

Nutritional and Hormonal Factors Influencing Follicle Development in Cattle

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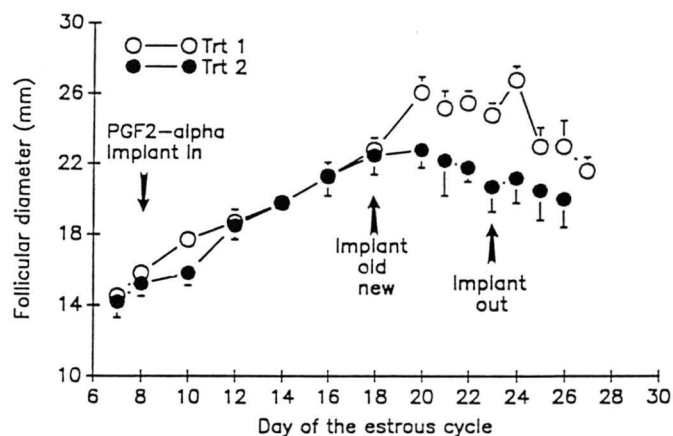
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Introduction

Ovarian antral follicular development in cattle proceeds through stages of follicular recruitment, selection and dominance. This is a reoccurring process during the estrous cycle when either one or two dominant anovulatory follicles develop prior to development of the ovulatory follicle. These changes in follicle development can be monitored by ultrasonography (Savio *et al*, 1988; Sirois and Fortune, 1988; Knopf *et al*, 1990). Apparently, dominant follicles control development of other follicles through the production of factors that act locally and/or systemically. This regulatory process is currently under intensive investigation. The process of dominance causes a single ovulation which is characteristic of cattle. We believe that growth of anovulatory dominant follicles during the estrous cycle is halted due to the negative feedback effect of progesterone from the CL on LH secretion. Insufficient LH secretion will not support the continued growth and function of the follicle. Consequently, the dominant follicle undergoes atresia and a new follicle is recruited and becomes the dominant follicle of the next wave. The importance of LH secretion was tested (Savio *et al*, 1990a) by giving PGF_{2 α} (Lutalyse; Upjohn Co., Kalamazoo, MI) on day 8 of the estrous cycle and maintaining a weak progestin environment by the insertion of a norgestomet implant into the ear (Synchro-mate-B; Ceva Laboratories, Overland Park, KS). The implant was either removed on day 23 post estrus (Treatment 1) or replaced with a new implant on day 18 which also was removed on day 23 (Treatment 2). It is clear that the first wave follicle was sustained until the implant was removed on day 23 (Figure 1). Furthermore, growth of this single follicle wave was greater in cows of treatment 1 that retained the original implant. Indeed basal LH secretion was greater on days 10 (5 pulses per 8 hours) and 19 (7 pulses per 8 hours) postestrus in treatment 1 compared to day 10 of the estrous cycle (control animals with an intact CL; .75 pulses per 8 hours). Insertion of a new implant on day 18 (treatment 2) reduced the pulse frequency of LH on day 19 (3 pulses per 8 hours). This experimental model demonstrated that alterations in

Figure 1. Growth of first wave dominant follicle in cycling dairy cattle injected with PGF_{2 α} and receiving a norgestomet implant on day 8. Implant was removed on day 23 postestrus (Treatment 1) or replaced with a new implant on day 18 that was also removed on day 23 (Treatment 2).



basal LH secretion modulate follicular development during the estrous cycle. This has profound implications relative to the management of ovarian follicles for the synchronization of estrus and subsequent fertility at this estrus. Furthermore, this endocrine scenario may account for the occurrence of ovarian follicular cysts in the early postpartum period. Indeed the early postpartum period is characterized by early growth of ovarian follicles in the absence of a CL (low progesterone; Savio *et al.*, 1990b). Perhaps a sustained basal secretion of LH and the inability to have a preovulatory surge of LH (hypothalamic-pituitary dysfunction) leads to the development of dominant and estrogenic follicular cysts. Our basic understanding of ovarian follicular dynamics will lead to rationale systems of follicular management to improve reproductive efficiency.

Association of Energy Balance with Dynamics of Follicle Development

Energy balance has been defined as the net energy intake of the animal minus the net energy required for maintenance and minus the net energy secreted in the milk. Dairy cattle undergo a period of energy deficiency in early lactation because maximum milk production is attained prior to maximum feed consumption. In order for cattle to ovulate in the early postpartum period, ovarian follicle activity needs to be re-established and this is dependent upon restoration of LH secretion following calving (Fernandes, *et al.*, 1977). Recently, Canfield and Butler (1991) reported that the postpartum restoration of pulsatile LH secretion did not occur until the maximal decrease in postpartum energy balance was observed and animals began to reverse their decrease in energy balance.

