Factors Influencing Reproductive Efficiency

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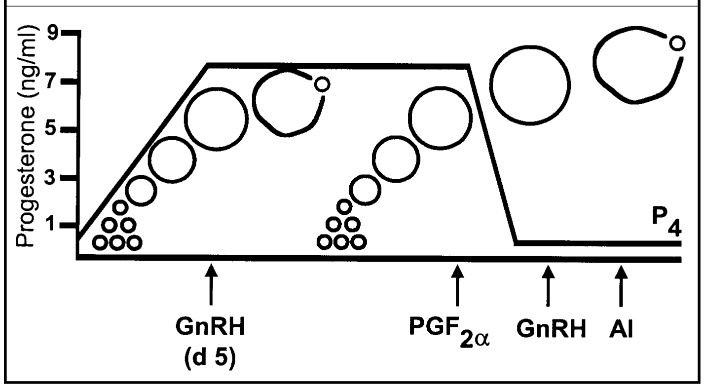
Introduction

The goal of maintaining herd pregnancy rates in current production systems is a challenge due to large herds, confinement systems that are not necessarily conducive to heat detection, cow identification, and the challenge of implementing nutritional management systems that meet individual cow requirements during both the transition and lactation periods that ultimately impact reproductive performance. Under our current production systems, the heat detection rate in high-producing cows is 50% at best. Objectives of this presentation are to examine potential impacts of reproductive technology to increase herd reproductive performance, and to emphasize that use of these new practices has to be founded on an understanding of the technology and its integration with good nutritional management.

Principles and Limitations of Implementing a Timed Insemination Program

At the present time, there are only two drugs available for dairy producers in the USA for use in lactating dairy cows. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) drugs (e.g., Lutalyse®, Pharmacia Upjohn) can be used effectively to regress the corpus luteum (CL) but are ineffective on CL that are developing on days 1 to 5 of the estrous cycle. The other major drug is the Gonadotrophin Releasing Hormone (GnRH) like drugs (e.g., Cystorelin®, Merial Co.) that release both LH and FSH from the pituitary of the cow. GnRH has the ability to ovulate a mature follicle to form a CL, and induce recruitment of a new follicle. Injection of GnRH recruits development of a new dominant follicle, which will induce the cow to express estrus when $PGF_{2\alpha}$ is injected 7 days later. An additional treatment with GnRH after injection of $PGF_{2\alpha}$ induced a timed ovulation. This procedure is known as the Ovsynch Program because it synchronizes ovulation and permits a timed insemination. Pregnancy rates are normal following a timed insemination (see review by Risco et al., 1998). However, the challenge is to further optimize this system.

Figure 1. Plasma progesterone and follicle dynamics during on Ovsynch program started on day 5 of the estrous cycle.



The Ovsynch protocol is idealized in Figure 1. In this example, concentrations of progesterone are monitored to document presence of a CL (the CL produces progesterone), and an idealized description of follicle development is presented. In this example, the cow is injected with GnRH (Monday, 5:00 PM) on day 5 of the estrous cycle. At this time, the cow has a dominant and healthy follicle that ovulates in response to the GnRH-induced release of LH; furthermore, the increase in FSH induced by the GnRH injection induces recruitment of a new pool of follicles in approximately 2 days (day 7) and one of the follicles is selected to become the dominant follicle. On day 12 of the cycle (7 days after the injection of GnRH), $PGF_{2\alpha}$ is injected (e.g., Monday, 5:00 PM) to regress both the original CL present at day 5 of the cycle and a newly formed CL that was induced by the injection of GnRH on day 5 of the cycle. The decrease in progesterone associated with regression of the CL accelerates growth of the newly recruited dominant follicle and a second injection of GnRH is made 2 days after the injection of $PGF_{2\alpha}$ (e.g., Wednesday, 5:00 PM). The second injection of GnRH induces ovulation 24 to 32 hours later. Knowing that ovulation will occur at this time, the timed insemination is given at approximately 16 hours after the injection of GnRH (e.g., 9:00 AM on Thursday). This permits sufficient time for sperm to develop the capacity to fertilize the egg so that when it ovulates, a fertile population of sperm is present to

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carry out fertilization or initiate a pregnancy. This is an idealized scenario and the timing of injections is considered critical to the success of the program. If an interval of less then 7 days is used between GnRH and $PGF_{2\alpha}$ injection, the ability to effectively regress a newly developed CL is reduced. If the second injection of GnRH is delayed to longer than 48 hours or 2 days, then more cows are detected in heat prior to injection of GnRH, cows become asynchronized, and the timing of insemination is not correct. With our present availability of drugs for lactating dairy cows, it is essential that producers not alter the protocol. One commonly asked question is, can cows be inseminated at the time of the GnRH injection or at 24 hours after the injection of GnRH to make the insemination process more convenient? Pregnancy rates will be lowered at the 24 hour insemination, and optimal insemination times appear to be between 12 to 18 hours after the GnRH injection.

