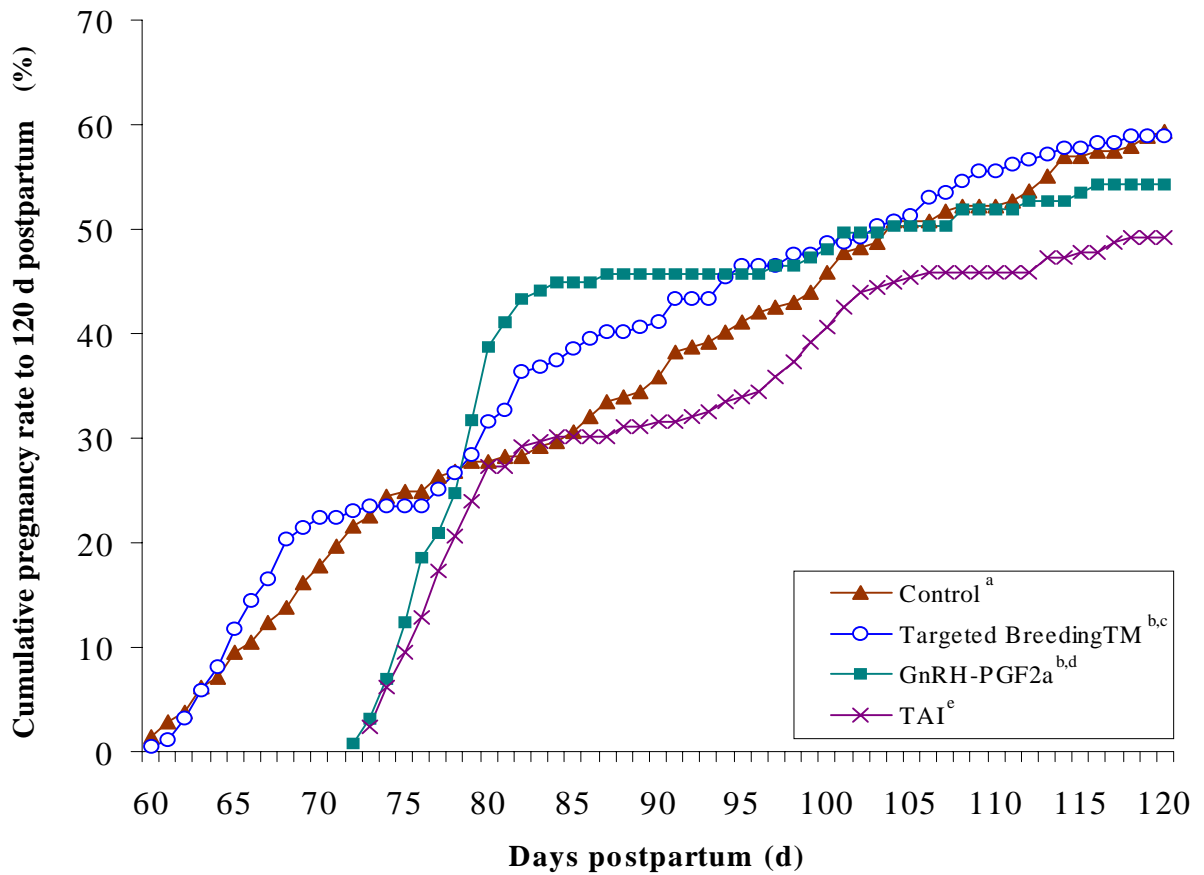


Figure 2. Cumulative pregnancy rates (the occurrence of pregnancy for experimental cows specified by protocol) up to 120 d postpartum.

^a Control cows were inseminated based upon the first spontaneously occurring estrus period following 60 d postpartum.

^b Cows inseminated > 10 d post PGF_{2α} were removed from analysis.

^c PGF_{2α} administered at 14-d intervals.

^d GnRH, 86 μg, - 7 d, PGF_{2α}, 500 μg.

^e TAI consisted of GnRH, 86 μg, - 7 d, PGF_{2α}, 500 μg, - 2 d, GnRH, 86 μg, followed by AI 6 to 18 h later.

of the control group, were evident at 20 d intervals as should be expected, since these increases indicated a time span of one estrous cycle. For the 14-d PGF_{2α} protocol increases in pregnancy corresponded with PGF_{2α} administrations. The control group showed a more linear response in pregnancy rates because no hormones were administered to synchronize estrus; therefore, cows randomly came into estrus between 60 and 120 d postpartum. It was anticipated that cumulative pregnancy curves would approach each other later in lactation, as was shown. However due to different culling rates between treatment groups, actual pregnancy rates for all experimental cows was not possible.

Because conception rates resulting from first AI and cumulative pregnancy rates at 120 d postpartum did not differ among the control, 14-d PGF_{2α} and GnRH-PGF_{2α} groups, the improved reproductive performance for cows receiving hormonal synchronization may be attributed to a greater synchronization of estrus for treated cows, resulting in improved estrus detection.

Estrus Characteristics

Characteristics of estrus were obtained from HeatWatch[®] herds (n=8) to evaluate the expression of estrus for the three systematic breeding programs. A profile of estrus characteristics for estrus periods (n=169) identified by the HeatWatch[®] electronic estrus detection system is presented in Table 5. As indicated in Table 5, estrus periods consisting of only one standing event were removed from the final analysis of estrus duration and estrus intensity.

It was determined within the HeatWatch[®] herds, that the number of standing events had no effect on conception rate ($p = 0.29$). It has generally been shown that synchronization programs elicit increased standing activity as compared to spontaneously occurring estrus periods, usually due to increased numbers of cows in estrus simultaneously (74). However, data obtained from this study were similar to those obtained by a recent field study (17) analyzing spontaneously occurring estruses, which showed that lactating cows expressed an average of 8.5 ± 6.6 standing events with an estrus duration of 7.1 ± 5.4 h. Estrus activity in the present study did not represent typical activity displayed by synchronized cows because only a small proportion of the cows were treated with hormones at any given time. Additionally, there was no evidence that any of the three protocols altered the duration of estrus periods or had an influence on the intensity of estrus.

Almost 50 % of all eligible cows express signs of estrus that go undetected when conventional methods of estrus detection are used (4). One possible explanation for this

TABLE 5. Characteristics of estrus periods in cows identified by the HeatWatch[®] ^a electronic estrus detection system for HeatWatch[®] herds (n=8).