Since growth of ovarian follicles is dependent on LH secretion, we would anticipate that ovarian follicle dynamics would be related to differences in postpartum energy balance in lactating dairy cows. A group of 52 multiparous Holstein cows were fed one of six diets consisting of a totally mixed ration (corn silage, corn grain, soybean meal, dried distillers grains, and whole cottonseed) plus either alfalfa hay, alfalfa cubes, or bermuda grass hay fed chopped as a component in the mixed ration or separate as long hay (Lucy *et al.*, 1991a). Predicted energy balance was calculated from dry matter intake, milk yield and composition, and body weight. On day 25 postpartum, ovarian status was programmed by injecting 25 mg of prostaglandin $F_{2\alpha}$ (PGF $_{2\alpha}$) and treating cows for 15 days with an intravaginal device that releases progesterone (CIDR-B device; Carter-Holt Plastics Molding Co., New Zealand). Throughout the postpartum period (days 7 to 60), number and size of ovarian follicles were determined by ultrasonography.

Between days 7 to 25, the number of class 1 follicles (3 to 5 mm in size) decreased with increasing days postpartum, and the number of class 3 (10 to 15 mm) and class 4 (>15mm) follicles increased (Figure 2). When day postpartum was ignored and energy balance was considered, the number of class 1 and class 2 (6 to 9mm) follicles decreased with increasing energy balance, and the number of class 3 follicles increased (Figure 3). Indeed, any differences due to diets in this early period (< day 25) were accounted for by energy balance. These follicular responses indicate that as energy balance increases follicle growth is being stimulated and follicles are being recruited from smaller classes and move into larger classes in which one follicle will be selected and developed as the ovulatory follicle. In fact by day 25, 37 of the 52 cows had formed a corpus luteum.

Multiple ovulation (presence of two or more corpora lutea) occurred in 12 cows by day 25 postpartum. Those cows experiencing multiple ovulations had a higher average energy balance than did cows having a single or no ovu-

Figure 2. Average number of follicles within different size classes (Class 1: 3 to 5 mm; Class 2: 6 to 9 mm; Class 3: 10 to 15 mm; Class 4: >15 mm) in cattle (n = 52) from day 7 to 25 after calving.

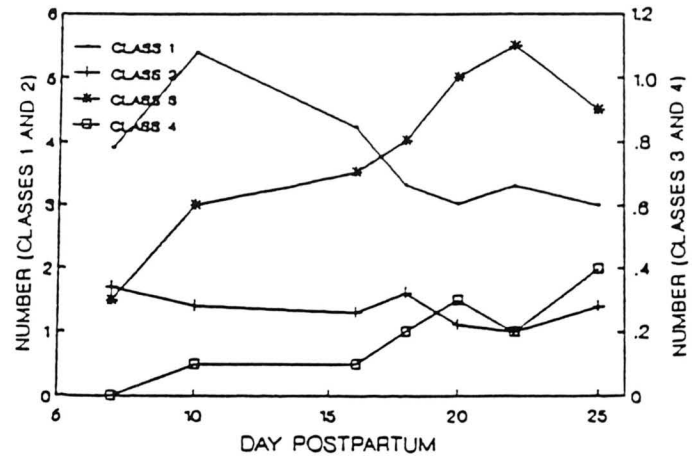
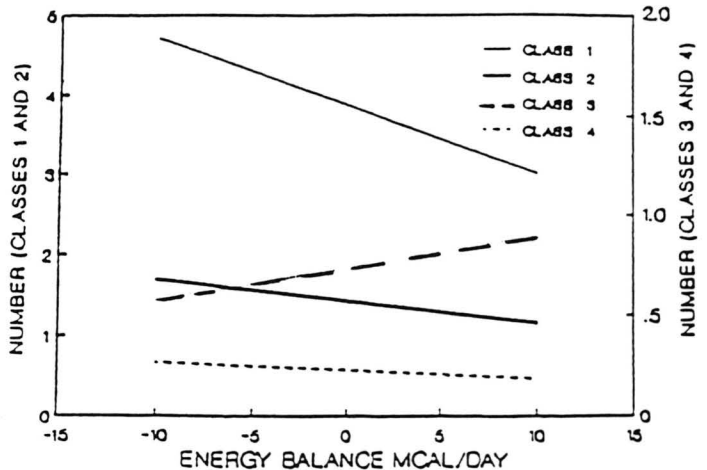


Figure 3. Predicted regression lines for predicted energy balance and number of follicles in Classes 1, 2, 3 and 4 during the period of day 7 to 25 after calving.