Success of the program is dependent on whether lactating dairy cows are cycling as well as stage of the estrous cycle at the time the Ovsynch protocol is initiated in cycling cows. Clearly, if cows in the group are not cycling then it is a given that pregnancy rates of the group will be somewhat less even though the Ovsynch protocol itself may induce some cows to begin to cycle and perhaps conceive. If a cow initiates the program at day 15 of the estrous cycle when a normal second wave follicle is under development. This follicle may or may not ovulate depending upon how mature it is. In many instances the second wave follicle is too small to ovulate and a new CL does not develop. At 2 to 4 days after the injection of GnRH, the cow spontaneously induces regression of the CL by releasing $PGF_{2\alpha}$ from the uterus. Thus, at the time of the $PGF_{2\alpha}$ injection given 7 days after the GnRH injection, the cow has already regressed the CL and may even be in heat. Such cows will be asynchronized in that they will ovulate prematurely and, if we follow the protocol, the insemination is made too late and the cow does not conceive. A second problematic stage of the cycle is in the early phases of the estrous cycle (e.g., day 2). In this scenario, the cow already has been in heat, ovulated and is recruiting a new dominant follicle. This is a small follicle and injection of GnRH on day 2 does not induce a new dominant follicle. As a consequence, at the second GnRH injection, the dominant follicle is considered aged and has expressed dominance for 5 days or longer. Follicles that have periods of dominance longer than 5 days or cows that initiate the Ovsynch program in early stages of the estrous cycle are less fertile. Follicles may ovulate but oocytes are less fertile, or some cows may fail to ovulate their follicle in response to GnRH. We can project what the success rate of the Ovsynch program will be in an idealized situation in which all cows are cycling and are at random stages of the estrous cycle when the program is initiated. Assuming a 20-day estrous cycle, we would expect 5% of the cows to be at each day of the estrous cycle. Thus, for a group of 100 cows, the percent of cows in early stages of the cycle (problematic), early diestrus, late diestrus (problematic) and proestrus have expected pregnancy rates of 20 to 50% at various stages for the reasons described above such that an overall pregnancy rate of 36% is anticipated. However, it is possible to manipulate the estrous cycle of cows such that they are in the ideal stage of the estrous cycle (i.e., days 5 to 10) when the Ovsynch program is initiated. One idealized scenario is to inject all cows twice with $PGF_{2\alpha}$ at an interval of 14 days and to initiate the first GnRH injection of the Ovsynch program on day 12 after the second injection of $PGF_{2\alpha}$. If all cows were cycling, we would expect 90% of the cows to be in the ideal stage of the estrous cycle, between 5 to 10 days, when the Ovsynch program is initiated 12 days after the second injection of $PGF_{2\alpha}$. With this scenario, an expected pregnancy rate to the Ovsynch program is 48%. Such a proposed treatment program prior to implementation of the Ovsynch program is called pre-synchronization with a standard protocol that is practiced in the industry. Effectiveness of such a program will be described later. However, it is imperative that the producer and veterinarian have a thorough understanding of the principles of ovarian manipulation in order to understand how the system functions when they make herd management decisions as to how to implement the program.

Optimization of Reproductive Performance with Pre-Synchronization Prior to the Ovsynch Program and Use of Bovine Somatotropin

A field trial was conducted with the objectives of: determining whether pre-synchronization of lactating cows prior to the initiation of the Ovsynch program would improve pregnancy rates; to verify prior results indicating that bST increased pregnancy rates to the Ovsynch program; to determine whether the possible beneficial effect of bST on pregnancy rates occurred prior to or after timed artificial insemination. Measuring the impact of any therapy or management system on pregnancy rates is a challenge because the experimental response is pregnancy rate in which a considerable number of cows are needed to detect potential differences, and the investigator has to cope with numerous management factors. At least with the Ovsynch program, the management errors associated with heat detection are eliminated and the precise timing of insemination can be controlled tightly. Of concern to dairy producers is whether Bovine Somatotropin (bST®; Posilac®, Monsanto, St. Louis, MO; 500 mg) treatment can be initiated in the ninth week of lactation and be continued without compromising reproductive performance. Our previous research findings indicated that first service pregnancy rates to the Ovsynch protocol were increased when cows received bST treatment at day 63 postpartum concurrently with first injection of GnRH given as part of the Ovsynch program. An additional challenge is to document whether pregnancy rates to the Ovsynch program can be improved with prior implementation of a pre-synchronization program.

Experimental design and treatment of lactating dairy cows:

