

# Effect of Short- and Long-Term Changes in Energy Balance on Reproduction

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## INTRODUCTION

Genetic selection for milk yield alters concentrations of metabolic hormones that direct the flow of nutrients to various tissues and organs (Bauman and Currie, 1980). An example of how selection for milk yield changed blood concentrations of somatotropin, a potent stimulator of lactation, and insulin, a hormone that favors fattening is shown in table 1 (Bonczek et al., 1988). In this selection experiment, Holstein cows were mated with frozen semen from 20 bulls that were in AI service in 1964 and whose merit was near breed average (controls), or to AI bulls that had the highest transmitting abilities for milk during subsequent years (selected). The data in table 1 reveal that five generations of selection for milk yield changed the ratio of insulin to somatotropin from 6.1 to 4.2. Thus genetic selection changed the homeorhetic signals in selected cows to favor higher milk production (increased somatotropin) and to decrease fat deposition (decreased insulin).

Metabolic hormones act through tissue-specific receptors to regulate the ability of cells to release nutrients to or acquire nutrients from the supply available in blood (Bauman and Currie, 1980). For example, incorporation of glucose into lipid within adipocytes (lipogenesis) is decreased by administration of somatotropin to lactating cattle; whereas breakdown of lipids

(lipolysis) to form fatty acids and glycerol is increased by somatotropin. Thus various tissues release stored nutrients or take up available nutrients because of their responses to the action of various hormones. Tissue response is affected by the number of receptors for the hormone on the cell membrane or within a cell and by the amount of hormone presented to the tissue. The amount of hormone presented to the tissue is influenced by blood flow to the tissue and by concentration of the hormone in blood.

The reproductive system competes with other physiological systems for nutrients because the same metabolic hormones that control processes such as growth and lactation also influence the function of reproductive target tissues such as the hypothalamus, ovary and uterus. Nutrient requirements for reproduction are small compared with other physiological processes. For example, the amount of energy required for follicular development, transport of sperm, production of uterine secretions and early embryonic development is negligible compared to the amount required by the liver, heart, central nervous system and other tissues to function. Nevertheless, the reproductive system will cease to function during periods of nutrient deprivation, even when these other tissues appear to function normally.

Table 1. Hormones in selected and control Holstein cows<sup>1</sup>

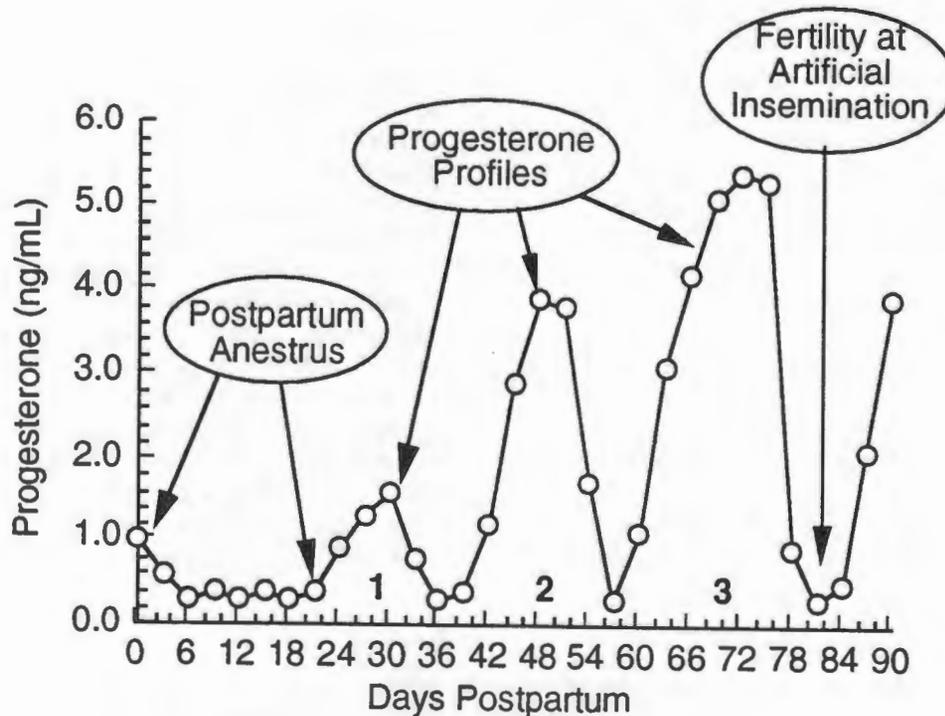
Trait	Control Generation = 5.3	Selected Generation = 4.8
Milk, kg (305 d)	7,401	9,878
Insulin, $\mu$ IU/ml	22.5	18.8
Somatotropin, ng/ml	3.7	4.5