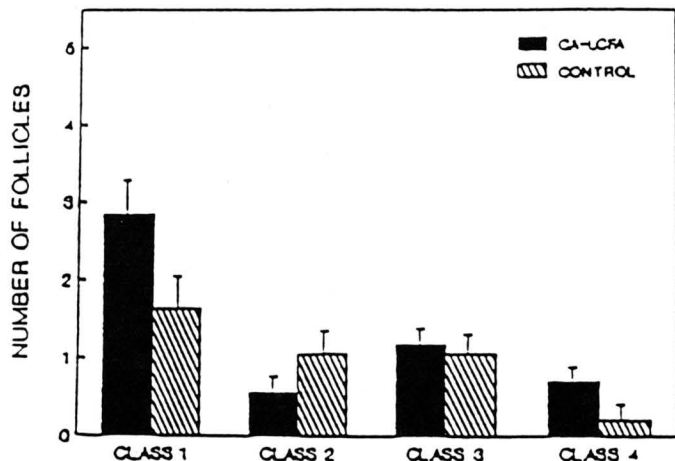
lation. Once again this implies that energy balance was associated with aspects of follicle development. As predicted from the results of Canfield and Butler (1990), the greater degree of follicle development with an increase in energy balance likely is due to the increase in LH secretion. In fact, the amplitude of LH peak concentrations was associated positively with both energy balance and size of the largest follicle on day 10 postpartum in the present experiment.

Effects of Fatty Acid Supplementation on Ovarian Activity

Perhaps, it is possible to minimize the effects of postpartum negative energy balance on ovarian activity by increasing caloric density of the diet. To evaluate the effects of feeding calcium salts of long chain fatty acids (CaLCFA;

Megalac®, Church and Dwight Co., Inc.; Princeton, NJ) on postpartum ovarian activity (ovarian follicular dynamics and corpus luteum activity), multiparous Holstein cows (n=18) were fed a total mixed ration containing corn silage, corn grain, whole cottonseed, soybean meal, dried distillers' grains, and chopped bermudagrass hay (control) or same diet plus calcium salts of long chain fatty acids (2.2% of dry matter; Megalac) for the first 60 days postpartum (Lucy *et al.*, 1991b). During the progesterone treatment period (CIDR-B device) and the post-progesterone treatment estrous cycle (Figure 4), cows fed fat had greater numbers of Class 1 (3 to 5 mm) and Class 4 (> 15 mm) follicles and a lower number of Class 2 (6 to 9 mm) follicles. Feeding CaLCFA increased the size of the largest follicles after progesterone treatment (Table 1).

Figure 4. Average number of follicles within different size classes (Class 1: 3 to 5mm; Class 2: 6 to 9 mm; Class 3: 10 to 15 mm; Class 4: >15mm) during an estrous cycle (days 6, 12 and 18) after removal of the CIDR-B device from lactating dairy cows fed diets with and without calcium salts of long-chain fatty acids (Ca-LCFA; Megalac).



Feeding CaLCFA influenced the number of follicles within each size class at all times during the experiment. During the first 25 days postpartum, changes in follicle numbers were consistent with the theory that a more positive energy balance causes movement of follicles from smaller to larger size classes. Indeed prior to day 25, cows fed CaLCFA had more Class 2 and fewer Class 1 follicles. This movement probably is responsible for earlier ovulation in cows having more positive energy balance. After day 25, the effects of CaLCFA feeding were such that these cows had more Class 1 and Class 4 follicles. This indicates that once estrous cycles have been initiated, effects of CaLCFA are quite different in that all follicle classes are affected. Of particular interest was the higher proportion of Class 4 follicles and the greater size of the largest follicle after the CIDR-B period (Table 1).

The larger follicle, observed during the estrous cycle

Table 1. Diameter of largest follicle determined by ultrasonography in lactating dairy cows fed without (control) or with calcium salts of long chain fatty acids (Megalac).

Period	Size of Largest Follicle (mm)	
	Control	CaLCFA ^a
During CIDR-B Treatment		
Day Postpartum		
30	17.3	18.3
35	15.9	19.1
40	17.7	19.9
After CIDR-B Treatment		
Day of Cycle		
6	11.6	15.4
12	12.1	21.0
18	13.4	18.3

^aSize of largest follicle greater in CaLCFA-fed cows (P < .05).

following removal of the CIDR-B device, appears to be physiologically active. This is because the number of follicles in Class 2 is reduced within the CaLCFA fed group (Figure 4). The ability of larger follicles to influence growth of smaller follicles is a phenomenon known as follicular dominance and is characteristic of physiologically functional follicles. This phenomenon seems to be more operative in the large dominant follicles of cows fed the CaLCFA in this trial and may account for the reduction in number of Class 2 follicles during the estrous cycle (Figure 4). It is clear from this experiment that feeding CaLCFA to alter energy balance in lactating dairy cows did indeed alter ovarian function in a manner to increase ovarian follicle development.

