

Effects of energy balance on follicular development and first ovulation in postpartum dairy cows

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As milk production has increased during the past four decades, conception rates in lactating cows have declined. Although reduced reproductive performance has been associated with high milk yields, measures of postpartum ovarian activity have been more closely related to energy balance. The relationship between daily energy balance and postpartum reproductive activity is confirmed by longer intervals to first ovulation in cows with greater body condition loss. Patterns in daily energy balance, such as improvement from nadir, have been correlated with enhanced follicular function and a shorter interval to first ovulation. Such observations are consistent with increased LH pulse frequency following the energy balance nadir in lactating dairy cows. Evidence indicates a primarily hypothalamic locus for the modulation of LH secretion during negative energy balance. Formation of follicular waves after parturition begins synchronously in response to increased FSH in the first week postpartum, and is typically not a limiting factor in reproductive recrudescence. Altered follicular responsiveness to gonadotrophic support through changes in metabolic hormones such as insulin-like growth factor I (IGF-I) and insulin may contribute to impaired function of dominant follicles early postpartum. Positive relationships between changes in energy balance, peripheral IGF-I and function of dominant follicles support the identification of IGF-I and the day of the energy balance nadir as metabolic modulators of postpartum ovarian activity in dairy cows.

Introduction

The reproductive process in the ruminant female entails a complex series of physiological events that may be impacted at many levels to influence overall fertility. Although a myriad of regulatory mechanisms remain to be elucidated, certain fundamental factors mediating reproductive activity have become apparent. One of the earliest factors to be recognized is the often profound influence of nutritional status. In the USA, apparent metabolic influences on reproductive performance have been reflected in declining conception rates (66% to 40% since 1951) coincident with increasing yearly milk production per cow (218% increase since 1950; National Agricultural Statistics Service, USDA). Conception rates after artificial insemination (AI) in nonlactating heifers have remained at 70–80% during this period (Butler and Smith, 1989), indicating that there is no direct genetic trend against fertility. Understanding the metabolic constraints on postparturient ovarian function is an important step towards the development of management practices that positively influence postpartum reproductive efficiency. The focus of this review is on current knowledge of the resumption of cyclic ovarian activity after parturition in high producing dairy cows, with an emphasis on patterns of follicular development and their relationship to energy balance and metabolic hormones. This review is not intended to be comprehensive for all nutritional interactions. Current reviews describe the effects of supplemental fat (Staples *et al.*, 1998) or the level of dietary protein (Butler, 1998) on postpartum reproduction in dairy cows.

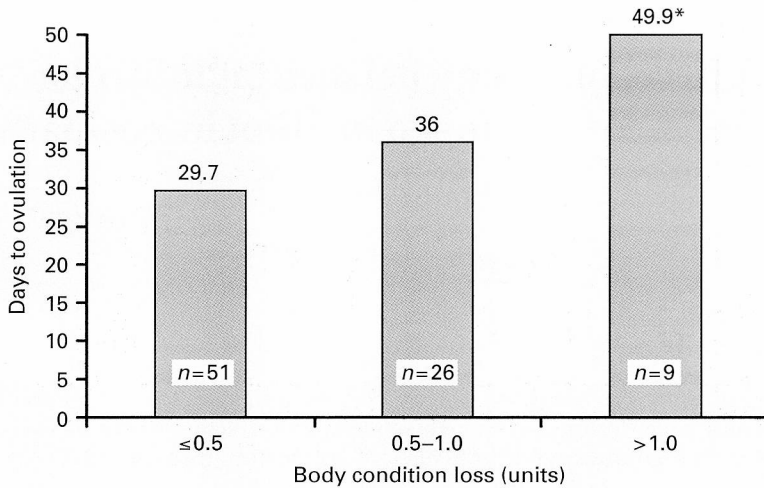


Fig. 1. The number of days to first ovulation in dairy cows with different body condition score loss during the first 30 days postpartum. Scores of 1 = emaciated and 5 = obese. Pooled data from Beam and Butler (1997, 1998). * Significantly different from other two means ($P < 0.05$).