Protocol	Cows	Characteristics of estrus ^{b,c}		
		Standing events (no.)	Estrus intensity ^d	Estrus duration ^e (h)
Control ^f	64	6.7 ± 4.5	5.5 ± 16.7	6.9 ± 6.9
14-d PGF _{2α} ^g	53	6.3 ± 3.5	3.3 ± 8.4	5.8 ± 4.8
GnRH-PGF _{2α} ^h	36	8.0 ± 5.5	2.6 ± 5.1	7.2 ± 5.3
TAI ⁱ	15	9.3 ± 8.6	1.4 ± 0.9	7.4 ± 4.9

^a DDX, Incorporated (Denver, CO).

^b Mean ± standard deviation.

^c Estrus periods consisting of only one standing event were removed from analysis.

^d Estrus intensity was defined as the number of standing events per estrus duration.

^e Estrus duration was defined as the time from first to last standing event as identified by HeatWatch[®].

^f Control cows were inseminated based upon the first spontaneously occurring estrus period following 60 d postpartum.

^g PGF_{2α} administered at 14-d intervals.

^h GnRH, 86 µg, - 7 d, PGF_{2α}, 500 µg.

ⁱ TAI consisted of GnRH, 86 µg, - 7 d, PGF_{2α}, 500 µg, - 2 d, GnRH, 86 µg, followed by AI 6 to 18 h later.

low rate of estrus detection has classically been thought to be the occurrence of a substantial amount of standing activity taking place during late night to early morning hours, when estrus detection is least likely to be conducted (31). Distribution for the onset of standing activity for estrus periods (n=202) identified by HeatWatch[®] is shown in Table 6. The onset of estrus was recorded as the occurrence of the first standing event identified by HeatWatch[®]. Results indicate that 28.7% of estrus onsets occurred between 0001 to 0006 h, which is similar to recent findings by Dransfield et al. (17) that showed 25% of onsets occurred during the same 6-h period. Overall, the onset of estrus was nearly evenly distributed into the four 6-h time periods.

The distribution of estrus periods having ≥ 2 standing events (n=168) is characterized by duration and intensity in Table 7. Low intensity was defined as an estrus period consisting of < 1.5 standing events per h, and high intensity was defined as ≥ 1.5 standing events per hour. Short duration was defined as < 7 h from first to last standing event, and long duration was any estrus period ≥ 7 h from first to last standing event. A majority of the cows (n=96) were classified into low intensity with short or long duration. Whereas, only 6 cows were classified as high intensity, long duration, and only 2 of those 6 cows conceived. Conception rates ranged from 33.3 % for cows that had estrus periods with high intensity and long duration to 46.2% for estrus periods with low intensity and long duration. The importance of estrus detection has been well established. This distribution of estrus periods reinforces the difficulty in accurately and efficiently detecting estrus. Because a majority of the cows were in one of the low

TABLE 6. Percentage distribution for onset of standing activity for estrus periods (n=202) identified by the HeatWatch[®]^a electronic estrus detection system (herds=8).

Time	Cows	Onset of estrus ^b
(h)	(no.)	(%)
0001 to 0600	58	28.7
0061 to 1200	43	21.3
1201 to 1800	43	21.3
1801 to 2400	58	28.7

^a DDX, Incorporated (Denver, CO).

^b Occurrence of first standing event identified by HeatWatch[®].

TABLE 7. Distribution of estrus periods (n=169) categorized by intensity and duration as identified the HeatWatch[®]^a electronic estrus detection system.

Estrus category ^{b,c}	Cows	Distribution	Conception rate
	(no.)	—————(%)—————	
Low intensity, short duration	31	18.3	38.7
Low intensity, long duration	65	38.5	46.2
High intensity, short duration	37	39.6	38.8
High intensity, long duration	6	3.6	33.3

^a DDx, Incorporated (Denver, CO).

^b Low intensity was defined as an estrus period consisting of < 1.5 standing events/h, and high intensity was defined as ≥ 1.5 standing events/h. Short duration was characterized as < 7 h from first to last standing event, and long duration was any estrus period ≥ 7 h from first to last standing event.

^c Estrus periods consisting of only one standing event were removed from analysis.

intensity groups, this indicates that cows experienced few standing events over the duration of estrus, be it long or short.

A profile of estrus detection efficiency across all herds (n=16) was calculated prior to initiation of the systematic breeding study and again after incorporation of the breeding protocols into each herd's reproductive management practices. These data are presented in Table 8. Estrus detection efficiency before the study ranged from 51% to 90% for the visual observation herds, with an average of 60%. The average estrus detection efficiency for HeatWatch[®] herds was 68% and ranged from 27% to 100%. An estrus detection efficiency of 100% may be explained by the fact that cows in Herd 1 were being inseminated before they were actually in estrus due to non-estrus related standing events detected by the HeatWatch[®] system software. This is evident because the estrus detection efficiency for Herd 1 dropped to 87% after incorporation of the breeding protocols, meaning this study forced the herd manager to follow the protocols in conjunction with use of HeatWatch[®] and only inseminate cows that expressed signs of estrus, allowing for a more accurate account of estrus detection efficiency for Herd 1. Additionally, Herd 2 experienced a decrease in estrus detection efficiency, most likely for the same reasons as Herd 1. Estrus detection efficiency close to 100% would also result if the interval between inseminations were exactly 21 days, or less.

Herd 4 experienced the greatest increase in estrus detection efficiency following the study because the herd manager was not using HeatWatch[®] to its full potential prior to the study due to lack of an adequate supply of transmitters. Herd 4 also had the lowest estrus detection efficiency prior to beginning the study, therefore allowing for the

TABLE 8. Profile of estrus detection efficiency across all herds (n=16) calculated prior to initiation of the systematic breeding study and after the incorporation of systematic breeding programs into reproductive management practices.

Herd	Estrus detection ^a	Estrus Detection Efficiency		
		Before ^b	After ^c (%)	Difference ^d
1	HW	100.0	89.0	-11.0
2	HW	94.3	87.0	-7.3
3	HW	77.8	92.8	15.0
4	HW	26.8	90.8	64.0
5	HW	40.2	86.5	46.3
6	HW	68.1	93.8	25.7
7	HW	63.9	99.8	35.9
8	HW	73.8	91.3	17.5
\bar{x}	HW	68.1	91.4 ^e	23.3
9	VO	51.3	83.7	32.4
10	VO	84.0	86.3	2.3
11	VO	43.1	77.8	34.7
12	VO	70.0	91.8	21.8
13	VO	42.6	76.5	33.9
14	VO	90.0	89.0	-1.0
15	VO	46.7	77.0	30.3
16	VO	53.7	79.5	25.8
\bar{x}	VO	60.2	82.7 ^f	22.5
\bar{x}	All herds	64.1	87.0	22.9

^a Method of estrus detection was either visual observation (VO) or was determined by the HeatWatch[®] (HW) electronic estrus detection system (DDx, Inc., Denver, CO).