Negative Energy Balance Alters Growth of Preovulatory Follicle

Of particular concern is whether an acute energy deficit specifically would alter the growth of the preovulatory follicle in lactating dairy cows. This is a concern to us because we know that lactating dairy cows have a lower degree of estrous intensity and are less fertile than nonlactating dairy cows or heifers when we precisely synchronize estrus in dairy animals (Van Cleeff, 1991). The objective of the present experiment was to examine changes in key hormones and growth factors during a period of induced energy deficit when the preovulatory ovarian follicle is in a final growth phase leading to estrus and ovulation.

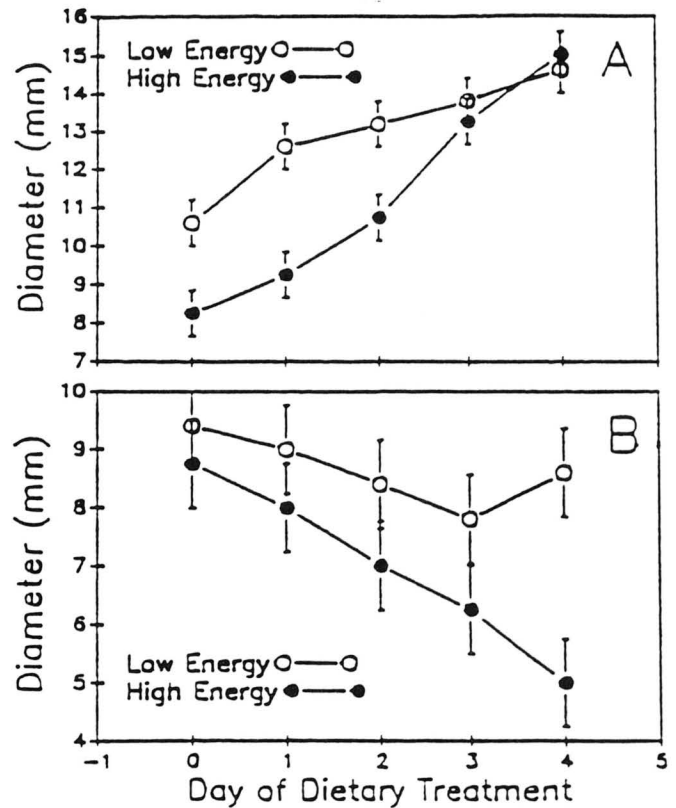
Ten lactating dairy cows were fed a high energy diet (HE; 40% forage: 60% concentrate) and five of the cows were switched to a low energy diet (LE; 100% forage) for 4 days during the preovulatory period when a follicle was un-

dergoing final growth (Lucy *et al.*, 1990). All of the cows were synchronized with a CIDR-B device for 9 days, Lutalyse (25 mg) given on day 7 of CIDR-B exposure, and a GnRH agonist (8 μ g Buserelin; Receptal, Hoechst-Roussel Agri-Vet Co., Somerville, NJ) injected on day 0 when CIDR-B devices were inserted. This system programs ovarian follicle development and corpus luteum regression. On day 6 of CIDR-B treatment (1 day prior to Lutalyse injection), the switch to a LE diet was implemented for a 4 day period. After 4 days of dietary treatments (HE versus LE), ovaries were removed by surgery. Ovarian follicle dynamics and growth of the preovulatory follicle were monitored on a daily basis by ultrasonography. Blood samples were collected daily for measurement of hormones and various nutritional metabolites.

Energy balance averaged -7.3 Mcal/day in cows fed the LE diet and +3.7 Mcal/day in cows fed the HE diet during the period in final maturation of the preovulatory follicle. During the dietary treatment period, cows fed the LE diet had higher concentrations of nonesterified fatty acids (NEFA) and lower concentrations of Insulin Like Growth Factor I (IGF-I) than cows fed the HE diet. Plasma concentrations of NEFA are a good indication of adipose tissue mobilization for energy, and the increase of NEFA in cows of the LE diet reflected the dietary induction of a negative energy balance. Plasma IGF-I averaged 65.3 ng/ml for HE-fed cows on day 0 and 71.4 ng/ml on day 4. In contrast, IGF-I declined from 64.7 ng/ml (day 0) to 42.0 ng/ml (day 4) for cows fed the LE diet. IGF-I is considered critical to the development of ovarian follicles and definitely was decreased by the LE dietary treatment.

