# Effect of Calcium and Energy Status During the Postpartum Period on Reproductive Performance in Dairy Cows

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#### Introduction

Pregnancy rate, a function of heat detection and conception rate, determines the number of days postpartum at which cows become pregnant from the voluntary waiting period.<sup>67</sup> Cows expressing one or more estruses during the first 30 days postpartum had improved pregnancy rates compared with anestrous cows.83 This observation indicates that the physiological and hormonal events associated with estrus help restore uterine and ovarian function to a state conducive to pregnancy. A number of studies have demonstrated the relationship between hypocalcemia and parturient disorders such as dystocia, retained fetal membranes (RFM), and ketosis.<sup>13,30,68</sup> These disorders predispose the cow to metritis, which can affect the energy status of the cow. This influences ovarian activity and resumption of cyclicity during the postpartum period.

The energy status of the lactating dairy cow has differential effects on early follicular development and competence, ovulation and subsequent corpus luteum (CL) function during the postpartum period. As our understanding of the endocrinology and reproductive physiology of the postpartum cow has advanced, it is clear that reproductive and nutritional management systems need to be integrated to achieve optimal reproductive efficiency.

Objectives of this presentation are 1) to describe the association between calcium status and periparturient disorders 2) to relate the differences in ovarian activity to energy status in postpartum dairy cows 3) to examine the effects of protein and fat on reproductive function during the postpartum period, and 4) to document the beneficial effects of bovine somatotropin (bST) on first-service pregnancy rates when integrated into a reproductive management program for timed insemination.

#### Association of Calcium Status to Periparturient Problems

During calving or shortly thereafter, hypocalcemia is inevitable in the dairy cow and is characterized by a blood calcium concentration < 8.0 mg/dl.<sup>28,59</sup> Hypocalcemia develops as a result of the sudden drain of calcium to colostrum at the onset of lactation, resulting in a tremendous challenge to the cow's ability to maintain calcium homeostasis. A cow producing 10 L (Kg) of colostrum (2.3 g of Ca/kg) will lose 23 g of calcium in a single milking. This represents about 9 times as much calcium as that present in the cow's entire plasma calcium pool.<sup>28</sup> At parturition, 30 g or more of calcium must be replenished daily into the calcium pool to maintain normocalcemia.

Parturient paresis or milk fever is the clinical manifestation of hypocalcemia, or decreased plasma calcium content in affected cows. In a study involving 39 hypocalcemic parturient cows, paresis was associated with plasma calcium levels below 5 mg/100 ml.<sup>50</sup> The degree of hypocalcemia appeared to be more critical for the development of paresis than did the duration of hypocalcemia. In 10 cows with milk fever, calcium levels were evaluated for 7 days postpartum.<sup>68</sup> Calcium levels were lower only in the sample prior to calcium therapy. After a single intravenous treatment with a 23% 500 ml calcium gluconate solution, clinical signs regressed in all cases, indicating that calcium administration was sufficient to restore plasma calcium levels. Calcium deficits in a mature dairy cow with parturient paresis have been reported to be around 8 g. A standard dose of 500 ml of a 23% calcium gluconate solution provides 10.8 g of calcium.

Hypocalcemia may affect organs that have smooth muscle function such as the uterus, rumen and the abomasum. A significant association between parturient hypocalcemia, dystocia and retained fetal membranes (RFM) in dairy cows has been reported.<sup>12,13,30</sup> Cows with parturient hypocalcemia were 6.5 times more likely to have dystocia, 3.2 times more likely to have RFM and 3.4 times more likely to have a left-displaced abomasum.<sup>13</sup> After evaluating the lactation and health records of over 61,000 dairy cows in Finland, Grohn, et al.<sup>30</sup> found that parturient hypocalcemia was a significant risk factor for dystocia, RFM and clinical ketosis. The latter was associated with silent heats, cystic ovaries and infertility.