A total of 543 cows were assigned randomly to the experiment in which half of the cows received the pre-synchronization program. The pre-synchronization program was initiated on a weekly basis such that cows 34 to 40 days postpartum (37±3 days) received an injection of $PGF_{2\alpha}$ (Lutalyse, Pharmacia-Upjohn Co.; 25 mg; i.m.) and this was followed 14 days later (51±3 days) with a second $PGF_{2\alpha}$ injection. In contrast, control cows (no pre-synchronization) did not receive the two injections of $PGF_{2\alpha}$. The rationale for the pre-synchronization program is described above. On day 63±3 days, the first injection of GnRH of the Ovsynch program was initiated, and this was 12 days after the second injection of $PGF_{2\alpha}$ of the pre-synchronization program. The pre-synchronization program will place cows between days 5 to 10 of the cycle at the time of the GnRH injection depending upon what day they expressed estrus after the second injection of $PGF_{2\alpha}$. Days 5 to 10 of the cycle are considered as an optimal time to begin the Ovsynch program as discussed above. On day 7 following the first GnRH injection (70±3 days postpartum), all cows received an injection of $PGF_{2\alpha}$ and the preovulatory injection of GnRH on day 72±3 days postpartum. All cows were time inseminated at day 73±3 days postpartum. A series of blood samples were collected on days 51, 63, 70 72 and 79. Relative comparisons of progesterone concentrations in plasma allow us to determine if cows are cycling (samples on days 51 and 63), stage of the cycle at the beginning of the Ovsynch program (samples on days 63 and 70), whether CL regression was successfully induced (samples on days 70 and 72) and whether cows had a synchronized ovulation (samples on days 72 and 79). All cows were examined by ultrasonography for pregnancy on day 32 after timed insemination and pregnant cows re-examined for pregnancy by rectal palpation on day 74 after timed insemination. This allowed us to characterize fetal losses between 32 and 74 days of pregnancy. All cows diagnosed open at day 32 after the first timed insemination were injected with GnRH and the Ovsynch program repeated with second insemination occurring at 115 days of lactation. The other factors tested in this experiment are the initiation of bST treatment at day 63 (time of the GnRH injection of the Ovsynch program), day 73 (time of artificial insemination as part of the Ovsynch program) or bST-control in which first injection of bST was not given until 147 days of lactation (well after first and second services).

Impact of Anestrous Cows:

With our ability to measure plasma progesterone in two plasma samples collected 12 days apart (on days 51 and 63 postpartum), it is possible to identify exactly what cows are anestrus when the Ovsynch program is initiated. If cows had progesterone ≤1ng/ml in both samples they were considered to be anestrus. It was important to determine which cows are cycling since pre-synchronization treatment effect would not be evaluated properly in cows that are not cycling. Furthermore, this assessment of anestrous status will allow us to document the frequency of this condition and its impact on reproductive performance of the herd. For the assessment of anestrous status, 499 cows had blood samples collected on both days 51 and 63 postpartum. It is interesting that overall 23.4% of the cows were anestrus or had not started to cycle by 63 days postpartum. Not surprising is the observation that the frequency of anestrus was greater for primiparous or first-calf heifers (35.6%) than multiparous cows (16.9%). The occurrence of anestrus decreased as body condition scores recorded at initiation of the Ovsynch improved. Body condition scores accounted for 7.8% of the variation in occurrence of anestrus. Thus, body condition score is not an absolute predictor of what cows are cycling. Some cows with body condition scores of 3.0 were anestrus. As anticipated, anestrous cows did not perform as well as cyclic cows in terms of pregnancy rates to the first-service Ovsynch protocol. Pregnancy rate at 74 days after insemination was only 22.4% for anestrous cows, which was lower than the 41.7% pregnancy rate at 74 days after insemination for cyclic cows. Postpartum management of lactating dairy cows is of extreme importance and will greatly affect reproductive performance. Efforts to maximize cow health, comfort, and nutritional status following parturition (e.g., enhance dry matter intake) will be reflected later in the lactation in terms of a higher incidence of cycling cows and improved reproductive performance.

In the present experiment, an interesting pattern of ovarian responses (induced ovulation pattern) and preg-

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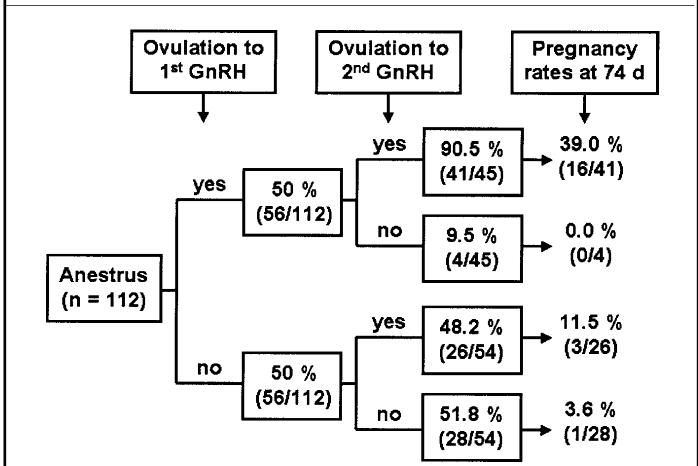
nancy rates for anestrous cows were documented to the Ovsynch protocol (Figure 2). Anestrous cows (n = 112) were classified according to P_4 concentrations. Results indicated that 56 anestrous cows (50.0%) ovulated to the first injection of GnRH of the TAI protocol, as indicated by P_4 concentrations greater than 2.5 ng/ml 7 d later (i.e., LOW-HIGH cows), whereas 56 cows (50.0%) failed to respond to the first GnRH injection and were classified as LOW-LOW. When cows were classified as having either complete or incomplete CL regression based on a 2.0 ng/ml plasma P4 concentration at the time of the second GnRH injection (48 hours after injection of $PGF_{2\alpha}$), only 3.6% (2/55) of LOW-HIGH cows had incomplete CL regression. Neither of the two cows classified as having incomplete CL regression were diagnosed pregnant at 74 days after insemination.