^b Estrus detection efficiency prior to initiation of the study was calculated using the equation: [(100) (average estrous cycle, 21 d) (services per conception - 1) / (average days open - days to first service)] from Fetrow et al. (20).

^c Estrus detection efficiency as a result of the study was calculated using the formula: {(21 / (days to second AI - days to first AI)) x 100} for all services. Only cows inseminated according to one of the four systematic breeding protocols were used in this analysis.

^d Difference was calculated as (estrus detection efficiency prior to the study) - (estrus detection efficiency as a result of the study); a "-" sign indicates a decrease in estrus detection efficiency.

^{e,f} Means with different superscripts differ ($P < 0.01$).

greatest improvement. The present study allowed Herd 4 to maximize the use of HeatWatch[®] by designating when HeatWatch[®] transmitters were to be applied. Additionally, systematic breeding helped decrease the interval to first AI which, in turn, allowed for earlier postpartum diagnosis of conception, making those transmitters available for other cows.

Estrus detection efficiency as a result of the systematic breeding protocols differed ($P < 0.01$) between visual observation herds, 83%, and HeatWatch[®] herds, 95%, which was obtained from the HeatWatch[®] system software. Both groups experienced a similar increase in efficiency as a result of the study. Barr (4) reported that estrus detection efficiency has been shown to have a high correlation with calving interval and a strong effect on reproductive management parameters. Use of hormonal intervention to synchronize estrus and possibly ovulation provided the opportunity to improve estrus detection efficiency, and hence, reproductive performance for the 16 herds involved in this study.

Although estrus detection efficiency, based on first, second, and third inseminations, may have been higher than average reported values, it should be expected because cows were programmed to come into estrus, thus increasing estrus behavior, allowing for increased estrus detection efficiency. Reproductive performance, conception rates, interval from parturition to first AI, and pregnancy rates, were well within acceptable ranges.

Estrus response to PGF_{2α} treatment

For purposes of this study, detection of estrus within 10 d after PGF_{2α} administration was considered a positive response to treatment for both the 14-d PGF_{2α} and GnRH-PGF_{2α} protocols. Cows inseminated more than 10 d after PGF_{2α} administration in either protocol were not included in the analyses for first AI conception rates or pregnancy rates.

For the 14-d PGF_{2α} protocol, there was no difference in the number of PGF_{2α} administrations between the visual observation herds (n=99, 1.6 administrations) and the HeatWatch[®] herds (n=88, 1.5 administrations). Therefore, the average number of required PGF_{2α} administrations prior to insemination for all herds was 1.6 for the 14-d PGF_{2α} protocol. The effect of the interval from PGF_{2α} administration to estrus on first AI conception rates is presented in Figure 3. A majority of the cows, 81.2%, were inseminated within the first 5 d following PGF_{2α} administration; however, conception rates remained similar up to 10 d after PGF_{2α}. The mean interval from administration of PGF_{2α} to estrus was 4.1 ± 2.2 d for the 14-d PGF_{2α} protocol and 3.9 ± 1.8 d for the GnRH-PGF_{2α} protocol. Similar results were reported by LeBlanc et al. (38), that showed the interval from PGF_{2α} to AI was 3.2 d. In the present study, all cows were inseminated following PGF_{2α} administration (n=187); 57% of the cows were inseminated after the first administration of PGF_{2α}, 30% after the second administration, and the remaining 13% were inseminated following the third PGF_{2α} administration. Distribution of

conception rates by the number of PGF_{2α} administrations for cows (n=187) in the 14-d PGF_{2α} protocol is shown in Table 9. There was no difference in fertility for cows receiving 1, 2, or 3 administrations of PGF_{2α}.

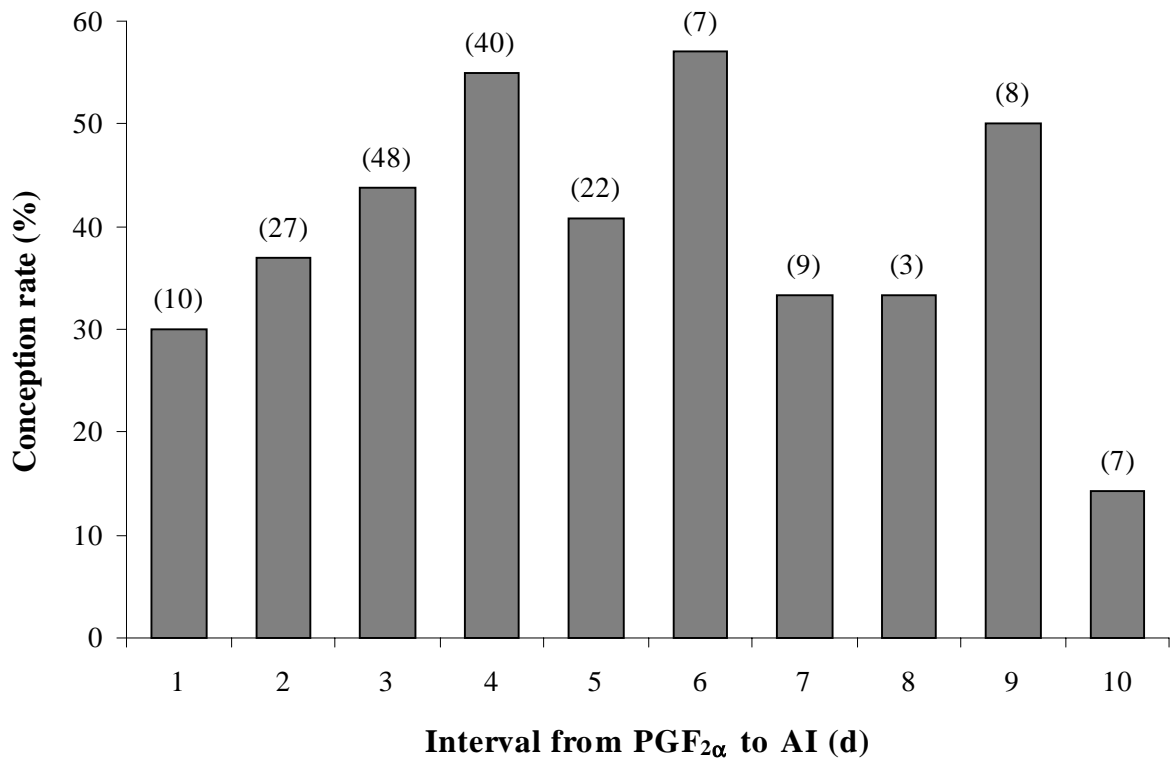


Figure 3. Effect of interval from PGF_{2α} administration to estrus and first postpartum AI on conception rate in lactating dairy cows for the 14-d PGF_{2α}^{b,c} protocol.