Reductions in blood calcium content may affect normal function of the uterus, rumen and abomasum, without causing the animal to become recumbent and unable to rise. This condition has been referred to as subclinical hypocalcemia and has been associated with various periparturient disorders.<sup>30,68</sup> In a California study, hypocalcemia without paresis was more common in cows affected with uterine prolapse than controls.<sup>69</sup> The prolapsed uterus was related to uterine atony, a delay in cervical involution and continued abdominal presses soon after parturition. Parturient hypocalcemia has been shown to delay cervical involution and cause uterine inertia.<sup>57</sup> In the study mentioned above,<sup>69</sup> there was no difference in serum calcium concentrations between 9 pairs of first parity cows which had prolapsed and their control contemporaries. This supports the clinical finding that primiparous cows seldom experience milk fever.

Hypocalcemia has been associated with displaced abomasum and reduced rumen contraction. Cows that had abomasal displacement in an Iowa study had abnormally low blood calcium content preceding displacement.<sup>36</sup> Cows affected with hypocalcemia without paresis (total Ca < 8 ng/ml) were 4.8 times more likely to develop left displacement of the abomasum.<sup>49</sup> In a study involving sheep, Huber, et al.<sup>35</sup> demonstrated a true cause-and-effect relationship between hypocalcemia and normal smooth muscle contractility in the ruminant stomach. The major conclusions of this study were: 1) ruminal contractions ceased long before signs of hypocalcemia were observed, 2) ruminal dysfunction may occur substantially before the clinical signs of hypocalcemia. In a study<sup>15</sup> that compared total serum calcium concentrations of cows diagnosed with abomasal displacement or volvulus with that of unaffected cows from the same herds, hypocalcemia occurred in over twothirds of affected cows, suggesting that calcium administration at the time of correction of these conditions may be beneficial.

#### **Energy Status**

Postpartum dairy cows undergo a marked change in energy status as they return to normal ovarian

cycles. Energy status has been defined as the net energy intake of the animal minus the net energy required for maintenance and minus the net energy necessary for milk production. Dairy cattle undergo a period of negative energy status in early lactation because energy output via milk production exceeds energy intake via feed consumption.

Any periparturient disorder that predisposes the cow to go off feed will further contribute to the negative postpartum energy status. Prolonged hypocalcemia after calving may suppress feed intake, and may exacerbate the negative energy status of the cows during early lactation. Cows with milk fever have been reported to have a lower dry matter intake postpartum than nonparetic cows.<sup>48</sup> Further, hypocalcemia prevents secretion of insulin, preventing tissue uptake of glucose which would enhance lipid mobilization and increasing the risk for ketosis.<sup>44</sup> In a study that evaluated uterine involution in cows with milk fever, mean uterine horn diameter was larger between days 15 to 32 postpartum when compared to non-milk fever postpartum cows.<sup>68</sup> The slower rate of uterine involution was attributed to a more severe negative energy balance reflected by a greater loss of body condition score body condition score (BCS) during 30 days postpartum in milk fever cows, and not to hypocalcemia.<sup>68</sup> Beede, et al.<sup>4</sup> have suggested that postpartum disorders associated with hypocalcemia may have major consequences on the health and productivity of the postpartum cow. Hypocalcemia may result in the "droopy cow" syndrome sometimes observed early postpartum, even in cows that did not show clinical milk fever at calving. Goff, et al.<sup>26</sup> have reported that 10% to 50% of cows remained subclinically hypocalcemic (plasma calcium < 7.5 mg/dl) up to 10 days postpartum. Similarly, in cows with RFM and uterine prolapse without signs of milk fever, hypocalcemia was observed during the first 7 days postpartum.<sup>68</sup> Calcium treatment early postpartum, particularly in cows affected with dystocia or RFM, would help restore blood calcium concentration and promote normal function of calcium-dependent organs. This treatment would aid the cow in making a smoother transition during the early postpartum period. Intravenous calcium gluconate products can be given as in milk fever cases. Alternatively, peroral calcium products are available for treatment of hypocalcemia.27,28,58,65