Short- and long-term changes in energy balance exert profound effects on reproduction. The three reproductive stages at which energy balance affects reproduction in the cow are illustrated in Figure 1. This paper will focus on how short- and long-term changes in energy balance in the postpartum cow influence duration of postpartum anestrus, magnitude of progesterone profiles and conception rate at insemination.

### Folliculogenesis in the Cow

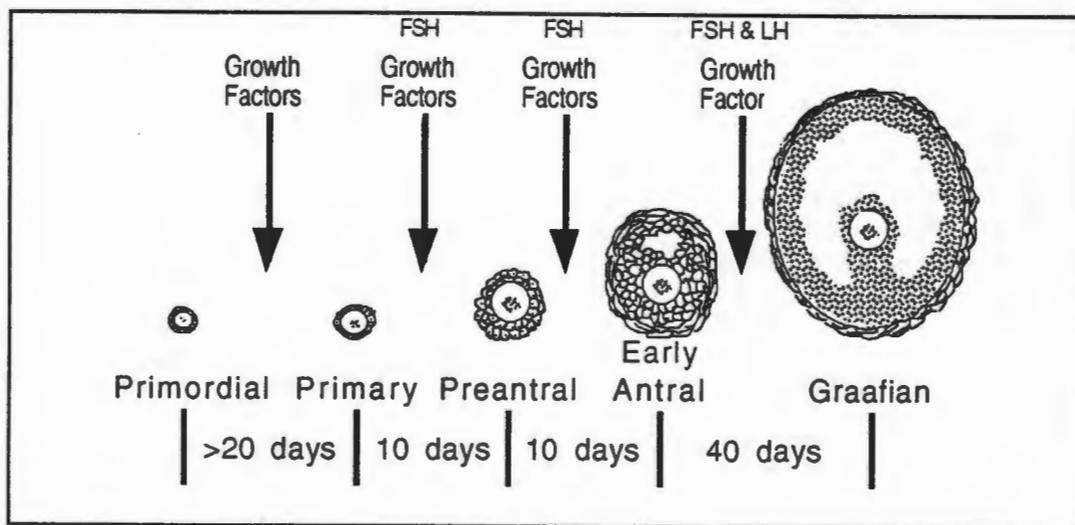
In order to understand how some of the long-term effects of energy balance are mediated, it is important to understand the process of folliculogenesis in the cow. The ovary of the bovine fetus is fully endowed with oogonia and primordial follicles by about mid-gestation, and

follicular growth commences before birth (Erickson, 1966; Marion et al., 1968). Follicular atresia occurs throughout the life of the cow, but even cows more than 15 years old have thousands of residual follicles (Erickson, 1966). The time-course of folliculogenesis from the primordial stage to ovulation is only partially known for the cow. Preantral development has not been studied, but once the antrum is formed, approximately two estrous cycles are required for a follicle to reach ovulatory size (Lussier et al., 1987). By extrapolation from Lussier et al. (1987) one can estimate that it takes about 80 to 100 days for an activated primordial follicle to reach ovulatory size (figure 2). This would be consistent with our estimate of the time required for folliculogenesis to occur in the pig (Morbeck et al., 1992).



**Figure 1. Reproductive processes affected by changes in energy balance in the postpartum cow. Duration of postpartum anestrus is affected by interval from calving to the energy balance nadir. Magnitude of progesterone profiles is influenced by energy balance during the first few weeks postpartum as well as by the temporal change in energy balance during four weeks before each ovulation. Fertility at AI is influenced by both short- and long-term energy balance because of effects on quality of follicles and progesterone secretion by the corpus luteum.**

**Figure 2. Folliculogenesis in the bovine.** This illustration is based on studies of antral follicle growth in the bovine (Lussier et al., 1987) and studies of effects of growth factors on follicle growth in laboratory animals.