^a Numbers in parenthesis represent number of cows inseminated on specified day.

^b PGF_{2α} administered at 14-d intervals.

^c Cows inseminated > 10 d post PGF_{2α} administration were removed from analysis.

TABLE 9. Distribution of conception rates by the number of PGF_{2α} administrations for cows (n=187) in the 14-d PGF_{2α}^a protocol.

PGF _{2α} administrations	Cows	Conception rate ^b
(no.)		(%)
1	106	48.1
2	56	50.0
3	25	36.0

^a PGF_{2α} administered at 14-d intervals.

^b Cows inseminated > 10 d post PGF_{2α} administration were removed from analysis.

Estrus response to TAI

Although use of the TAI protocol eliminated the need for estrus detection for the first postpartum AI, HeatWatch[®] herds participating in this study were asked to apply transmitters to cows in the TAI group for evaluation of estrus expression. Results from this phase of the experiment are shown in Table 10. It was interesting that 76 cows expressed no standing events associated with signs of estrus, while 21 cows did express standing activity and experienced more than a two-fold increase ($P < 0.05$) in the first service conception rate, 19.7% versus 42.9%, respectively.

It has been suggested (65) that as intensity of estrus increases, the occurrence of conception also increases. Using HeatWatch[®], Nebel et al. (44) found that as the number of standing events increased, pregnancy rates also increased. Conception rates from cows in the TAI group, in comparison with cows that showed no visible signs of estrus activity, supported both of the aforementioned reports.

Enrollment of 734 lactating dairy cows into one of the four systematic breeding programs yielded overall changes among most herds. These changes included fewer days to first AI and increased estrus detection rates because cows were expected to come into estrus within a given time period which, reduced the prevalence of uninseminated cows during a later stage of lactation.

Disadvantages of utilizing systematic breeding programs include additional expense for hormone administrations and increased handling of cattle. In addition, all

estrus synchronization programs reported herein were effective in decreasing days to first postpartum AI and achieving adequate conception rates; however, results from this and other field studies suggest that herd reproductive management practices must be adequate for these programs to benefit the producer. Systematic breeding programs should be implemented as a tool to improve reproductive efficiency through more efficient use of labor in conjunction with programmed estrus induction.

TABLE 10. Analysis of first AI conception rate and estrus activity, as identified by the HeatWatch[®]^a electronic estrus detection system, for the TAI protocol within HeatWatch[®] herds (n=8).

Periods	Standing events	Conception rate
(no.)		(%)
76	0	19.7 ^c
21	≥ 1	42.9 ^b

^a DDX, Incorporated (Denver, CO).

^{b,c} Percentages with different superscripts differ ($P < 0.05$).

Economic evaluation of systematic breeding protocols

The 16 commercial dairy herds participating in the present study were surveyed to establish average drug costs for PGF_{2α} and GnRH and their respective analogues. Cost for labor associated with estrus detection was also calculated. The average wage paid by the 16 herds was \$7.85/h. With information obtained from the survey (Appendix, Figure 4), cost per pregnancy using estrus detection efficiency associated with the 14-d PGF_{2α}, GnRH-PGF_{2α}, and TAI protocols was calculated and is shown in Table 11. Pregnancy rates for each protocol were obtained as a result of the first postpartum insemination from the present study. The TAI protocol contained a fixed-time AI 6 to 18 h after the second GnRH administration, thus achieving a 100% submission rate. The average price paid for PGF_{2α} was \$3.50 per administration and \$6.75 per administration of GnRH. The cost for drugs ranged from \$2.75 to \$5.42 per PGF_{2α} administration and from \$4.50 to \$10.00 per GnRH administration. Cost of hormone administration, or cow handling, was \$0.50 per administration and was lowest for the 14-d PGF_{2α} protocol and highest for the TAI protocol. An average of 1.6 PGF_{2α} administrations were used for the 14-d PGF_{2α} protocol based on results obtained from the 16 herds. Labor savings and costs associated with sorting cows for administration of hormones varies by the number of cows being synchronized and the type of housing and handling facilities available in each herd. Cost per pregnancy was calculated at \$19.39 for 14-d PGF_{2α}, \$45.00 for GnRH-PGF_{2α}, and \$61.67 for the TAI protocol.

A survey conducted in 1997 (43) evaluated the cost effectiveness of 14-d PGF_{2α}

TABLE 11. Cost per pregnancy using the 14-d PGF_{2α}, GnRH-PGF_{2α}, and TAI protocols.

Protocol	Drug cost ^d	Cost for hormone administration ^e	Pregnancy rate ^f	Cost per pregnancy
	—————(\$)—————		(%)	(\$)
14-d PGF _{2α} ^a	5.60	0.80	33	19.39
GnRH-PGF _{2α} ^b	10.25	1.00	25	45.00
TAI ^c	17.00	1.50	30	61.67

^a PGF_{2α} administered at 14-d intervals.

^b GnRH, 86 μg, – 7 d, PGF_{2α}, 500 μg.

^c TAI consisted of GnRH, 86 μg, – 7 d, PGF_{2α}, 500 μg, – 2 d, GnRH, 86 μg, followed by AI 6 to 18 h later.

^d Costs obtained from survey of 16 herds participating in the systematic breeding program study. Average cost for a single PGF_{2α} administration was \$3.50 and \$6.75 per single administration of GnRH. An average of 1.6 PGF_{2α} administrations was obtained from the 16 commercial dairy herds.

^e Cost for cow handling was \$0.50 per hormone administration.

^f Pregnancy rates were a result of first AI according to specified protocol.