Postpartum reproductive function can be divided into recrudescence of ovarian follicular activity, induction of a spontaneous LH surge that induces ovulation, and formation of fully functional corpora lutea that will maintain a pregnancy. The effect of energy status upon integrated ovarian activity during early lactation was assessed by examination of plasma progesterone profiles of 54 multiparous Holstein cows.<sup>77</sup> Based on their plasma P<sub>4</sub> concentrations (<1 ng/ml), 28% of the cows (n=15) were anestrus (no ovarian cycles) for the 9-week postpartum period. These cows were compared with 2 cycling groups: a group of 25 cows showing CL activity within 40 days of parturition and a second group of 14 cows showing CL activity between 40 and 63 days postpartum. The energy status during the first 2 weeks postpartum was very important. Both the later-cycling and non-cycling cows were in progressively negative energy states; that is, their energy state was more negative in the second week than in the first week. This was especially true for the anestrous cows. Intake of feed by anestrous cows continually lagged behind that of cycling cows. Not only did anestrous cows eat less at week 1 postpartum, but the difference between them and cycling cows became greater as time went on.

On the average, anestrous cows ate between 5.5 and 7.9 lb (2.5 and 3.6 kg) less feed per day than cycling cows. The cows returning to CL activity the earliest started their recovery to a positive energy state immediately after the first week. The movement back toward positive energy status appears to be important in initiation of estrus. Butler and Smith<sup>10</sup> calculated that ovulation occurred approximately 10 days after the negative energy status nadir.

Concentrations of insulin-like growth factor I (IGF-I) in plasma were related closely to the recrudescence of CL activity among cycling and anestrous cows.<sup>78</sup> Cows that cycled early in the postpartum period experienced an early increase in IGF-I concentrations (2 weeks postpartum), whereas cows that were anestrous through 8 weeks of lactation did not exhibit a rise in plasma IGF-I concentrations until 5 to 6 weeks postpartum. Cows beginning to cycle between 40 and 63 days postpartum showed an intermediate pattern in IGF-I concentrations in plasma. These changes in IGF-I were related to metabolic differences among cows that began to cycle at different times and were critical to follicle development and subsequent formation of the CL. The marked deficit in early energy status for the anestrous cows exerted a marked carry-over effect on conception. Only 33% (5/15) of anestrous cows eventually conceived compared to 84% (21/25) and 93% (13/14) for early and late cycling cows, respectively.

In order for cattle to ovulate in the early postpartum period, ovarian follicular activity must be re-established and followed by normal CL activity. Lucy, et al.<sup>47</sup> examined ovarian follicular dynamics in a group of 52 multiparous Holstein cows during the postpartum period. Between day 7 to 25 postpartum, the number of class 1 follicles (3 to 5 mm in size) decreased with increasing days postpartum, whereas the number of class 3 (10 to 15 mm) and class 4 (>15 mm) follicles increased. These changes are characteristic of cows developing dominant follicles early in the postpartum period. When day postpartum was ignored and energy status was considered, the number of class 1 and 2 (6 to 9 mm) follicles decreased with increasing energy status, whereas the number of class 3 follicles increased. This interaction between energy status and follicle size classes was not significant when cows receiving a diet containing calcium salts of long-chain fatty acids were removed from the analysis. Nevertheless, these follicular responses indicate that as the postpartum period increased, follicle growth was stimulated and may be associated partially with energy status. Follicles were recruited from smaller classes and moved into larger classes from which one follicle was selected and developed into the ovulatory follicle. In fact, 37 of the 52 cows had formed a CL by day 25. Double ovulations had occurred in 12 cows by day 25 postpartum. Those cows had a more positive energy status than did cows having a single or no ovulation.

## **Body Condition**

Body condition scores (BCS) postpartum are related with the magnitude and severity of negative energy balance. Cows that lose more than 0.5 BCS postpartum have been reported to have compromised reproductive performance.<sup>16</sup> Furthermore, pregnancy rates to first service are lower in cows with a BCS < 2.5 during the first 100 days postpartum.<sup>7,8,54</sup> Because nearly all cows lose body condition postpartum, cows should be in good body condition at calving. A score of 3.25 to 3.75 at calving is recommended.