The daily growth rate (1.8 versus .8 mm/day) and total growth (6.8 versus 4.0 mm) of the dominant follicle during the dietary treatment period was greater in HE-fed cows than LE-fed cows (Figure 5, panel A). The lower growth rate of the preovulatory follicle in LE-fed cows indicates that acute growth of preovulatory follicles can be affected by short-term changes in energy balance. It is not clear what hormonal or metabolic factors may be causing this difference. Clear differences in LH secretion were not detected. However, IGF-I concentrations were reduced, and this may have contributed to the reduction in growth rate. We feel that the physiological competence of the preovulatory follicle in LE-fed cows was compromised. The second largest follicle became smaller and decreased in size at a faster rate (-.9 versus -.3 mm/day) in cows fed the HE diet compared to cows fed the LE diet (Figure 5, panel B). A decrease in size of the second largest follicle during a follicular preovulatory wave is associated with the development of a physiologically active dominant follicle. Therefore, when compared to LE-fed cows, cows fed the HE diet developed dominant follicles which more effectively controlled growth of other follicles on the ovary. These dietary differences in follicle dynamics during the preovulatory period provide clear evidence linking undernutrition with suboptimal follicular development as deter-

Figure 5. Size (mm) of the preovulatory follicle (Panel A) and second largest follicle (Panel B) in cows fed the high energy and low energy diets.



mined by ultrasonography.

Effect of Diet, Energy Balance and BST on Follicle Development

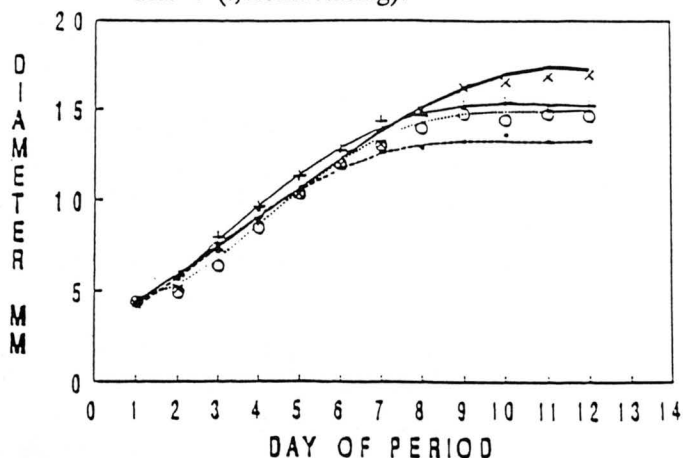
From the above series of experimental observations, we know what a negative energy balance is associated with: a delay to first ovulation, fewer ovulatory sized follicles, slowed growth of the preovulatory follicle, and that increasing caloric density of the diet with CaLCFA will stimulate ovarian follicle development. An additional experiment was conducted to quantitatively evaluate several nutritional and physiological states on ovarian function in an integrated manner (Lucy *et al.*, 1991; De La Sota *et al.*, 1991). We wanted to examine ovarian follicle dynamics in response to: cows that were lactating versus dry; dietary differences due to CaLCFA per se or the additional energy provided by CaLCFA in lactating cows; BST, as to whether a BST induced decrease in energy balance would alter ovarian follicle development or perhaps directly stimulate follicle growth; possible interactions that may exist between diet, BST administration and lactational state on ovarian follicle dynamics during the estrous cycle.

Eighteen lactating dairy cows, between 60 to 100 days in milk, and six dry cows were synchronized for estrus (day 0) and treated with PGF₂ α (25 mg of Lutalyse on day 12) and a CIDR-B device (1.9 grams of progesterone; day 12

to 17). All cows were treated with either saline or BST (25 mg per day; USAN, methionyl bovine somatotrophin; Monsanto Co., St. Louis, MO) during one of two estrous cycles. The lactating dairy cows (6 cows per group) were fed one of three diets: Diet 1 = control without CaLCFA, NEL (net energy for lactation) of 1.68 Mcal/kg; Diet 2 = CaLCFA fed at 2.2% of the diet dry matter but energy density the same as Diet 1 by adjusting the forage to concentrate ratio; Diet 3 = CaLCFA added to diet 1 at 2.2% of diet dry matter and NEL=1.78 Mcal/kg. Nonlactating cows were fed solely perennial peanut hay (Diet 4; NEL = 1.2 Mcal/kg). Cows were fed diets starting 30 days prior to the first period and maintained on the same diet throughout the experiment. Cows received either BST or saline during period 1 and were switched to saline or BST during period 2. Each 19 day period constituted an estrous cycle orchestrated by use of the CIDR-B device and Lutalyse treatment so that each cow would have two follicle waves per cycle. Development of all follicles was monitored daily by ultrasonography and blood samples collected to collate ovarian function with hormonal and metabolite responses.