Energy Balance and Postpartum Reproduction: General Relationships

In the context of the postpartum dairy cow, energy balance is the difference between the dietary intake of utilizable energy and the expenditure of energy for body maintenance and milk synthesis. Owing to a lower rate of increase in feed intake compared with that of milk production, high producing dairy cows typically experience a variable period of negative energy balance during early lactation that is characterized by the loss of body weight and mobilization of body fat stores. Negative energy balance may persist for 10–12 weeks of lactation (Bauman and Currie, 1980), and the level of energy deficit is often related directly to milk yield (Butler *et al.*, 1981). High milk yields have in turn been associated with lower reproductive efficiency. Data summarized by Nebel and McGilliard (1993) from over 4550 herds of Holstein cows have shown an inverse relationship between first-service conception rate and per cow yearly milk yield (52% for cows producing 6300–6800 kg per year versus 38% for cows producing > 10 400 kg per year). However, despite the apparent negative relationship between conception rate and milk production, measures of reproductive performance, in several studies, have been related more closely to energy balance than to milk yield (Butler and Smith, 1989). Staples *et al.* (1990) reported lower milk production, lower feed intake and more negative energy balance in anoestrous dairy cows compared with cows that returned to cyclic ovarian activity before day 60 postpartum. Likewise, multiparous dairy cows ovulating after day 42 postpartum consumed less feed, but had similar fat corrected milk production, compared with cows ovulating before day 42 postpartum (Lucy *et al.*, 1992a). Furthermore, variation in energy balance among cows within a herd is due largely to variation in energy intake ($r = 0.73$) as compared with milk yield ($r = -0.25$; Villa-Godoy *et al.*, 1988), and increased utilization of body energy reserves for milk production has been associated with longer intervals to cyclic ovarian activity (Staples *et al.*, 1990). Effects of a given lactation on reproduction are dependent upon concomitant patterns of feed energy intake and subsequent energy availability to support proper postparturient function of the hypothalamic–pituitary–ovarian (HPO) axis. The inverse relationship between energy balance and postpartum ovarian function is represented most clearly by changes in body condition; cows losing more body condition during the first month postpartum experience longer intervals to first ovulation (Fig. 1).

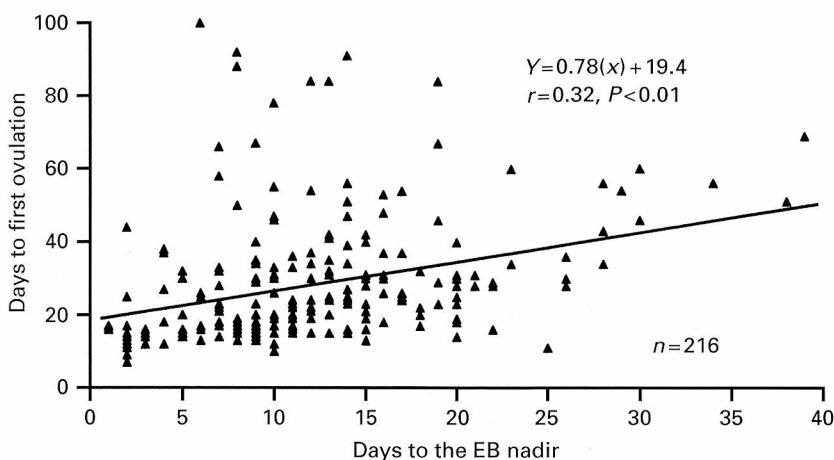


Fig. 2. Linear regression of the number of days to the first postpartum ovulation on the number of days to the energy balance (EB) nadir in dairy cows. Data include observations from five separate studies: Canfield *et al.* (1990); Canfield and Butler (1990, 1991); Beam and Butler (1997, 1998).