Cows that are over-conditioned at calving are also candidates for excess body condition loss postpartum. Over-conditioned cows are unable to increase their dry matter (DM) intake quickly postpartum. As a result, body reserves are relied upon heavily to help support milk production. Heifers carrying approximately 48 lb (21.8 kg) more fat at calving (BCS of 3.72) ate 2 lb (0.95 kg) less per day of DM and lost approximately 75 more lb (34 kg) more of body weight than controls.<sup>31</sup> Overconditioned cows were 2 weeks later in achieving a positive energy status than cows in good body condition and fed high-energy diets. One BCS unit (converted to U.S. system) was lost in order to support milk production by overconditioned cows compared to a slight gain in body condition for control cows over the 10-week period.<sup>39</sup>

The reproductive problems of fat cows may not be due solely to lowered feed intakes. Recent data from Florida indicates that fatter non-lactating cows were less able to maintain a persistent follicle in the absence of a CL, but exposed to progesterone from an intravaginal controlled internal drug releasing device (CIDR), whereas thinner cows maintained a persistent follicle.<sup>6</sup> This difference in ovarian follicular response could be due to greater clearance of supplemental progesterone from the blood stream by fat cows.

Changing body condition through dietary manipulation requires strategic planning and careful consid-

eration. Under-conditioned cows should put on condition during the late lactation period because they are more efficient at utilizing metabolizable energy during this time than during the dry period (75% vs. 60%). In addition, the dry period may be too short to fully recover condition needed prior to calving. Cows should not lose weight during the dry period, as the cow must gain 1 to 1.5 lb/day simply to meet the needs of the rapidly developing fetus.

The probability of conception to occur at first insemination can be determined by the loss of BCS during the postpartum period. Domecq, et al.<sup>16</sup> investigated the relationship between changes in BCS during the dry period, early lactation and conception to first service in 720 Holstein cows. This study<sup>16</sup> concluded: 1) cows that lost 1 point of BCS in the first month of lactation were 1.5 times less likely to conceive than were cows that did not lose 1 point of BCS; 2) energy balance during the dry period and early lactation, as monitored by BCS, was more important to conception to first service than were health disorders or other risk factors evaluated. In several Florida field trials with lactating dairy cows, body condition during the first 100 days postpartum was related to conception rate.<sup>7,8</sup> An experiment was designed that compared pregnancy rates to timed insemination using the Ovsynch protocol for the first service of lactating dairy cows with body condition scores < 2.5 (Low BCS group) versus  $\geq 2.5$  (Control group) using a 1-to-5 scale.<sup>54</sup> The Ovsynch timed insemination protocol has been tested against control treatments that employ insemination at detected estrus in dairy heifers and in lactating dairy cows. In general, the program has been producing pregnancy rates that are either similar or greater when compared to pregnancy rates for the control groups.<sup>2,5,8,14,23,63,64,70</sup>

This is an excellent reproductive management system to evaluate specific nutritional and hormonal effects on pregnancy rates and was used to examine the effect of BCS on pregnancy rates. At 63±3 days postpartum, cows were assigned to experimental groups (n=81 for the Low BCS group and n=126 for the Control group) and began the 9-day Ovsynch timed insemination protocol. Cows returning to estrus were re-inseminated at detected estrus. Cows were examined by ultrasonography for pregnancy at 27 days after insemination and by rectal palpation at 45 days. Pregnancy rates were less for the Low BCS group compared to the Control group at day 27  $(18.11\% (\pm 6.10) < 33.83\% (\pm 4.55); P < 0.02)$  and at day 45 (11.14% (+5.49) < 25.64% (±4.10); P<0.02). Rates of cumulative pregnancies through either 120 or 365 days postpartum were lower for Low BCS cows (P<0.01). Thatcher, et al.<sup>80</sup> used a dynamic programing model to determine the additional revenue per cow per year in dollars of various scenarios in which the percentage of the herd with low body condition score (<2.5)

varies (Figure 1). For example there is an increase in net revenue per cow per year of \$10.33 between a herd of cows with a low body condition rate of 10% versus 30%. The data presented in Figure 1 is unique to this commercial herd of cows in which the study was completed. However, it gives the relative costs under various herd management scenarios.