Lactational status definitely influenced ovarian follicle responses. Dry cows had smaller dominant follicles during the first follicle wave (Figure 6), and the frequency of large follicles (> 15mm) was lower in dry cows throughout the estrous cycle until development of the preovulatory follicle between days 17 to 19 (Figure 7). In dry cows, the developing preovulatory follicle is much more estrogenic or functionally active based on the higher plasma estradiol concentrations detected in the peripheral circulation (Figure 8). Thus the hormonal and metabolic changes associated with lactation do indeed appear to alter terminal development of the preovulatory follicle in our experimental model.

Figure 6. Mean diameter (mm) and fourth-order fitted curves for the first dominant follicle in cows fed diet 1 (o), diet 2 (x), diet 3 (+), or diet 4 (.; nonlactating).



As discussed earlier, follicle growth is dependent upon recruitment of a pool of small follicles from which a

Figure 7. Average number of class 4 (>15 mm) follicles for cows fed diet 1 (o—o), diet 2 (x—x), diet 3 (+—+), or diet 4 (.-.-.-; nonlactating).

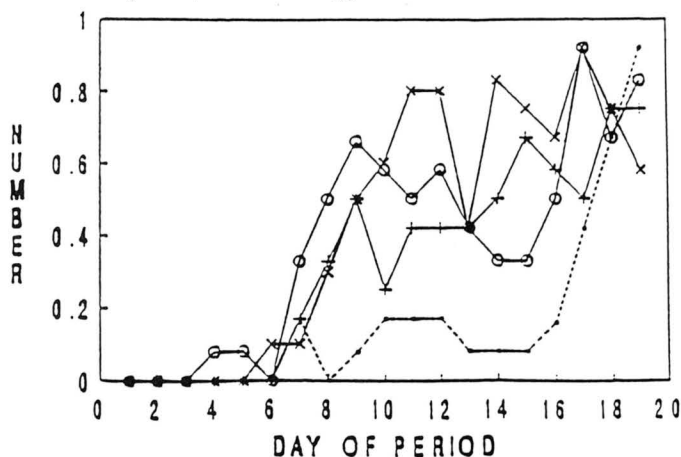
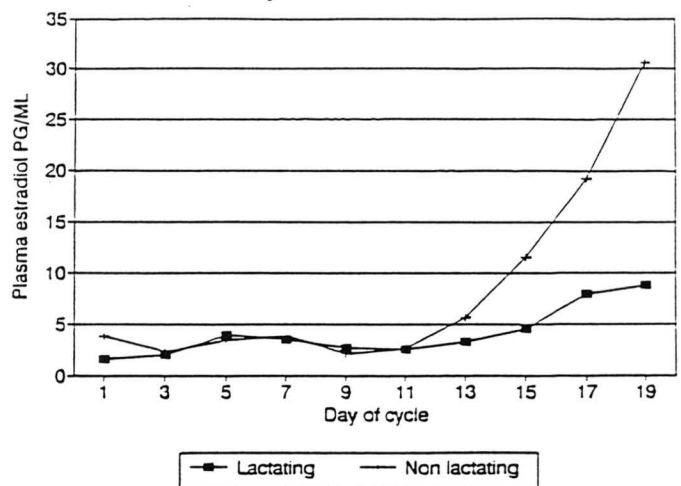


Figure 8. Plasma estradiol of lactating (diets 1, 2 and 3) versus nonlactating (diet 4) dairy cows during the estrous cycle.



follicle is selected and undergoes continued growth to a large dominant follicle. The dominant follicle suppresses the growth of subordinate and smaller follicles. Ovarian follicle dynamics differed between dry and lactating cows in their responses to BST. For example dry cows had a greater number of Class 2 follicles (6 to 9 mm) than lactating cows between days 3 to 6 of the estrous cycle. However, BST stimulated the number of Class 2 follicles in lactating cows to the higher level found in dry cows. Class 2 follicles of dry cow were not responsive to BST (Figure 9). This same stimulation by BST in lactating cows was evident for Class 3 follicles (10-15 mm) during the second phase of the estrous cycle (from 12 to 18 days). In contrast, dry cows had a greater number of Class 3 follicles and a further stimulation by BST was not detected (Figure 10). Collectively, these results indicate that the hormonal and metabolic responses associated with lactation attenuate follicle

Figure 9. Mean number of Class 2 follicles in lactating (diets 1, 2 and 3) versus nonlactating (diet 4) cows treated with BST versus Saline.