BreedingTM and TAI protocols in Virginia, representing 877 dairy herds. Veterinarians reported an average cost of \$3.30 per dose of PGF_{2α}, and a range of \$2.50 to \$5.50 per dose. The cost for GnRH ranged from \$4.50 to \$14.00 per dose with an average price of \$7.27 per dose. Cost per pregnancy was calculated at two levels of estrus detection, 40% and 70%, and included the mean and least costs for drug prices obtained from the survey. Estimated cost per pregnancy ranged from \$16.27 for 14-d PGF_{2α} with a 70% estrus detection rate at the lowest drug cost to \$46.10 for the TAI protocol at the mean drug cost. Costs per pregnancy were similar for the TAI protocol at the lowest drug cost (\$30.25) and the 14-d PGF_{2α} program with 40% estrus detection rate at the mean drug cost (\$29.61).

Kristula et al. (35) prepared a partial budget analysis comparing cows inseminated after PGF_{2α} administration based on rectal palpation and a protocol that required weekly PGF_{2α} administration to open cows. Results indicated that the cost for cows receiving PGF_{2α} weekly was \$3.73 more per cow than those receiving PGF_{2α} based on rectal palpation, but median days open per cow was 13 d less for cows receiving PGF_{2α} weekly.

Cost for estrus detection for the first postpartum AI according to 14-d PGF_{2α}, GnRH-PGF_{2α}, TAI, and the untreated control protocol, was calculated to determine the cost per pregnancy for each protocol within both methods of estrus detection (Table 12). The cost for estrus detection was calculated by multiplying the number of days required for detection of estrus by \$7.85, the average wage per hour paid by the 16 herds. Results

TABLE 12. Cost per pregnancy based on estrus detection for first AI according to protocol using the 14-d PGF_{2α}, GnRH-PGF_{2α}, and TAI protocols versus an untreated control for a breeding program utilizing visual observation for detection of estrus or the HeatWatch[®] electronic estrus detection system.

Protocol	Estrus detection ^e (\$)	Pregnancy rate ^f (%)	Cost per pregnancy (\$)
Control			
Visual Observation	2865.00	49	19.10
HeatWatch ^{® d,g}	15.40	42	15.40
14-d PGF_{2α}^a			
Visual Observation	188.40	35	4.14
HeatWatch [®]	15.40	32	15.40
GnRH-PGF_{2α}^b			
Visual Observation	235.50	33	5.16
HeatWatch [®]	15.40	18	15.40
TAI^c			
Visual Observation	0.00	36	0.00
HeatWatch [®]	0.00	25	0.00

^a PGF_{2α} administered at 14-d intervals.

^b GnRH, 86 μg, – 7 d, PGF_{2α}, 500 μg.

^c TAI consisted of GnRH, 86 μg, – 7 d, PGF_{2α}, 500 μg, – 2 d, GnRH, 86 μg, followed by AI 6 to 18 h later.

^d DDx, Incorporated (Denver, CO).

^e Estrus detection cost was calculated using the formula: (number of days required for estrus detection) x (\$7.85, the average wage/h for estrus detection paid by 16 commercial dairy herds). Control cows require estrus detection 365d/yr, 14-d PGF_{2α} required 24 d at the specified pregnancy rate, GnRH-PGF_{2α} required 30 d at the specified pregnancy rate because only 50% of cows submitted for the protocol actually responded to hormonal intervention, and TAI required 0 d for estrus detection. Herd size was 130 cows, based on Virginia State average (October 1998).

^f Pregnancy rates were a result of first AI according to specified protocol. Rates with a line through them were not used in calculations.

^g Cost of estrus detection for HeatWatch[®] herds was calculated using \$10.00/cow for system costs and \$5.40/cow for labor and disposable costs. Disposable costs included \$3.75/patch, \$1.00/glue application, and \$0.65 for labor, calculated at 5 min at \$7.85/h.

from the survey indicated that the average time spent on detection of estrus was 1 h per day. Because estrus detection usually involves observing the entire herd, rather than individual cows, cost associated with estrus detection were calculated using an average herd size of 130 cows, the Virginia State average (October 1998). The number of days required for observation of estrus would be variable for different herds, depending upon the number of cows cycling at any given time. The cost for estrus detection for the 14-d PGF_{2α} protocol was calculated by multiplying the number of days required for detection of estrus following PGF_{2α} administration (5 d) by the average number of PGF_{2α} administrations, 1.6, which resulted in 8 d for estrus detection. However, at a pregnancy rate of 35% for first AI, approximately 24 d of estrus detection would be necessary for most cows to conceive within 1 yr following PGF_{2α} administration. For the GnRH-PGF_{2α} protocol, the number of days necessary for detection of estrus was also 5 d after PGF_{2α} administration. However, because nearly 50% of the cows in the GnRH-PGF_{2α} group were not inseminated according to protocol, the number of days was doubled to 10 d for estrus detection to achieve a 33% first AI pregnancy rate. Therefore, approximately three 10-d periods of estrus detection would be necessary for cows in the GnRH-PGF_{2α} group to conceive within 1 yr. There was no cost of estrus detection for the TAI protocol because detection of estrus was eliminated for the first postpartum insemination. However, it is important to point out that estrus detection was still required for identification of subsequent expression of estrus by cows that did not conceive following the initial TAI protocol. The cost for estrus detection for the HeatWatch[®] herds was

calculated on an individual cow basis, using \$10.00 for system costs, assuming payment for the system was over a 5-yr period, and \$5.40 for labor and disposable costs. Disposable costs were defined as \$3.75/patch and \$1.00/glue application. Labor cost, \$0.65, was estimated at 5 min (based on unpublished data collected by our laboratory) at \$7.85/h. Pregnancy rates were a result of the first postpartum insemination according to each specified protocol within both methods of estrus detection. For the visual observation herds, the cost per pregnancy was highest for untreated controls, due to variability in days to first postpartum AI, and lowest for the TAI protocol because detection of estrus was eliminated for the first insemination. Costs per pregnancy for HeatWatch[®] herds was \$15.40 for all protocols. Once again, there was no cost per pregnancy for the TAI protocol due to the elimination of estrus detection. Cost per pregnancy for visual observation herds ranged from \$4.14 for the 14-d PGF_{2α} protocol to \$19.10 for the untreated control group. The GnRH-PGF_{2α} protocol was originally designed to be a more economical approach to systematic breeding than TAI. However, because of the low response rate, 56%, to GnRH-PGF_{2α} versus 100% submission for TAI, the GnRH-PGF_{2α} protocol resulted in a higher cost per pregnancy for estrus detection efficiency.