Factors that regulate growth of preantral follicles are only beginning to be elucidated, and most of the experimental data have been derived from rodents. Nevertheless, regulatory mechanisms that control activation and development of preantral follicles are likely to be similar among species. Oocytes in developing preantral follicles are highly active in terms of synthesis of nucleic acids and protein and such oocytes double in size during the preantral growth period (Roy and Greenwald, 1991). During this same period, number of granulosa cells surrounding the oocyte increases linearly and thecal cells are first observable by histological techniques (Erickson, 1966; Marion et al., 1968). Microscopic cellular connections between the vitelline membrane of the oocyte and plasma membrane of granulosa cells indicate that cellular components of developing follicles communicate, and thickening of the zona pellucida between the oocyte and its surrounding cells indicates that there is active secretion of glycoproteins.

Experimental data support the concept that early development is dependent on a variety of growth factors, particularly epidermal growth factor (EGF), insulin-like growth factor-I (IGF-I), basic fibroblast growth factor (FGF) and transforming growth factor(s). Roy and Greenwald (1991) cultured isolated preantral follicles from hamsters with various combinations

of growth factors and measured DNA synthesis. They found that EGF was the most potent stimulator of DNA synthesis, and that IGF-I robustly stimulated DNA synthesis and acted synergistically with EGF to promote incorporation of labeled thymidine into DNA. Significantly, EGF and IGF-I stimulated greater incorporation of thymidine into DNA in follicles of stages 1-3 than in follicles at latter stages; stages 1-3 represent follicles with 1 to 3 layers of granulosa cells surrounding the oocyte.

Boland et al. (1993) reported the successful culture of mouse follicles from the primordial stage through ovulation in vitro. Growth of preantral follicles in media containing insulin, transferrin, pyruvate, glutamine and 5% mouse serum devoid of gonadotropins paralleled growth of follicles in the same milieu supplemented with FSH, indicating that gonadotropins are apparently not essential for normal development to the preantral stage. However, FSH is clearly required for formation of the antrum (Boland et al., 1993) and it will stimulate incorporation of thymidine into DNA in early stage follicles (Roy and Greenwald, 1991). Although insulin and IGF-I can directly influence early follicular growth (DNA synthesis), there is little evidence that somatotropin can exert such a direct effect. Bovine ovarian stromal tissue and the preantral follicles therein do not express the somatotropin receptor, but the bovine CL and particularly large

luteal cells strongly express the somatotropin receptor (Lucy et al., 1993).

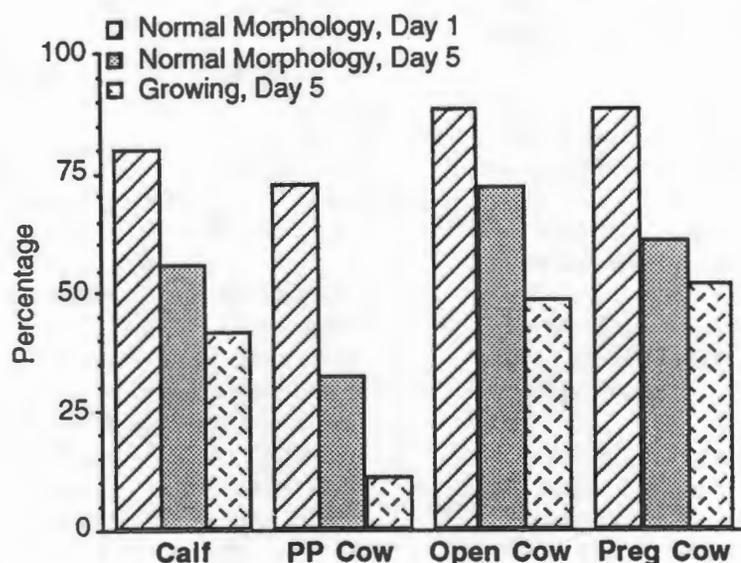
Because there is substantial evidence that metabolic factors can influence early follicular development, it is conceivable that changes in metabolism in the early postpartum period could influence preantral follicles destined to ovulate weeks later during the breeding period (figure 2). Although little is known about development of preantral follicles in the postpartum cow, there is good evidence that the population and health of the earliest antral stages change in the first 5 wk postpartum. For example, Dufour and Roy (1985) found that the number of smallest-sized, non-atretic antral follicles decreased from 28 per ovary on day 15 to 1.5 per ovary on day 35 in postpartum Holstein cows. In contrast, there was an increase in number of non-atretic follicles in the next two larger-sized categories over the 20-d sampling period. These data indicate that follicles that have reached the antral stage by 2 wk postpartum continue to develop normally during the next few weeks, but replenishment of the