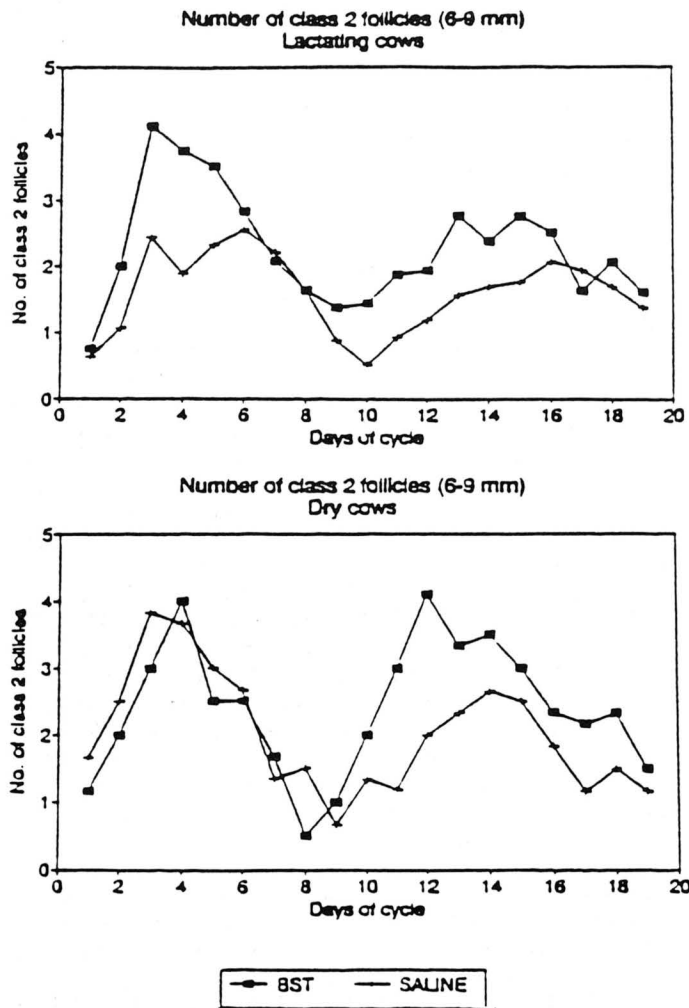
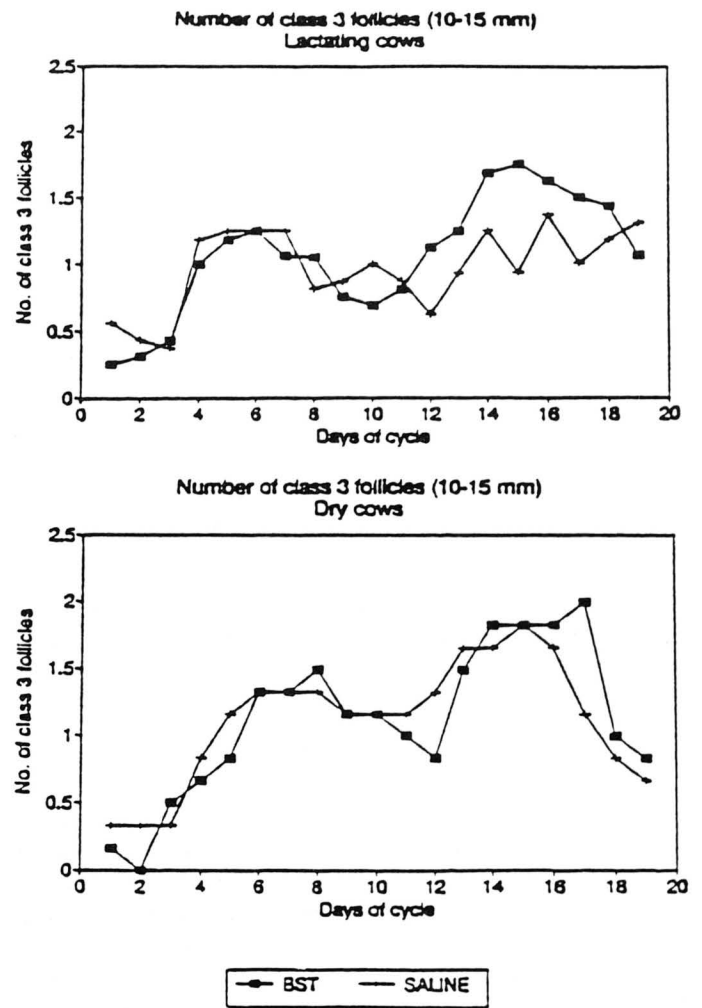


Figure 10. Mean number of Class 3 follicles in lactating (diets 1, 2 and 3) versus nonlactating (diet 4) cows treated with BST versus Saline.