Tables 11 and 12 represent best and worst case scenarios for all protocols. Although 14-d PGF_{2α}, GnRH-PGF_{2α} and TAI protocols may have had higher costs per when considering drug and labor costs, cost per pregnancy in terms of estrus detection, make these protocols appear more favorable than the untreated control group. Utilization

of the HeatWatch[®] system does not appear to be as cost effective as systematic breeding programs in the present study because all of the participating herds, HeatWatch[®] and visual observation, were very well managed herds prior to initiation of the study. Therefore, significant differences between the two groups are not evident in the data obtained from this study.

It is important to realize that the decision of which protocols to implement must be made on an individual herd basis. The need to identify cows in estrus could be reduced for the 14-d PGF_{2α} protocol to a few days weekly and may even be eliminated with the TAI protocol. However, cows diagnosed open following TAI will still need to be observed for subsequent estrus expression and AI. Although, TAI may be a profitable alternative for managing large commercial dairy herds during periods of heat stress. Improved reproductive performance during the summer months with the TAI protocol appear to have a significant economic impact, \$359.00 per cow versus \$476.00 per cow with the 14-d PGF_{2α} protocol (16). It must also be considered that although cows must be handled three times according to the TAI protocol, there is no labor involving estrus detection for the first postpartum insemination.

CONCLUSIONS

The dairy industry has been faced with the problem of breeding cows back in a timely manner for numerous years. It was and still is believed that cows that do not breed back right away suffer from infertility and reproductive tract problems. However, in today's dairy industry management factors should also be considered when the average number of days open for any herd is much higher than the optimum. Throughout the nation herd sizes have increased considerably, but a proportional increase in labor has not always been simultaneous. Therefore, herd management continues to be a factor contributing to the reproductive success on any dairy.

The detection of estrus is often difficult, especially when external signals expressed are vague. As a consequence, estrus may go unnoticed, or cows may be inseminated at a less than optimal time, leading to lower conception rates. Fourteen-day $\text{PGF}_{2\alpha}$, GnRH- $\text{PGF}_{2\alpha}$, and TAI protocols vary in effectiveness; however, these protocols have the potential to increase the reproductive performance of lactating dairy herds while maintaining AI as the dominant insemination option. Systematic breeding programs may not be beneficial to all herds, especially those on the extreme ends of efficient reproductive performance. These programs are beneficial only when reproductive management is properly maintained prior to implementing a systematic breeding program. Well-managed herds will probably not benefit from systematic breeding protocols to the same degree as herds with lower reproductive performance. Likewise, inefficiently managed herds probably have underlying problems that need to be

addressed before implementing a systematic breeding protocol, which could further complicate herd management. If estrus detection efficiency is low to begin with, the 14-d $\text{PGF}_{2\alpha}$ protocol may be implemented. With 14-d $\text{PGF}_{2\alpha}$ many cows will be in estrus simultaneously and estrus activity will increase, allowing for more efficient detection of estrus. If the 14-d $\text{PGF}_{2\alpha}$ protocol is unsuccessful in decreasing days open and increasing estrus detection efficiency, the TAI protocol is the second alternative because detection of estrus is eliminated.

Reduced labor costs from less time spent on estrus detection may be offset by the cost of the drug protocols. In addition, response to hormonal induction of estrus and potentially low conception rates should also be taken into consideration when deciding which protocol to implement. Systematic breeding programs should be considered tools for convenience and efficiency of estrus detection; however, cost effectiveness must be calculated for each herd to decide whether a systematic breeding program is the appropriate management decision.

The VWP and average days to first AI are two important components necessary to achieve efficiency in reproductive management. The VWP should be targeted between 60 and 80 d postpartum; however low producing cows may be inseminated earlier. A goal for producers should be to have every cow in the herd inseminated by 100 d postpartum. If conventional methods of estrus detection and AI are not successful, the HeatWatch[®] electronic estrus detection system should be explored as an option for increasing estrus detection efficiency, thereby, decreasing days to first AI and potentially

decreasing days open. Systematic breeding programs should also be explored as methods for maintaining a desired interval from parturition to first AI and achievement of efficient reproductive performance. Although HeatWatch[®] herds in the present study did not benefit over the visual observation herds by implementing systematic breeding programs in conjunction with the HeatWatch[®] system, herds with lower estrus detection efficiency ($\leq 40\%$) may consider using HeatWatch[®] and systematic breeding programs together.

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APPENDIX

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1. Estimate how much time per day you spend observing cows for signs of estrus.
 2. On an hourly basis, how much do you pay for detection of estrus?
 3. What price do you pay for $\text{PGF}_{2\alpha}$ or its analogues (per dose)?
 4. What price do you pay for GnRH or its analogues (per dose)?
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Figure 4. Questionnaire given to 16 commercial dairy herds participating in the systematic breeding study, evaluating 14-d $\text{PGF}_{2\alpha}$, GnRH- $\text{PGF}_{2\alpha}$.

TABLE 13. First, second, and third service conception rates and overall pregnancy rate across all herds (n=16) divided by method of estrus detection.

	Protocol	Cows	First AI ^{a,b}	Cows	Second AI ^a	Cows	Third AI ^a	Pregnancy Rate ^c
		(no.)	(%)	(no.)	(%)	(no.)	(%)	(%)
Herd 1	Control ^d	14	36	4	50	0	0	44
	14-d PGF _{2α} ^{e,h}	10	50	2	0	0	0	50
	GnRH-PGF _{2α} ^{f,h}	4	50	0	0	0	0	50
	TAI ^g	15	40	3	33	0	0	44
Herd 2	Control	14	21	7	29	0	0	31
	14-d PGF _{2α}	10	50	3	33	0	0	55
	GnRH-PGF _{2α}	9	22	4	0	0	0	22
	TAI	16	0	9	11	1	0	6
Herd 3	Control	14	43	5	40	0	0	50
	14-d PGF _{2α}	16	50	5	80	0	0	60
	GnRH-PGF _{2α}	10	30	4	75	1	0	46
	TAI	15	33	5	80	0	0	47
Herd 4	Control	4	50	1	100	0	0	60
	14-d PGF _{2α}	10	20	4	50	0	0	33
	GnRH-PGF _{2α}	4	0	2	0	0	0	0
	TAI	9	11	2	100	0	0	27
Herd 5	Control	12	58	2	100	0	0	64
	14-d PGF _{2α}	7	86	0	0	0	0	86
	GnRH-PGF _{2α}	6	67	0	0	0	0	67
	TAI	7	14	4	50	0	0	33
Herd 6	Control	18	56	2	50	1	0	58
	14-d PGF _{2α}	13	15	8	38	1	100	35
	GnRH-PGF _{2α}	10	60	4	75	1	0	69
	TAI	10	40	6	50	0	0	54
Herd 7	Control	12	42	4	50	1	0	50
	14-d PGF _{2α}	13	38	4	25	2	50	47
	GnRH-PGF _{2α}	7	14	3	0	1	0	14
	TAI	11	18	5	0	0	0	18
Herd 8	Control	14	43	4	25	1	100	50
	14-d PGF _{2α}	9	56	2	0	0	0	56
	GnRH-PGF _{2α}	5	40	1	0	1	100	50
	TAI	14	36	7	57	1	100	53
HW Herds ⁱ	Control	102	43	29	45	3	33	50
	14-d PGF _{2α}	88	38	28	39	3	67	50
	GnRH-PGF _{2α}	55	36	18	33	4	25	44
	TAI	97	24	41	71	2	50	37