**Figure 1.** Economical estimates of additional revenue using different scenarios based on the percentage of cows with low body condition in the herd (From: Thatcher et al., Proceedings SW Nutritional Conference, Phoenix, AZ, 1999).

#### Elevated Crude Protein Intake and Reproduction

Postpartum changes in uterine involution, restoration of ovarian activity and increased milk production are accompanied by dramatically changing energy and protein states of the animal. Changes in these nutritional conditions have been shown to influence physiological changes associated with reproduction. If more crude protein (CP) is fed than can be utilized by the cow, urea concentrations in body tissues can be elevated. Feeding of diets containing 19% to 21% CP result in elevated BUN concentrations and frequently in lowered conception rates compared with cows fed 15% to 16% CP diets. Older cows are more likely to be affected negatively by elevated dietary CP than younger cows. Not only has the total CP content of a diet proven important for reproductive performance but also the dietary concentration of degradable intake protein (DIP). Replacing soybean meal with a less ruminally degradable protein feedstuff such as fish meal, corn gluten meal, etc. often alleviated some reproductive inefficiency, including delayed first ovulation, lowered conception rates, and elevated embryonic deaths. In general, feeding of excess protein leading to elevated BUN concentrations resulted in some reduction in reproductive performance of lactating dairy cows.

High protein feeding may adversely affect reproductive performance by increasing energy costs to the animal for detoxification of ammonia resulting in a "weakening" of the cow's energy state. The need to detoxify ammonia by animal tissues can be energetically costly. Feeding 100 g of un-utilized CP results in a loss of 0.2 Mcal of energy.<sup>81</sup> If 500 to 1000 g of excess protein is consumed, energy costs could be a quite substantial 2 Mcal/day (up to 7% of NEL requirement for maintenance and production of 66 lb (30 kg) of milk. With energy status averaging about -11 Mcal/day during the first three weeks postpartum,<sup>77</sup> an additional 1 to 2 Mcal/day cost is not small. 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<sup>®</sup>).<sup>21,22</sup> Crude protein intake was 1100 g greater than required for milk produced.<sup>55</sup> Treatments continued through 120 days in milk. Cows fed the highly degradable protein diets had greater BUN values (22.0 vs. 17.3 mg%; P=.01). 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; P=.002). 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 recrudescence 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 body weight of cows fed 15.7% DIP diets. In addition, body condition loss was greater and more prolonged by cows fed the CaLCFAfree, 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 were reduced by 6 days when CaLCFA was fed with 15.7% DIP diets. Accumulated progesterone concentrations throughout the postpartum period are depicted in Figure 2.

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 (interaction among dietary treatments, P<.0001). Results indicate that dynamics of postpartum



**Figure 2.** Regression curves of accumulated plasma progesterone concentrations from lactating Holstein cows fed diets containing 11.1% and 15.7% degradable intake protein and/or 0% and 2.2% CaLCFA. The standard error of the mean was 0.9. The interaction of DIP and CaLCFA differed among dietary treatments (P=0.0001). (From: Thatcher et al., Proceedings SW Nutritional Conference, Phoenix, AZ, 1999).

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.

The fact that elevated intakes of protein may exert its effect through increased energy costs to the animal is supported by the work of Elrod and Butler.<sup>17</sup> They demonstrated that feeding excess CP (21% vs. 15% of diet) lowered conception rates of heifers from 82% to 61% when heifers were fed an energy-deficient diet (70% of ME requirements).