Figure 2. Ovulatory and pregnancy responses of anestrous cows to Ovsynch protocol

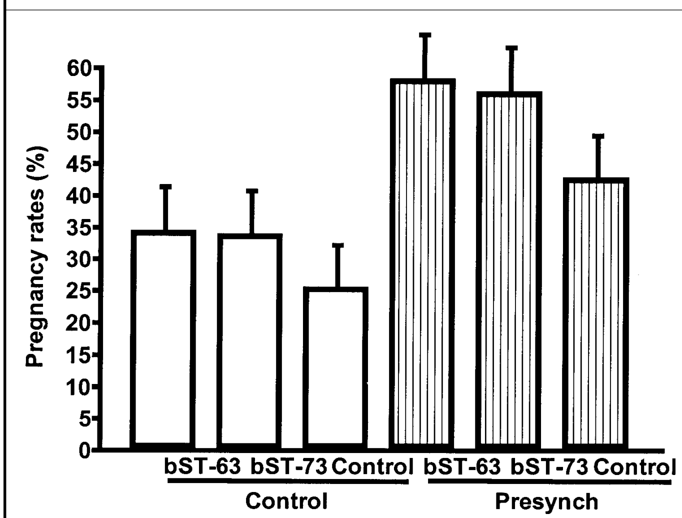


Among LOW-HIGH anestrous cows, which completely regressed their CL upon injection of $PGF_{2\alpha}$ (n = 45), 90.5% were classified as ovulating to the second injection of GnRH in the TAI protocol, whereas ovulation rate to the second GnRH injection for LOW-LOW cows (n = 54) was 48.2% ($P<0.01$). Pregnancy rates for cows ovulating to the second GnRH were higher ($P<0.01$) for LOW-HIGH cows than for LOW-LOW cows at 74 days after TAI (39.0%>11.5%). Only 9.5% of LOW-HIGH cows, which had complete CL regression (4/45) failed to ovulate to the second injection of GnRH and none of those cows conceived to the timed insemination. Among LOW-LOW cows, 51.8% (28/54) were classified as having failed to respond to the second GnRH injection. Among the 28 cows not ovulating to the second GnRH injection of the TAI protocol, only one cow conceived to the timed insemination. Overall, the TAI protocol was able to induce cyclicity in 74.5% (82/110) of anestrous cows, based upon the number of anestrous cows which were classified as ovulating to either the first and/or second injection of GnRH. Pregnancy rates for cows that ovulated to both first and second GnRH injections were enhanced compared to pregnancy rates of cows, which ovulated only after the second GnRH injection. Cows ovulating to both GnRH injections would have had a greater exposure to P_4 before insemination. Based on present results, pre-exposure to P_4 may be limited to a period of a few days (i.e., about 5 days of plasma P_4 greater than 1.0 ng/ml) to achieve acceptable pregnancy rates following a timed insemination in anestrous cows. Furthermore, approximately 75% percent of anestrous cows ovulated to at least one of the two GnRH injections of the TAI protocol. Since an ovulation rate of 90.5 % occurred after the second GnRH (if anestrous cows also ovulated to the first GnRH) and pregnancy rate (39.6%) was as high as pregnancy rate of cyclic cows (41.7%), the TAI protocol may stimulate anestrous cows to cycle earlier. The fact that pregnancy rates to the second service for anestrous cows were similar to those obtained for cyclic cows, constitutes further evidence for a beneficial effect of the Ovsynch protocol when used on anestrous cows in response to two GnRH injections over a 9 day period.

Reproductive performance of Cyclic Cows:

Since anestrus had such a highly significant affect on pregnancy rates, reproductive performance was examined only in cyclic cows. First-service pregnancy rates to the Ovsynch protocol were affected by both bST pre-synchronization and bST treatments (Figure 3). Cows initiating bST treatment at 63 or at 73 days postpartum had increased pregnancy rates compared to controls among cows not pre-synchronized and also among cows pre-synchronized. The fact that a similar stimulation in pregnancy rates was

Figure 3. First-service pregnancy rates diagnosed at day 74 after insemination for cyclic cows (n = 375) following the Ovsynch protocol.



observed in cows treated with bST at 63 (day of first GnRH injection) and at 73 days postpartum (day of timed insemination) indicates that bST is probably enhancing embryonic development and survival following insemination and also may be having effects on the reproductive tract. Concentrations of bST are elevated throughout the 14-day period between injections such that bST injection at the time of the second GnRH injection will still induce elevated concentrations of bST 14 days later during the period of embryo development and this is followed by continued bST injections at 14 day intervals.

Increased pregnancy rates at 74 days after insemination were detected when cows were pre-synchronized (Figure 3; vertical bars; 52.3%) compared to cows not pre-synchronized (white bars; 31.1%). An additional comparison is the effect of pre-synchronization in the two groups that did not receive bST in which pre-synchronized cows had a 42.6% pregnancy rate compared to 25.3% for the control group (Figure 3). This difference associated with pre-synchronization (17.3%) approximates the predicted differences (12.0%) discussed above. The reason for increased pregnancy rates to the Ovsynch protocol in cows pre-synchronized was related to the frequency of cows initiating the synchronization protocol at favorable stages of the estrous cycle. As indicated above, it was hypothesized that pregnancy rates to the Ovsynch protocol would be increased if cows received the first GnRH injection between days 5 to 10 of the cycle, and that pre-synchronization would synchronize approximately 90% of the cycling cows such that these cows would be between days 5 and 10 of the cycle when the Ovsynch protocol was initiated. By collecting blood samples at the first injection of GnRH (at day 63) and again when $PGF_{2\alpha}$ was injected (at day 73), we were able to indirectly identify cows that initiated the synchronization program at the early luteal phases of the estrous cycle (e.g., between days 5 to 10 of the cycle). Cows with high plasma progesterone (> 1.0 ng/ml) at both day 63 and day 73 (i.e., HIGH-HIGH cows) probably initiated the Ovsynch protocol at the optimal stage of the estrous cycle. Results from the frequency of cows classified as HIGH-HIGH indicated that approximately 87.4% of pre-synchronized cows were classified as HIGH-HIGH versus only 71.7% of cows not pre-synchronized were HIGH-HIGH cows. Therefore, we were successful in programming the cows to be in the optimal stage of the cycle to begin the Ovsynch program. As a consequence, pre-synchronization increased first-service pregnancy rates by enhancing the rate of synchronized ovulations and this increased the percentage of inseminated cows to respond to bST treatments.