	Protocol	Cows (no.)	First AI ^{a,b} (%)	Cows (no.)	Second AI ^a (%)	Cows (no.)	Third AI ^a (%)	Pregnancy Rate ^c (%)
Herd 9	Control ^d	12	42	2	100	0	0	50
	14-d PGF _{2α} ^{e,h}	14	57	2	50	0	0	60
	GnRH-PGF _{2α} ^{f,h}	12	50	0	0	0	0	50
	TAI ^g	15	60	2	100	0	0	65
Herd 10	Control	12	42	6	33	2	100	56
	14-d PGF _{2α}	13	38	5	100	0	0	56
	GnRH-PGF _{2α}	4	75	1	0	0	0	75
	TAI	14	36	5	60	1	0	47
Herd 11	Control	14	43	2	0	2	50	47
	14-d PGF _{2α}	12	33	2	100	0	0	43
	GnRH-PGF _{2α}	9	11	5	0	0	0	11
	TAI	13	38	4	100	0	0	53
Herd 12	Control	15	47	6	33	0	0	53
	14-d PGF _{2α}	12	42	2	50	0	0	46
	GnRH-PGF _{2α}	11	36	4	25	0	0	42
	TAI	12	25	5	80	0	0	44
Herd 13	Control	13	38	7	43	1	100	53
	14-d PGF _{2α}	14	50	5	100	0	0	63
	GnRH-PGF _{2α}	8	88	1	100	0	0	89
	TAI	15	40	3	33	0	0	44
Herd 14	Control	15	53	6	33	3	33	61
	14-d PGF _{2α}	13	38	6	50	2	0	50
	GnRH-PGF _{2α}	10	80	1	0	1	0	80
	TAI	14	29	6	67	0	0	44
Herd 15	Control	13	85	0	0	0	0	85
	14-d PGF _{2α}	11	45	1	0	0	0	45
	GnRH-PGF _{2α}	10	60	2	50	0	0	64
	TAI	15	20	4	75	0	0	33
Herd 16	Control	13	54	2	0	0	0	54
	14-d PGF _{2α}	10	20	7	43	2	50	43
	GnRH-PGF _{2α}	10	40	5	20	0	0	45
	TAI	14	29	6	50	0	0	41
Visual	Control	107	50	31	35	8	63	57
Observ.	14-d PGF _{2α}	99	41	30	67	4	25	52
Herds	GnRH-PGF _{2α}	74	53	19	21	1	0	55
	TAI	112	35	35	39	1	0	46

^a Conception rate for specified insemination number.

^b Conception rate for first AI includes all cows inseminated regardless of day to first AI (i.e.

>120 d postpartum).

^c Pregnancy rate includes only cows diagnosed pregnant to AI on or before 120 d postpartum.

^d Control cows were inseminated based upon the first spontaneously occurring estrus period following 60 d postpartum.

^e PGF_{2α} administered at 14-d intervals.

^f GnRH, 86 μg, – 7 d, PGF_{2α}, 500 μg.

^g TAI consisted of GnRH, 86 μg, – 7 d, PGF_{2α}, 500 μg, – 2 d, GnRH, 86 μg, followed by AI 6 to 18 h later.

^h Cows inseminated >10 d post PGF_{2α} administration were removed from analysis.

ⁱ HeatWatch[®], DDX, Incorporated (Denver, CO).

TABLE 14. Analysis of variance table testing the effects of protocol and method of estrus detection on the number of days to first postpartum AI.

Source	N	Type III sum of squares	Mean square	F Value	P > F
Method ^a	1	33.45	33.45	0.22	0.6470
Protocol ^b	3	3846.80	1282.27	10.20	0.0001
Protocol*Method ^b	3	28.31	9.44	0.08	0.9731
Herd(Method)	14	2138.02	152.72	1.48	0.1112
Protocol*Herd(Method)	42	5281.05	125.74	1.22	0.1632
Residual Error	670	68659.37	102.92		

^a Hypothesis tested using Herd*Method as the error term.

^b Hypothesis tested using Protocol*Herd(Method) as the error term.

TABLE 15. Analysis of variance table testing the effects of protocol and method of estrus detection on conception rates (0,1) resulting from first postpartum AI.

Source	N	Type III sum of squares	Mean square	F Value	P > F
Method ^a	1	1.02	1.02	2.37	0.1458
Protocol ^b	3	3.65	1.22	4.42	0.0087
Protocol*Method ^b	3	1.16	0.39	1.40	0.2548
Herd(Method)	14	6.02	0.43	1.86	0.0272
Protocol*Herd(Method)	42	11.56	0.28	1.19	0.1921
Residual Error	670	154.57	0.23		

^a Hypothesis tested using Herd*Method as the error term.

^b Hypothesis tested using Protocol*Herd(Method) as the error term.

TABLE 16. Analysis of variance table testing the effects of protocol and method of estrus detection on pregnancy rates resulting from first postpartum AI.

Source	N	Type III sum of squares	Mean square	F Value	P > F
Method ^a	1	1.73	1.73	4.83	0.0452
Protocol ^b	3	3.05	1.02	5.33	0.0033
Protocol*Method ^b	3	0.38	0.13	0.67	0.5756
Herd(Method)	14	5.02	0.36	1.67	0.0560
Protocol*Herd(Method)	42	8.02	0.19	0.89	0.6692
Residual Error	856	183.47	0.21		

^a Hypothesis tested using Herd*Method as the error term.