## **Nutritional-Reproductive Management**

The example above demonstrates that the detrimental effects of feeding a high DIP diet on reproduction can be alleviated by supplemental fat feeding (CaLCFA). 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.76 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.<sup>34,72,75</sup> Nevertheless, feeding supplemental fat has proven effective in improving reproductive performance of lactating dairy cows. Conception rates were improved by feeding prilled fat<sup>20</sup> or calcium salts of longchain fatty acids.<sup>21,22,71,73,74</sup>

Integration of nutritional and reproductive management programs is essential for successful operation of the dairy operation. An example of this 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.<sup>1</sup> Cows were housed in an open-sided free-stall barn with grooved concrete floors. The 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<sup>®</sup>, 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. Cows were assigned randomly to

1 of 4 treatments (T) in a 2 x 2 factorial design. Treatments were WCS diet group (15 % of DM) with (+WCS +bST; T3) or without (+WCS -bST; T1) bST and no WCS diet groups with (-WCS +bST; T2) or without bST (-WCS -bST; T0). All cows received PGF<sub>2a</sub> (25 mg im , Lutalyse<sup>®</sup>, Pharmacia-Upjohn Co., MI) at  $30 \pm 3$  days postpartum. Blood samples were collected 3 times a week from calving until initiation of the Ovsynch timed insemination protocol. The Ovsynch protocol was initiated on  $65 \pm 3$ days postpartum, and cows were time 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 timed insemination 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.

Whole cotton seed (WCS) is a common supplemental fat source that is fed in the southeastern USA. Since increases in IGF-1 appear to be stimulatory to follicle and ovarian development as described earlier, we were interested in administering bST at a low dose to evaluate ovarian activity and subsequent fertility. 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, we routinely inject PGF<sub>20</sub> at day 30 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 predisposes the uterus to infection.

Feeding WCS diets clearly stimulated ovarian activity based upon a greater accumulation of progesterone during the postpartum period up to 62 days postpartum when the Ovsynch timed insemination program was initiated (Figure 3; P<.02). 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, P<.05), and a higher peak progesterone elevation during the rise after  $PGF_{2\alpha}$  injection (11.4 > 9.25) ng/ml, P<.05). 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



**Figure 3.** Effect of whole cottonseed on plasma progesterone (P<0.02). (From: Thatcher et al., Proceedings SW Nutritional Conference, Phoenix, AZ, 1999).

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 timed insemination 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.

Table 1.Least square means for pregnancy rates at<br/>day 45 after insemination for cows fed diets<br/>of 0 or 15% WCS and injected with 0 or 208<br/>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

From Thatcher et al., Proceedings of the SW Nutritional Conference, Phoenix, AZ, 1999.

Field experiments with Ovsynch, conducted at the University of Florida, indicate a lower fertility rate in cows identified to be anestrous and in lower BCS as described above. 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 - 100 days). However, an Ovsynch program permits all inseminations to be made at  $70 \pm 3$  days if implemented on a weekly basis. Furthermore, an assessment of pregnancy rates for cows that underwent Ovsynch between 76-100 days postpartum was greater than cows that received Ovsynch between 50-75 days (47% vs. 35%; Pursley et al.,<sup>64</sup> 1995). Thus, it may be an advantage to delay first inseminations until a period of greater fertility, using the Ovsynch timed insemination program to ensure that there will be no net loss in time to first service by controlling the time of insemination for all cows.

# Effect of Bovine Somatotropin on Reproduction

The role of bovine somatotropin (bST) in ovarian folliculogenesis, ovulation and embryo development has gained considerable interest since its approval for use in the dairy industry. A number of reports have indicated that bST reduces fertility in dairy cattle.<sup>9,11,19,32,33,51,52,84</sup> while other investigations failed to observe deleterious effects of bST on dairy herd fertility.  $^{18,56,60,61,66}$  A common feature of studies where bST did not affect fertility was the small number of cows used for comparison, and the fact that bST treatment was initiated late during the postpartum period (after 84 days postpartum, except for two studies where bST was initiated at 60 days postpartum). It has been hypothesized that poor reproductive performance of dairy cows treated with bST may be related to a temporary negative energy balance associated with high milk production.<sup>40</sup> In addition, recent studies have indicated that bST may also affect fertility of lactating dairy cattle by reducing estrus expression and increasing the frequency of undetected ovulations.41,43,52,82