Results from our field experiments indicate for the second time that bST increased first-service pregnancy rates to the Ovsynch protocol. Such an observation impels us to review previous reports of decreased reproductive performance in cows receiving bST and find explanations for such a discrepancy. It has been reported that use of bST may reduce the rate of estrous detection that may reduce reproductive performance of lactating cows. However, when estrous detection was eliminated though the use of the Ovsynch protocol, the inefficiency of heat detection possibly associated with bST may have been eliminated. We surely found no evidence that bST had any detrimental effect on reproductive performance in our studies and dairy producers should not be inhibited to begin a bST program in concert with an Ovsynch program. Indeed, pregnancy rates were increased at first service to timed inseminations as part of an Ovsynch program.

Impact of Body Condition Score on Pregnancy Rates to the Ovsynch Program

There is the perception that pregnancy rates are lower in lactating dairy cows with poor body condition. Retrospective analyses of our field experiments indicate that as body condition score increases pregnancy rate increases to the Ovsynch program. We recently completed an experiment that examined pregnancy rates of the Ovsynch program in cows that had Body Condition Scores (BCS) of <2.5 versus ≥2.5 (Moreira et al., 2000c). Pregnancy rates at days 27 and 45 after insemination were 18.1% and 11.1% for cows with a low BCS (81 cows) versus higher rates of 33.8% and 25.6% for cows with BCS ≥2.5. The proportion of cows conceiving to the first synchronized service was lower for the cows in low body condition, and this was a temporary decrease since rates of cumulative pregnancies during the ensuing 120 days postpartum were similar). This demonstrates the importance of optimizing fertility to the first service. Utilizing the differences in pregnancy rates in cows with body condition scores <2.5 versus ≥2.5, dynamic modeling was used to estimate net revenue per cow per year when considering what percentage of the herd had a low BCS of <2.5. The difference in net revenue was $10.33 per cow per year as to whether 10% versus 30% of the herd had low body condition scores at the time the Ovsynch program was initiated. Thus, it is essential that producers try to nutritionally manage the dynamics of body condition postpartum to optimize fertility rates. Why does a low body condition score result in a lower pregnancy rate to the Ovsynch program? Is the rate of anestrus (non-cycling cows) responsible for the poor pregnancy rates or are pregnancy

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rates in cows cycling reduced due to defective eggs and/or the reproductive tract is unable to maintain a pregnancy? The encouraging result presented above in which anestrous cows ovulating twice within a 9 day period to the Ovsynch protocol had a normal pregnancy rate suggests a major constraint in anestrous cows is a failure of ovulation.

Integrated Nutritional and Reproductive Management

Successful management of lactating dairy cows needs to integrate the disciplines of reproduction and nutrition with standard postpartum herd health programs to optimize both milk and reproductive performance. The achievement of high energy intake, to bring cows out of a decreasing negative energy status as early as possible postpartum, is critical for both productivity responses. In the majority of lactating dairy cows, development of dominant follicles on the ovary occurs very early in the postpartum period. However, functional competence of these follicles varies in association with concentrations of IGF-1 in plasma and energy status in which the majority of these follicles emerged after the nadir in energy status. The ability of early dominant follicles to either ovulate, undergo turnover or to form follicular cysts influences length of the postpartum anovulatory period. Both regulation of IGF-1 and preovulatory surges of LH appear to be critical to the efficiency of this process. Subsequent timing of ovarian cycles, measured by formation of CL, also is related to postpartum concentrations of IGF-1 and energy status. It is clear that the anestrous condition impacts reproductive efficiency to timed insemination systems such as Ovsynch and that nutritional programs such as fat feeding may reduce the incidence of anestrus and thus benefit herd reproductive management.

Exciting strategies have developed to integrate nutritional and reproductive management. Fats (concentrated energy sources) can be incorporated into the diet of cows in early postpartum in order to try to minimize the differences between energy intake and energy output. Absorption of total fatty acids by the ruminant is linear up to 1200 g/day, which is about 6% of DMI. Typical nonfat-supplemented diets contain about 2 to 3% fat. Therefore it appears that there is significant room to increase the use of fat in diets without loss of efficiency. Because fat is an energy dense nutrient, it is natural to suppose that supplemental fat would improve energy status of the cow. However, this has not been the result in many cases. Oftentimes energy status is not affected by feeding fat because either DMI is depressed or milk production is increased. Nevertheless, feeding supplemental fat has proven effective in improving reproductive performance of lactating dairy cows. Conception rates were improved by feeding prilled fat or calcium salts of long chain fatty acids.