^b Hypothesis tested using Protocol*Herd(Method) as the error term.

TABLE 17. Analysis of variance table testing the effects of protocol and method of estrus detection on cumulative pregnancy rates resulting from inseminations up to 120 d postpartum.

Source	N	Type III sum of squares	Mean square	F Value	P > F
Method ^a	1	0.11	0.11	2.34	0.1485
Protocol	3	0.16	0.54	2.45	0.0319
Protocol*Method	3	0.08	0.03	1.20	0.3218
Herd(Method)	14	0.65	0.05	2.11	0.0318
Residual Error	42	0.92	0.21		

^a Hypothesis tested using Herd*Method as the error term.

TABLE 18. Analysis of variance table testing the effects of protocol and herd on standing activity associated with estrus periods detected by the HeatWatch^{® a} electronic estrus detection system.

Source	N	Type III sum of squares	Mean square	F Value	P > F
Protocol ^b	3	32.63	10.88	0.33	0.8065
Herd	7	230.49	32.93	1.42	0.2017
Protocol*Herd	17	567.08	33.36	1.44	0.1271
Residual Error	141	3269.25	23.19		

^a DDx, Incorporated (Denver, CO).

^b Hypothesis tested using Protocol*Herd as the error term.

TABLE 19. Analysis of variance table testing the effects of protocol and herd on estrus duration associated with estrus periods detected by the HeatWatch^{® a} electronic estrus detection system.

Source	N	Type III sum of squares	Mean square	F Value	P > F
Protocol ^b	3	57.12	19.04	1.78	0.1891
Herd	7	443.30	63.33	1.87	0.0789
Protocol*Method	17	181.87	10.70	0.32	0.9958
Residual Error	140	4742.44	33.87		

^a DDx, Incorporated (Denver, CO).

^b Hypothesis tested using Protocol*Herd as the error term.

TABLE 20. Analysis of variance table testing the effects of protocol and herd on estrus intensity associated with estrus periods detected by the HeatWatch[®] ^a electronic estrus detection system.

Source	N	Type III sum of squares	Mean square	F Value	P > F
Protocol ^b	3	177.49	59.16	1.53	0.2430
Herd	7	138.83	19.83	0.13	0.9959
Protocol*Method	17	657.45	38.67	0.26	0.9989
Residual Error	140	21221.69	151.58		

^a DDx, Incorporated (Denver, CO).

^b Hypothesis tested using Protocol*Herd as the error term.

TABLE 21. Analysis of variance table testing the effects of estrus duration and intensity on the outcome of pregnancy (0,1) from estrus periods detected by the HeatWatch[®] ^a electronic estrus detection system.

Source	N	Type III sum of squares	Mean square	F Value	P > F
Intensity	1	0.071	0.07	0.29	0.5935
Duration	1	0.002	0.00	0.01	0.9341
Intensity*Duration	1	1.073	0.07	0.29	0.5880
Residual Error	165	40.75	0.25		

^a DDx, Incorporated (Denver, CO).

TABLE 22. Analysis of variance table testing the effects systematic breeding programs on efficiency of estrus detection for both visual observation herds and HeatWatch[®] ^a herds.

Source	N	Type III sum of squares	Mean square	F Value	P > F
Method ^b	1	0.11	0.11	10.96	0.0052
Protocol	3	0.16	0.05	2.15	0.1099
Protocol*Method	3	0.02	0.01	0.30	0.8266
Herd(Method)	14	0.14	0.01	0.41	0.9619
Residual Error	38	0.95	0.02		

^a DDX, Incorporated (Denver, CO).

^b Hypothesis tested using Herd(Method) as the error term.

TABLE 23. Analysis of variance table testing the effects of method of estrus detection on the 14-d PGF_{2α} protocol on interval from PGF_{2α} administration to AI.

Source	N	Type III sum of squares	Mean square	F Value	P > F
Method ^a	1	15.64	15.64	3.12	0.0990
Herd(Method)	14	70.11	5.01	1.05	0.4075
Residual Error	165	787.32	4.77		

^a Hypothesis tested using Herd(Method) as the error term.

TABLE 24. Analysis of variance table testing the effects of method of estrus detection on the GnRH-PGF_{2α} protocol on interval from PGF_{2α} administration to AI.

Source	N	Type III sum of squares	Mean square	F Value	P > F
Method ^a	1	0.34	0.34	0.05	0.8272
Herd(Method)	14	96.57	6.90	2.30	0.0083
Residual Error	109	326.75	3.00		

^a Hypothesis tested using Herd(Method) as the error term.

TABLE 25. Analysis of variance table testing the effects of method of estrus detection and the number of PGF_{2α} administrations for the 14-d PGF_{2α} protocol on the outcome of pregnancy (0,1).

Source	N	Type III sum of squares	Mean square	F Value	P > F
Method ^a	1	0.06	0.06	0.47	0.5062
PGF _{2α} ^b	2	0.17	0.08	0.51	0.6040
PGF _{2α} *(Method) ^b	2	0.38	0.19	1.18	0.3220
Herd(Method)	14	1.76	0.13	0.48	0.9396
PGF _{2α} *Herd(Method)	26	4.20	0.16	0.62	0.9221
Residual Error	141	36.72	0.26		

^a Hypothesis tested using Herd(Method) as the error term.

^b Hypothesis tested using PGF_{2α}*Herd(Method) as the error term.

TABLE 26. Analysis of variance table testing the effects of method of estrus detection on the number PGF_{2α} administrations required before AI.

Source	N	Type III sum of squares	Mean square	F Value	P > F
Method ^a	1	0.48	0.48	1.04	0.3251
Herd(Method)	14	6.48	0.46	0.89	0.5729
Residual Error	171	89.11	0.52		

^a Hypothesis tested using Herd(Method) as the error term.

TABLE 27. Analysis of variance table testing the effects of standing activity associated with estrus periods detected by the HeatWatch^{® a} electronic estrus detection system on the outcome of pregnancy (0,1) for first AI according to the TAI protocol.

Source	N	Type III sum of squares	Mean square	F Value	P > F
Standing Events	1	0.88	0.88	4.86	0.0299
Residual Error	95	17.18	0.18		

^a DDx, Incorporated (Denver, CO).

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