antral pool is not sustained. More evidence for an effect of early postpartum metabolism on follicular development was recently provided by Figueiredo et al. (1994) who isolated preantral follicles from the ovaries of calves and cows at different reproductive stages and cultured these for 5 days in vitro. Overall, follicles from early postpartum cows had the poorest morphology at the time of isolation (Day 1) and after 5 days of culture (Day 5), and fewer of these follicles were still growing on Day 5 (figure 3). This could mean that negative EB and associated changes in metabolism during the early postpartum period curtail development of preantral follicles or cause more of them to become morphologically abnormal or atretic.

### Postpartum Anestrus and Cyclicity

Fertility in the postpartum cow is influenced by the number of estrous cycles that occur between parturition and first insemination (figure 1). Thatcher and Wilcox (1973) reported that cows that had experienced two or three periods of estrus before insemination had nonreturn rates of

**Figure 3.** Morphology and development of preantral follicles isolated from ovaries of cattle at different reproductive stages. Follicles from early postpartum cows (PP Cow) had the poorest morphology and poorest growth in vitro during a 5-day culture. Drawn from results of Figueiredo et al. (1994).



44 to 47% compared to 34% for cows that had not shown estrus before the breeding period beginning at 60 days postpartum. Thus, when inseminated at the same time postpartum, cows that have experienced two or more estrous cycles before being inseminated are more fertile than those inseminated at first or second cycle.

Studies of Butler and his colleagues (Butler et al., 1981; Butler and Smith, 1989; Canfield and Butler, 1991) revealed that commencement of postpartum estrous cycles is closely linked to the occurrence of the postpartum nadir in negative EB. Thus, when negative EB reaches its lowest point sooner and then begins returning toward a positive EB, cows resume cycles sooner and breed back quicker than when the negative energy nadir occurs later. In addition to its effect on cyclicity, postpartum EB may also influence expression of estrus in cyclic cows. For example, Harrison et al. (1989, 1990) and Spicer et al. (1990) found that cows experiencing more severe negative EB were detected in estrus at lower rates during the first two postpartum estrous cycles than herdmates that had higher EB.

The principal factor that affects postpartum EB is feed intake. Villa-Godoy et al. (1988) reported that variation in EB in postpartum Holstein cows was influenced most strongly by dry matter intake (DMI;  $r = .73$ ) and less by milk yield ( $r = -.25$ ). Thus differences among cows in the severity of negative EB is more related to how much they eat than it is with how much milk they produce. The effect of negative EB on duration of postpartum anestrus is mediated mainly through its effect on secretion of gonadotropins, because final maturation of an antral follicle and ovulation requires sustained episodic secretion of luteinizing hormone (LH; Stevenson and Britt, 1979; Stevenson and Britt, 1980). Gonadotropin-dependent, mid-antral follicles are present at the time of parturition, and dairy cows will ovulate as early as the second week postpartum (Fonseca et al., 1983). Prolonged anestrus is usually associated with fairly severe negative EB, and it is more likely to occur in primiparous than multiparous cows.

## Latent Effect of Nutrition and Energy Balance on Progesterone and Fertility

Folman and colleagues (1973) were the first to point out that early postpartum nutrition affected progesterone secretion during the breeding period in high-producing dairy cows. They fed 14 Holstein cows either high-energy rations (6 kg of hay plus free access to concentrates) or rations that included the same amount of hay but restricted amounts of concentrates. Cows were milked three times daily and inseminated at the first estrus beyond 56 days postpartum. Cows that conceived at first service had higher levels of progesterone during the luteal phase before first breeding than cows that failed to conceive. Moreover, among cows requiring more than one service, cows fed the higher energy diet had greater luteal phase progesterone concentrations than cows fed the lower energy diet. Cows fed the higher energy diets required fewer services per conception. The investigators also noted that weight change (an estimate of EB) during the estrous cycle preceding first service influenced progesterone secretion during the luteal phase preceding first service, so that cows that gained weight had higher levels of progesterone than cows that lost weight.