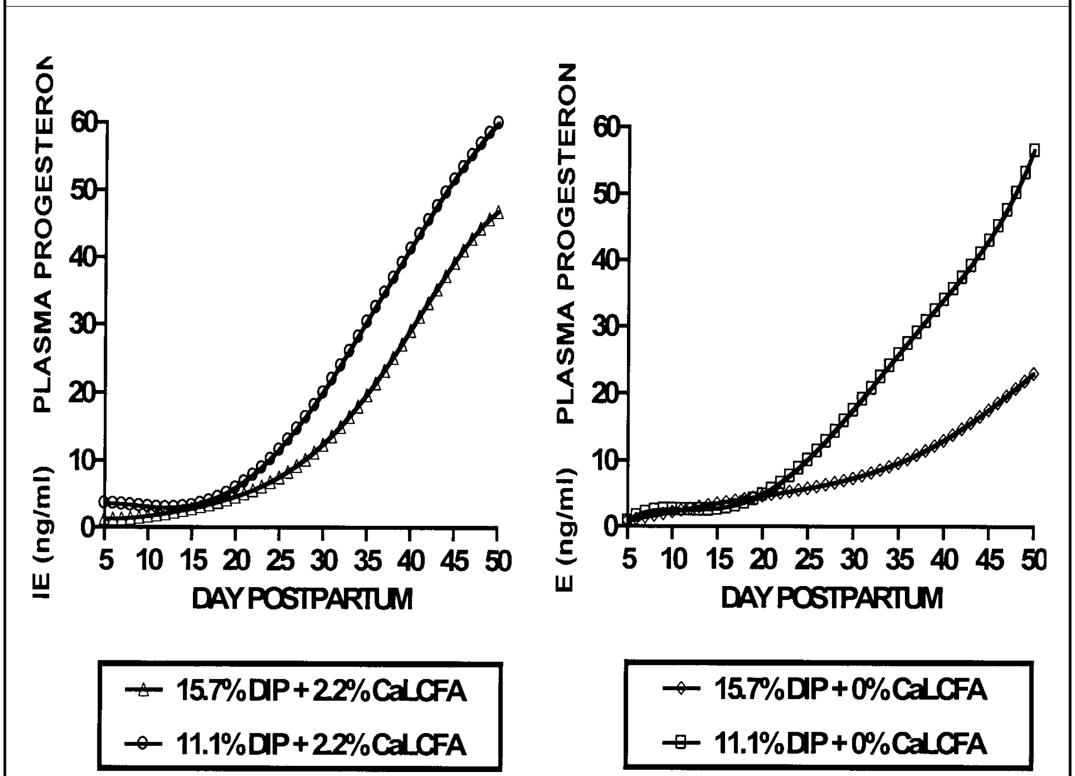
The detrimental effects of feeding a high degaradable intake protein (DIP) diet on reproduction can be alleviated by supplemental fat feeding (CaLCFA). One possibility of how high protein feeding may adversely affect reproductive performance is the increased energy costs to the animal for detoxification of ammonia resulting in a "weakening" of the cow's energy state. This energy cost is likely to push early postpartum cows even further into negative or less positive energy states, thus delaying return to normal ovarian activity. To test the effects of intake of energy and DIP on reproductive performance of lactating dairy cows, 45 cows were assigned at calving to 20% CP diets containing either 15.7% or 11.1% DIP and 0 or 2.2% CaLCFA (Megalac®). Crude protein intake was 1100 g greater than required for milk produced. Treatments continued through 120 days in milk. Cows fed the highly degradable protein diets had greater BUN values (22.0 vs. 17.3 mg%). Based upon progesterone concentrations of blood samples taken three times per week, cows fed the 15.7% DIP diets experienced more days to first luteal phase postpartum than cows fed other diets (39 vs. 25 days). All cows on experiment were synchronized to estrus between days 50 and 57. Cows not cycling prior to synchronization were assigned 50 days to first luteal activity. If cows had not been synchronized, the number of days to first luteal activity likely would have been even greater for cows fed the 15.7% DIP diets. Four out of 10 cows fed 15.7% DIP diet without CaLCFA were anestrus at synchronization compared with only three out of 35 cows fed the other dietary treatments. These prolonged days to restoration of ovarian activity and the anestrus condition were matched with greater loss of body weight and body condition by these cows. Cows fed 15.7% DIP diets lost more body weight and for a longer period of time compared with cows fed 11.1% DIP diets. The absence of CaLCFA resulted in a 10 kg greater loss in BW of cows fed 15.7% DIP diets. In addition, body condition loss was greater and more prolonged by cows fed the CaLCFA-free, 15.7% DIP diet.