Investigations at Florida examined whether the Ovsynch timed insemination program would provide an efficient system to manage the lactating cow in which bST is initiated on day 63 postpartum.<sup>54</sup> The objective was to determine if bST alters fertility when administered at the initiation of a Ovsynch protocol. Lactating dairy cows (n=403) with body condition score  $\geq 2.5$  were injected with gonadotropin-releasing hormone (GnRH, Cystorelin, Sanofi Inc., KS; 100 µg, im) at 63 days in milk followed 7 days later with  $\mathrm{PGF}_{2\alpha}(25~\mathrm{mg~im}$  , Lutalyse®, Pharmacia-Upjohn Co., MI). At 48 hours after PGF20, cows received a GnRH injection and were inseminated 16 hours later. Cows treated with bST at the initiation of the Ovsynch program had higher pregnancy rates than control animals at day 27 (46.9%[+6.2] > 29.6%[+4.5])and day  $45(37.7\%[\pm 5.8] > 22.1\%[\pm 4.2])$  post-breeding.

Little is known about the physiological window(s) and mechanism(s) by which bST affects reproductive efficiency in cattle. Reproductive tissues (i.e., ovary, oviduct, and uterus) contain receptors for both growth hormone (GH) and insulin-like growth factor - I.<sup>24,42,45,75,83</sup> Furthermore, the uterine endometrium produces IGFs and IGF binding proteins (IGFBPs) which may have an effect on embryonic development.<sup>3,25,75</sup> Thus, uterine function and embryonic growth and differentiation may be dependent partially on local (autocrine) and systemic (endocrine) IGF-I that is produced in response to exogenous bST. Alternatively, bST may have direct effects on the developing embryo, as bovine blastocysts are capable of responding to bST in vitro.<sup>37,38</sup> The current fertility results with bST are very encouraging and an additional study completed at the University of Florida (Thatcher and Moreira, unpublished observations) indicated that pregnancy rates were increased when bST was given at the time of the first GnRH injection or at the time of artificial insemination within the Ovsynch/ TAI protocol. From a management perspective, producers can be confident that bST will not suppress fertility when given at the recommended time of lactation. This fertility response was achieved utilizing the Ovsynch timed insemination protocol which eliminated the need for detection of estrus. The Ovsynch timed insemination protocol has permitted a careful examination of various nutritional and hormonal factors that influence reproductive efficiency. Figure 4 depicts the adverse effect of low BCS on pregnancy rate and that cows with a  $BCS \ge 2.5$  have a higher pregnancy rate when receiving bST at the first injection of GnRH within the Ovsynch timed insemination protocol. Such responses achieved under practical conditions within a commercial dairy are very encouraging.

## Conclusion

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. In addition to paresis, hypocalcemia appears to be a risk factor for dystocia, uterine prolapse, RFM and displaced abomasum, disorders which can negatively affect postpartum health and reproductive performance. Consequently, nutritional management strategies should be implemented during the last 3 to 4 weeks prepartum in order to promote a rapid return to normocalcemia early postpartum. 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 postpar-



**Figure 4.** Least square means and standard errors for pregnancy rates as diagnosed at 45 days after timed insemination (TI) of first service for treatment groups (Low BCS, n=81; TI, n=126; TI + bST, n=67). (From Thatcher et a., Proceedings of the SW Nutritional Conference, Phoeniz AZ., 1999).

tum period. Low body condition scores at the time of insemination are associated with lower pregnancy rates to a detected or timed insemination. Feeding of high degradable protein results in greater loss of body weight and body condition which is associated with a decrease in ovarian activity. In contrast, feeding of supplemental fat in the highly degradable protein diet restores ovarian activity. Feeding a diet containing whole cotton seed as a fat source stimulates postpartum ovarian activity, but this stimulation does not result in increased fertility when cows receive a programmed timed insemination for first and second services. Injection of bovine somatotrophin, beginning at  $63 \pm 3$  days postpartum in concert with a timed insemination program, increases pregnancy rates.

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