Subsequently, we (Fonseca et al., 1983) studied the relationship between progesterone secretion prior to first service and fertility in 212 Holstein and Jersey cows. In both breeds there was a positive relationship between the level of progesterone in blood during 12 days before first service and conception rate at first service. For Holsteins, each 1 ng/ml increase or decrease in average progesterone during the last half of the estrous cycle preceding first service was associated with a 12.4% difference in conception rate—for Jerseys a 1 ng/ml change in progesterone was associated with a 7.4% change in conception rate. We also noted that for Jerseys there was a positive relationship between weight change during the 28 days before first AI and progesterone during the 12 days before first AI. A 1 kg change in weight during this 4-wk period was associated with a .03 ng/ml change in average progesterone. Thus a cow that lost 20 kg during the 28 days before first AI would have an average progesterone 1.2 ng/ml lower than one that gained 20 kg, and this would result in a 9% difference in first service conception rate.

Villa-Godoy et al. (1988) were the first to point out that EB during 2 wk immediately postpartum exerted a profound latent effect on progesterone secretion. They showed that cows that experienced the greatest decline in EB during 9 d postpartum had the lowest levels of progesterone secretion during the second and third postpartum estrous cycles, 40 to 70 days after parturition. Even though cows differed substantially in EB and progesterone secretion, daily milk yield during the first 100 days postpartum did not differ. Their data support the concept that early postpartum feed intake, not milk yield, is the main factor that influences progesterone secretion during the breeding period. Ferguson et al. (1989) suggested that cumulative negative EB during the postpartum period was more important than nadir per se in influencing subsequent conception rate, but there is a strong correlation between the magnitude of the nadir and cumulative negative EB.

In the last several years, changes in body condition score (BCS) have been used to estimate changes in EB of the postpartum cow. On a 5-point scale, a 1 point change in BCS is equivalent to a 25 to 60 kg change in live weight, unadjusted for changes in gut fill (Otto et al., 1991). Both labile protein and fat are depleted when body condition changes, and a decrease in condition is associated with negative EB. Butler and Smith (1989) found that severity of body condition loss postpartum strongly influenced first service conception rate. Thus, cows that experienced a body condition loss of <.5 points during the first 5 weeks postpartum had a first service conception rate of 65% compared with conception rates of 53% and 17% for cows that lost .5 to 1.0 or >1.0 points of body condition. Ultimately, however, cows that lost higher amounts of body condition had cumulative pregnancy rates similar to cows that lost little condition, so the infertility associated with body tissue loss was temporary. A general observation by several researchers is that body condition loss is greater in cows that have a higher BCS at parturition than in cows that have lower scores. Fatter cows (those with higher BCS) have lower appetites after parturition, and this leads to less DMI and more loss of body tissue.