The additional energy costs of detoxifying ammonia from highly degradable dietary protein possibly led to a greater reliance on body energy stores for milk production. This resulted in a more severe energy state that delayed ovarian activity. By including CaLCFA in the diet, the energy shortage was somewhat alleviated, allowing cows to rely more on feed energy and less on body reserves for milk production. Days to first estrous was reduced by 6 days when CaLCFA was fed with 15.7% DIP diets. Accumulated progesterone concentrations throughout the postpartum period are depicted in Figure 4. The detrimental effect of 15.7% DIP diets was alleviated markedly by supplementation of CaLCFA, but supplementation of CaLCFA to the 11.1% diet was not stimulatory. Results indicate that dynamics of postpartum ovarian activity can be suppressed indirectly by feeding of high DIP (15.7%), but this adverse effect can be alleviated partially by feeding of CaLCFA. Also of interest was the observation that pregnancy rate by 120 days postpartum was increased from 52.3% to 86.4% when CaLCFA was supplemented and evaluated as a main effect across diets. This study demonstrated the specific benefit of feeding by-pass fat to increase ovarian cycles and reduce the incidence of anestrus in the postpartum period which is a major impediment to herd reproductive efficiency as described above.

Another example of integrated nutritional and reproductive management programs is a study involving 186 cows that evaluated effects of whole cotton seed (WCS) feeding and low doses of bST on reproduction during the postpartum period of lactating dairy cows. Diets were total mixed rations (TMRs) formulated according to the requirements for lactating Holstein cows. Within 24 hours after calving, cows received one of two experimental diets ad libitum. All cows that were on bST treatment received 208 mg (0.5 ml) of bST (Posilac®), Protiva Co., St. Louis, MO) subcutaneously every 2 weeks starting within 7 days of calving. This dose of bST is 50% of the standard commercial dose rate. Since increases in IGF-1 appear to be stimulatory to follicle and ovarian development as described above, we were interested in administering bST at a low dose to evaluate ovarian activity and subsequent fertility. Healthy cows were assigned randomly to one of four treatments (T). Treatments were WCS diet group (15% of DM) with (+WCS +bST; T3) or without (+WCS –bST; T1) bST and no WCS diet groups with (–W CS +bST; T2) or without bST (–W CS -bST; T0). Although early ovarian activity may be associated with subsequent increases in fertility, we feel that it is important not to sustain a long period of progesterone exposure during the period of uterine involution. Consequently, all cows received $PGF_{2\alpha}$ (25 mg im , Lutalyse®), Pharmacia-Upjohn Co., MI) at 30±3 d postpartum to regress any CL and reduce progesterone concentrations. This stimulates turnover of CL and ovarian follicles, permits clearance of uterine contents, and reduces exposure to progesterone that may inhibit uterine defense mechanisms and predispose the uterus to infection. Blood samples were collected three times a week from calving until initiation of the Ovsynch protocol. The Ovsynch protocol was initiated on 65±3 days postpartum, and cows were timed inseminated at day 75. On day 111 postpartum (36 days after insemination) cows were diagnosed for pregnancy by ultrasound examination. If cows were not pregnant the Ovsynch protocol was repeated and second insemination was made at day 121 postpartum. Thus all cows received their first insemination on day 75 postpartum and both inseminations required no heat detection. Following second service, cows were watched for heats for subsequent services.

Feeding WCS diets stimulated ovarian activity based upon a greater accumulation of progesterone during the postpartum period up to 62 days

Figure 4. Regression curves of accumulated plasma progesterone concentrations from lactating Holstein cows fed diets containing 11.1% and 15.7% degradble intake protein and/or 0% and 2.2% CaLCFA.



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postpartum when the Ovsynch program was initiated. The increase in accumulated progesterone associated with WCS diets was associated with an earlier occurrence of a progesterone rise following $PGF_{2\alpha}$ injection on day 30 (39.2<43.5 days), and a higher peak progesterone elevation during the rise after $PGF_{2\alpha}$ injection (11.4>9.25 ng/ml). The increase in ovarian activity as measured by accumulated progesterone concentrations may have been associated with higher plasma concentrations of HDL-cholesterol in the WCS treatment group (107.4>83.5 mg/100ml). Cholesterol is essential for the synthesis of progesterone. Although ovarian activity differed significantly between diets with and without WCS, pregnancy rates did not differ following timed inseminations to either the first, second, or accumulative pregnancy rate to first and second service (Table 1). Pregnancy responses demonstrate the advantage of integrating a reproductive management program with nutritional management. Although the diet without WCS was associated with a lower level of ovarian activity, implementation of the Ovsynch protocol stimulated and controlled ovarian activity such that there was no dietary treatment effect on fertility. Indeed the Ovsynch protocol permitted a very precise first service for all cows, and the re-synchronized Ovsynch procedure for cows that did not conceive to first service guaranteed a second service within a 46 day period for all open cows.

Our field experiments with Ovsynch indicate a lower fertility rate in cows identified to be anestrus and in lower BCS. With our ability to guarantee that all cows can be inseminated precisely at a designated time postpartum with the use of Ovsynch , producers can lengthen the voluntary waiting period, since the time of first insemination is controlled more precisely. If all cows are cycling, a normal program of inseminating at detected estrus, assuming a 50% estrus detection rate, would have to be started at day 40 to ensure that mean time of insemination will be day 70 (range 40 to 100 days). However, an Ovsynch program permits all inseminations to be made at 70±3 days if implemented on a weekly basis. Furthermore, an assessment of pregnancy rates for cows that underwent Ovsynch between 76 to 100 days postpartum was greater than cows that received Ovsynch between 50 to 75 days (47% vs. 35%). Thus, it may be an advantage to delay first inseminations until a period of greater fertility, using the Ovsynch program to ensure that there will be no net loss in time to first service by controlling the time of insemination for all cows.