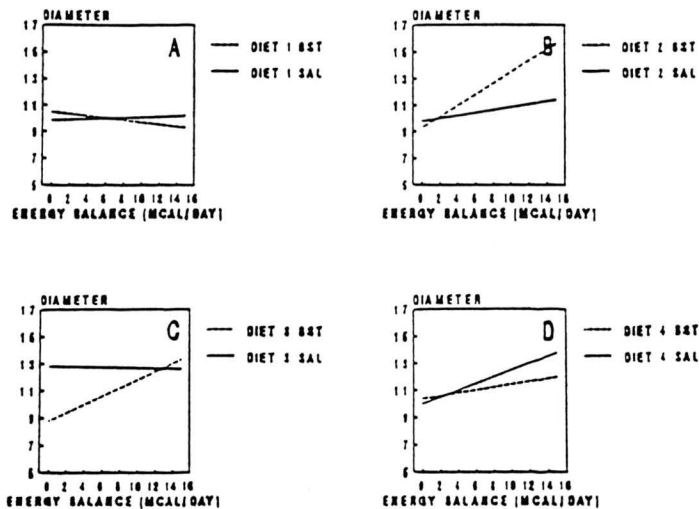


recruitment, and treatment with BST restores attenuated follicle development to a level comparable to the dry cow. However, the BST stimulation in follicle recruitment in lactating dairy cows was not associated with any increase in ovulation rate.

Of interest is whether BST effects on follicle development in lactating dairy cows was influenced by the various diets. BST altered mobilization of fats from adipose tissue based on higher concentrations of plasma non-esterified fatty acids (NEFA) throughout the estrous cycle (BST > Control; 283 > 182 uEq per liter, respectively). As expected, BST stimulated 4% fat-corrected milk production throughout the cycle compared to the control cycle. The stimulation in milk production was associated with a distinct decrease in energy balance during the second half of the estrous cycle when the preovulatory follicle was undergoing development. Cows receiving CaLCFA tended to produce more milk than control cows when BST was injected (12% versus a 6% increase). In this experiment, as reported in our earlier research with CaLCFA, maximum size of the dominant follicle on the day of estrus was larger

for cows fed CaLCFA (18.8 versus 16.6 mm). A clear interaction is detected between growth of the preovulatory follicle (mm) between BST and control treatments relative to the type of diet and energy balance during this period (12 to 19 days of the programmed estrous cycle). Basically, when cows were fed CaLCFA (Diets 2 and 3), the size of the preovulatory follicle increased with an increase in energy balance if they had been injected with BST. This positive increase was not evident in the saline treatment period (Figure 11). This stimulation by BST was not evident in cows fed the control Diet 1 without CaLCFA. Apparently, CaLCFA feeding altered responsiveness of the ovary to BST that permitted a greater growth or increase in size of the preovulatory follicle with increasing energy balance. Regardless of the effects of BST or CaLCFA feeding on development of the preovulatory follicle, these treatments failed to stimulate estrogenic activity of this follicle to a level comparable to what was detected in the dry cows during the same period (Figure 8). This difference between dry and lactating dairy cows, relative to estradiol concentrations, also was reflected in progesterone concentrations

Figure 11. Linear regression of EB on the size of the preovulatory follicle for BST- or Saline-treated cows fed diets 1 (A), 2 (B), 3 (C), or 4 (D, non-lactating).



which were greater in dry cows during the first 12 days of the estrous cycle when the corpus luteum was present ($4.58 > 3.72$ ng/ml).

Summary

The turnover of ovarian follicles and their ultimate growth response is influenced by secretion of LH that is limited by progesterone secretion from the CL. Based on the results from this series of experiments, there appear to be measurable effects of energy balance on growth and development of ovarian follicles in postpartum dairy cows. These effects may be mediated by several mechanisms including changes in circulating growth factors (eg. IGF-I) and LH secretion. Feeding strategies for postpartum cows to increase caloric density of the diet (eg. CaLCFA feeding) affected the population of ovarian follicles at all stages of the postpartum period examined. Furthermore, feeding of CaLCFA interacted with the effect of BST on growth of the preovulatory follicle across a range of positive energy balances. CaLCFA feeding consistently stimulated the development of large follicles in two experiments. Follicle function in lactating dairy cows was appreciably different than responses observed in cycling dry cows (eg. plasma estradiol concentrations during the preovulatory period were higher in dry cows). Such differences due to lactation may contribute to our inability to optimize fertility and re-

productive management of the lactating dairy cow versus the heifer or dry cow. In lactating cows, BST stimulated recruitment of Class 2 follicles in Wave I and Class 3 follicles in Wave II; however, the normal process of follicular dominance in BST treated cows prevented a higher frequency of multiple ovulations. The basic knowledge gleaned from these experiments provides the foundation to ultimately improve postpartum reproductive efficiency through physiological and nutritional management of the lactating dairy cow.

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Buiatrics is the study of cattle disease and management.