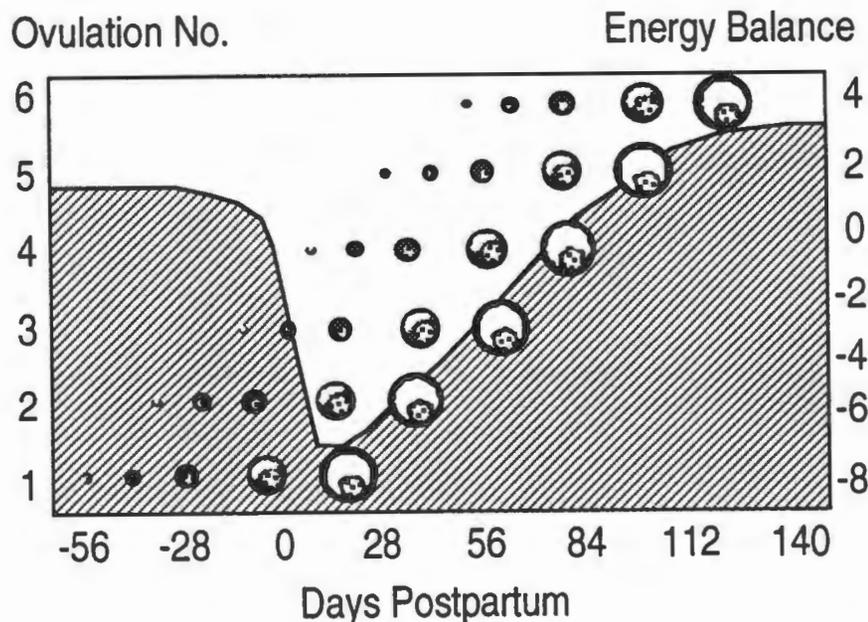
Feeding fat to postpartum cows may change the relationship between EB and fertility through ways that are not clearly understood. For example, Sklan et al. (1991) fed high-producing Holstein cows calcium soaps of fatty acids (CSFA) at the rate of 2.6% of DM for 4 mo postpartum. Cows fed CSFA lost more weight during the early postpartum period and their fertility at first service was similar to controls (39.2 vs 41.6%); however, for services 2-4, cows fed CSFA had higher conception rates (42.6 vs 25.0%). These data indicate that CSFA may somehow restore fertility sooner in cows losing substantial weight postpartum. Subsequently, Sklan et al. (1994) fed CSFA (2.5% of DM) until 120 days postpartum in primiparous and multiparous cows. They noted that milk yield increased in cows fed CSFA, but weight losses were greater in cows fed added dietary fat and these cows had lower fertility at first insemination. Ferguson et al. (1990) showed that feeding fat to high-producing postpartum cows improved first-service conception rate, although the magnitude of the difference in conception rate between cows fed diets with or without fat supplementation varied considerably among four herds. The effects of dietary fat on fertility may be through progesterone secretion, because cows fed CSFA or other fats had higher progesterone levels after breeding than controls (Ferguson et al., 1990; Sklan et al., 1991), or the fat may alter metabolic profiles in a way that favors conception. Some of the mixed results with added fat may be associated with imbalances between dietary energy and protein after the addition of fat to the diet. If dietary protein is not increased to match the increased energy from dietary fat, then available protein may limit any increase in milk yield and the additional calories will be directed toward tissues rather than toward milk synthesis. Lucy et al. (1991) studied the effect of dietary fat on development of the dominant follicle in Holstein cows. Cows were given prostaglandin F<sub>2</sub>-alpha to induce luteolysis and fitted with intravaginal progesterone releasing devices to deliver constant amounts of progesterone. One-half of the cows were fed control diets and one-half were fed diets containing CSFA. Cows fed CSFA had larger diameter dominant follicles than controls, indicating that CSFA may temporarily affect CL function by altering development of the preovulatory follicle.

### Theoretical Explanation for Latent Effect of Nutrition on Progesterone and Fertility

In an effort to understand how losses in body condition (negative EB) could affect fertility several weeks later, a theoretical model was developed (Britt, 1991; Britt, 1992) based on the rates of follicular growth estimated by Lussier and colleagues (1987). The theory proposed that preantral follicles exposed to adverse environmental conditions would have altered gene expression leading to impaired or altered development. Impaired developing follicles would then result in formation of dysfunctional mature follicles, which would produce poorer oocytes and result in the formation of weakened CL. Adverse environmental conditions that might exacerbate the metabolic perturbations accompanying severe negative EB include heat stress or toxic conditions associated with postpartum disease. The temporal scenario associated with exposure of developing follicles to metabolic perturbations is illustrated in figure 4.

One can visualize from figure 4 that the first two ovulatory follicles experience most of their development during the previous dry period when EB is positive. In contrast, the third, fourth and fifth ovulatory follicles experience much of their development when EB is most negative. During this time, the metabolic status would be characterized by elevated somatotropin, low IGF-I, low insulin, low glucose and high nonesterified fatty acids (Canfield and Butler, 1991; Harrison et al., 1990; Spicer et al., 1990; Whitaker et al., 1993). One might expect that follicles which begin to grow when EB is most negative would be exposed to adverse metabolic conditions that would render them less functional. Although the hypothesis states that adverse conditions can affect gene expression during folliculogenesis, little data are available to test this hypothesis directly. However, in a parallel system, the spermatozoa-producing seminiferous tubules of the testis, there is clear evidence that factors such as heat stress, altered nutrition or exposure to toxins can exert adverse effects on gene expression of

Figure 4. Theoretical growth and development of the first six ovulatory follicles in the postpartum cow. Most of the period of folliculogenesis of the first two ovulatory follicles occurs prepartum. Follicles 3-5 experience much of their development during the period of most severe negative energy balance postpartum (Mcal/d; shaded area).



developing germ cells, resulting several weeks later in ejaculation of poor quality sperm.