Improved Embryo Survival

Early embryonic losses have been documented in numerous studies in cattle. Factors such as dairy versus beef cattle, fertile versus repeat breeder cows, insemination to spontaneous versus synchronized estrus, season of year relative to heat stress conditions, and other factors such as parity, nutrition and disease contribute to the time of embryonic losses. It is clear that infertile cows (e.g., repeat breeder cows) or groups of animals in which the proportion of infertile cows is high experience appreciable embryonic losses (~30%) by day 7 of pregnancy. In more fertile groups of animals, embryonic losses (~40%) occur gradually between days 8 to 17 of gestation. When the transfer of morphologically normal embryos eliminated fertilization failure and early embryonic losses, 24.4% of recipient cows terminated their pregnancies between day 17 and 24. The rate of late embryonic mortality after day 27 in a fertile group of dairy heifers was estimated to be 10.6% and this agrees with an estimate of 10.5% in lactating dairy cows that were pregnant at 28 days and lost a pregnancy by day 42. Collectively, these reports indicate differential timing of embryonic losses and a failure of different mechanisms to sustain embryo development. Several investigators demonstrated lower progesterone concentrations in plasma of cows that failed to conceive and this was evident as early as day 6 after insemination. Development of the embryo is related to concentrations of progesterone and ability of the conceptus to secrete the antiluteolytic hormone, interferon-ϑ. In fact, exogenous progesterone stimulated embryo development (see review, Thatcher et al., 1994). Studies to supplement progesterone during the luteal phase after insemination (i.e., after day 5) with insertion of intravaginal progesterone releasing

Table 1. Least square means for pregnancy rates at day 45 after timed artificial insemination (TAI) for cows fed diets of 0 or 15% WCS and injected with 0 or 208 mg of bST at 14 days interval.

Treatment	Total cows	1st TAI (%)	2nd TAI (%)	1st and 2nd TAI (%)
0% WCS; no bST	50	37.1	23.6	51.3
15% WCS; no bST	45	33.6	26.4	51.8
0% WCS; +bST	43	27.1	35.8	51.1
15% WCS; +bST	48	27.3	26.2	46.8

devices for 6 to 12 days have had inconsistent effects on pregnancy rates. An alternative strategy to increase progesterone concentrations is to induce an accessory CL by ovulating the dominant follicle at day 5 of the estrous cycle with an injection of hCG (e.g., 3,300 IU, i.m.). The hCG induced CL elevates progesterone concentrations to a greater degree than observed with the use of GnRH.

A study was designed to determine the effects of hCG (3,300 IU i.m.) administered on d 5 after AI on accessory CL formation, plasma progesterone concentration, conception rate, and pregnancy loss in high producing Holstein dairy cows. Following synchronization of estrus (GnRH followed 7 d later by $PGF_{2\alpha}$) and AI at detected estrus, 406 cows were injected with either hCG or saline on d 5 after AI (203/treatment). Blood sampling and ultrasonography of ovaries were conducted once between days 11 and 16 after AI. Pregnancy diagnosis was performed by ultrasonography on d 28 and by rectal palpation on days 45 and 90 after AI. Treatment with hCG on d 5 induced formation of one or more accessory CL in 86.2% of the hCG-treated cows compared with 23.2% in the controls. Differences in progesterone concentrations between hCG and control cows were +6.3 ng/ml for primiparous cows and +3.1 ng/ml for multiparous cows. Accessory CL increased progesterone concentration in hCG-treated cows but not in controls. Treatment with hCG increased conception rates on days 28 (45.8>38.7%), 45 (40.4>36.3%), and 90 (38.4>31.9%) after AI. Pregnancy losses between days 28 and 45, 45 and 90, and 28 and 90 were similar between the two groups. Progesterone concentration and number of CL after AI affected conception rate such that pregnant cows had higher progesterone concentrations and a greater frequency of accessory CL. Body condition score at AI and milk yield affected conception rate, but no interaction between these variables and treatment were observed. However, hCG improved conception rate in cows loosing BCS between AI and day 28 after AI. Treatment with 3,300 IU of hCG on day 5 after AI induces the formation of one or more accessory CL, increases plasma progesterone concentrations, and improves conception rate of high producing dairy cows.

Multiple mechanisms may contribute to the increase in conception rate in response to hCG. Injection of hCG at day 5 after estrus increased progesterone concentrations and induced a three wave follicular cycle. In all hCG-treated heifers, the dominant follicle of the third follicular wave did not reach 9 to 10 mm in size until approximately day 20. Thus the potential estrogenic follicle for hCG treated heifers would not occur until d 20 versus day 14 in control heifers with a two-wave follicular cycle. Therefore, hCG treatment would decrease the estrogenic environment during the period of pregnancy recognition. Injection of hCG on day 7 has increased conception rates in lactating dairy cows with a slight increase in plasma progesterone concentrations. Systems of progesterone delivery are needed that increase substantially both the rate of progesterone rise and absolute luteal phase concentrations of progesterone after insemination. Such systems need to deliver progesterone in an amount equivalent to what the normal CL can produce at various physiological stages.

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