The theoretical model would predict that the first two postpartum CL would differ little in progesterone secretion between cows that differed in postpartum losses of body condition, whereas the third, fourth and fifth CL would differ because cows that lost more body condition should have dysfunctional ovulatory follicles that form CL which produce less progesterone. To determine whether this model was supported by experimental evidence, data from one of our earlier studies (Fonseca et al., 1983) were reanalyzed to determine the temporal relationship between loss in condition, development of preantral follicles and the secretion of progesterone from these follicles. Data from 76 Holstein cows were sorted into two groups based solely on changes in BCS between the first and fifth week postpartum. Cows above the mean were allotted to one group and those below the mean to the other. Changes in BCS for the two groups for 10 wk postpartum are summarized in figure 3 and table 2. Cows that lost condition during the first 5 wk gained more condition than the other group during the subsequent 5 wk. Milk production did not differ between the two groups, but cows that lost condition commenced estrous cycles about 6 d later than those that maintained condition (table 2). This difference in interval to ovulation was maintained for five ovulatory cycles.

Blood samples collected twice weekly were available for estimation of progesterone secretion during each postpartum estrous cycle (figure 5). One can see that the results support the theoretical model, because CL function, as manifested by progesterone during peak luteal function (days 6-13 of cycle 1; days 10-17 of cycles 2-5) was not different during the first two cycles, but cows that lost more condition produced less progesterone during cycles 3-5. Fertility of cows that differed in amounts of body condition loss also differed (table 3). These data indirectly support the hypothesis that early postpartum differences in metabolism can exert effects on follicular development and therefore influence the CL that are subsequently formed from those follicles. But questions remain about potential mechanisms and what might be the mediators of such effects.

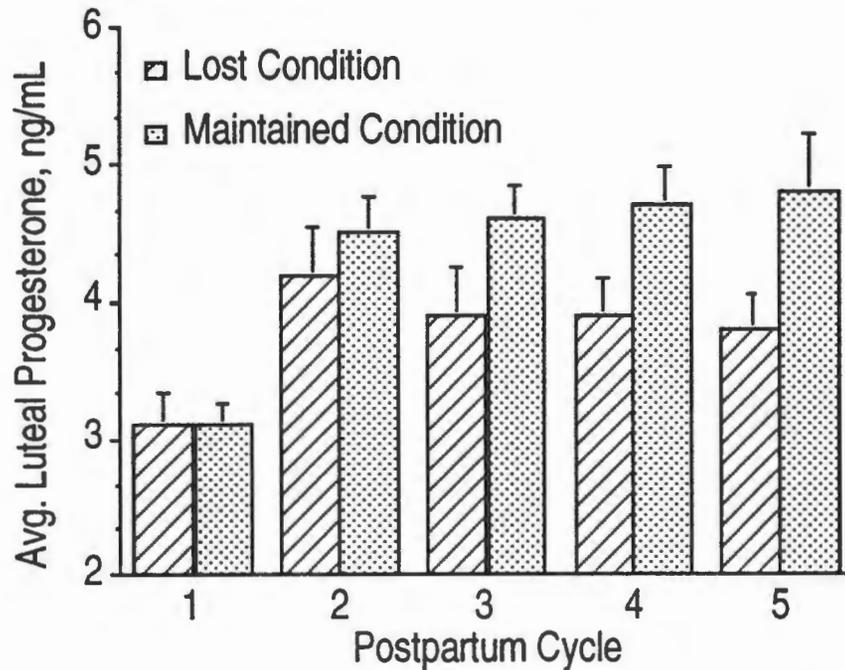
Two experiments with heifers may shed light on potential mechanisms through which changes in metabolism might affect subsequent progesterone secretion. Villa-Godoy et al. (1990) assigned Holstein heifers to two conditioning regimens (fat or moderate body condition) and then fed them to be in negative or positive EB for the next 3.5 estrous cycles. Progesterone was monitored daily throughout the study and CL were removed, dispersed and incubated during the mid-luteal phase of the fourth estrous cycle. Negative EB was associated with a decrease in average progesterone concentrations during the second,

**Table 2. Body condition scores, milk yield and intervals to ovulation for cows grouped according to change in body condition score from week 1 to 5 postpartum.**

Trait	Maintained Condition	Lost Condition
No. cows	46	30
Body condition score change:		
Weeks 1 to 5	+06	-.58*
Weeks 5 to 10	-.02	+17*
Milk yield:		
Yield/d first 70 d postpartum (kg)	26.4	27.3
Average yield for 305 d lactation (kg)	8,155	8,271
Days postpartum to ovulations:		
First	17.2	23.3*
Second	35.8	44.3*
Third	58.7	64.4*
Fourth	78.4	86.1*
Fifth	102.0	110.1

\* P < .05

Figure 5. Average progesterone during the luteal phase of cows divided into two groups based on changes in body condition during the first 5 wk postpartum. Progesterone was measured in blood samples collected twice weekly. Based on re-analyses of raw data of Fonseca et al. (1983).



third and fourth experimental estrous cycles in heifers in moderate body condition, but heifers that were in fat condition showed inconsistent changes in progesterone until the fourth cycle when their progesterone secretion differed from moderate conditioned heifers in positive EB. The ratio of small to large cells of the CL was increased from 5.8 to 9.5 by negative EB in the moderate-conditioned heifers, and such a shift would be consistent with the observed lower secretion of progesterone. Thus one to three estrous cycles elapsed before effects of EB on CL function were detected, and this would be consistent with an effect on mid-preantral and to mid-antral follicles.

Gong et al. (1991) gave heifers saline or bovine somatotropin (bST; 25 mg/d) for two consecutive estrous cycles and then removed ovaries and dissected all antral follicles >2 mm in diameter. The bST-treated and control heifers all had a single CL at slaughter, but the bST treated group had twice as many 2-5 mm follicles as controls (52.5 vs 25.8). Thus, treatment with bST doubled the number of small antral follicles without affecting ovulation rate. The bST-treated heifers also had higher blood levels of

matotropin and IGF-I. Subsequently Gong et al. (1993) inserted bST implants (320 mg) into heifers and then superovulated the heifers with equine chorionic gonadotropin (eCG) 5 d later. The bST-treated heifers ovulated more follicles in response to eCG than control heifers, indicating that bST or IGF-I or both influence late antral follicular growth, but that gonadotropin support beyond endogenous levels is essential for multiple ovulations to occur.

It is not clear from these studies whether bST acted directly to influence follicular development or whether its effect was through IGF-I. The later is most likely, because Echemkamp et al. (1990) showed that IGF-I was higher in blood and follicular fluid of cows that had repeatedly produced twins compared with controls that were monotocous, and Lucy et al. (1993) indicated that the somatotropin receptor is mainly localized in the CL of the ovary. While bST can exert both a short-term and long-term effect on antral follicle function, short-term fasting with an associated decline in blood levels of IGF-I does not affect the amount of IGF-I in follicular fluid (Spicer et al., 1992).

**Table 3. Interval to first insemination and conception rates in cows grouped according to change in body condition score from week 1 to 5 postpartum.**

Trait	Maintained Condition	Lost Condition
Days postpartum to first AI	84.9	82.9
Conception rate (%)		
First service	62*	25*
All services	61*	42*
3rd ovulation	63	27
4th ovulation	67	50
5th ovulation	53	44

\*P<.05

## CONCLUSIONS

Fertility during the 3rd and 4th month postpartum in high producing dairy cows has been associated with EB during the first month postpartum. Thus cows that experience greater losses in body condition after calving have lower fertility during the usual breeding period compared to cows that experience less loss in body condition. It is proposed that preantral follicles may be biologically imprinted by physiological changes that accompany negative energy balance in the early postpartum period and therefore at maturity produce oocytes that are of lower quality or form corpora lutea that secrete less progesterone. The end result is lower fertility.

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