Energy balance and days to first ovulation

Because the number of oestrous cycles preceding AI has been shown to influence conception rate (Thatcher and Wilcox, 1973; Lucy *et al.*, 1992a), the duration of the postpartum anovulatory interval can serve as a measure of potential energy balance mediated effects on reproductive performance. Although effects of energy balance on the number of days to first ovulation have been observed inconsistently, both the level of negative energy balance (Butler *et al.*, 1981; Staples *et al.*, 1990) and changes in energy balance over time (Canfield and Butler, 1990; Canfield *et al.*, 1990; Beam and Butler, 1997) have been implicated in the timing of the first ovulatory event after parturition. In modern dairy cows, the average day of first ovulation is approximately 25–30 days postpartum with a typical range between 17 and 42 days (Butler and Smith, 1989). Thus, energy balance during the first three to four weeks postpartum has been correlated with the interval to first ovulation (Lucy *et al.*, 1991; Beam and Butler, 1998). Average energy balance over longer periods (6 to 12 weeks) often has no significant relationship to the resumption of ovarian cycles (Staples *et al.*, 1990; Beam and Butler, 1998). In addition to the influence of the level of negative energy balance during the early weeks postpartum, recovery of daily energy balance from its most negative value (nadir) appears to provide an important signal for initiation of cyclic ovarian activity. The number of days to the energy balance nadir is positively correlated with the number of days to first ovulation (Fig. 2; Canfield and Butler, 1990; Canfield *et al.*, 1990; Beam and Butler, 1997). Evaluation of relationships between energy balance profiles and the day of first ovulation, although informative, does not reveal the nature of ovarian follicular activity within the anovulatory period. Examination of follicular development leading to the first ovulation postpartum has produced important insights into factors limiting reproductive recrudescence during negative energy balance.

Postpartum Follicular Development

The term 'follicular dynamics' has been defined by Lucy *et al.* (1992b) as the continual growth and regression of antral follicles leading to preovulatory follicle development. In cattle, this process is characterized by the formation of follicular waves; two or three waves typically occur during the

normal course of the oestrous cycle (Pierson and Ginther, 1984). Although follicular dynamics during the oestrous cycle have been extensively examined, there is less information on follicular development following parturition and before the first postpartum ovulation, particularly in modern high producing dairy cows. The use of transrectal ultrasonography to monitor follicular development has added greatly to the understanding of postpartum ovarian activity. Savio *et al.* (1990) examined follicular development by ultrasonography in dairy cows from day 5 postpartum and reported a variable period until the detection of a dominant (> 9 mm) follicle (range 5–39 days). Follicular development before dominant follicle formation was characterized by growth and regression of small (< 5 mm) and medium (5–9 mm) sized follicles; the first dominant follicle either ovulated (14/19 cows) or became cystic (4/19 cows) in all but one cow. Ultrasonography in dairy cows beginning on day 14 postpartum revealed first ovulation in eight of ten cows before day 25 postpartum; one cow developed a follicular cyst and one cow displayed multiple waves of anovulatory follicle development until first ovulation at day 55 (Rajamahendran and Taylor, 1990). More recent studies using ultrasonography revealed the regular formation of follicular waves, including the development of dominant follicles, throughout most of pregnancy in dairy heifers (Ginther *et al.*, 1996), and the rather synchronous initiation of a new follicular wave 5–7 days postpartum in multiparous dairy cows (Beam and Butler, 1997).

During the oestrous cycle in cattle, new waves of follicular development are preceded by peak increases in mean circulating concentrations of FSH that are believed to be responsible for initiating the growth of each new cohort of ovarian follicles (Adams *et al.*, 1993). This observation was extended to follicular waves occurring throughout most of pregnancy in heifers by Ginther *et al.* (1996), in which dominant follicles developed at 6–8 day intervals in association with peaks in plasma FSH. However, the size of dominant follicles observed by Ginther *et al.* (1996) decreased linearly during months 4–9 of gestation, and no follicles > 6 mm in diameter were detected during the last 3 weeks before parturition. This reduced follicular development during the final weeks of gestation was associated with altered FSH secretion patterns, including a longer interpeak interval, and was postulated to result from increased concentrations of gestational oestrogens (oestradiol and oestrone). This finding is in agreement with a report by Beam and Butler (1997) in which mean daily plasma FSH concentrations increased to peak values on days 4–5 postpartum after gestational oestradiol concentrations had declined. The FSH peak was followed immediately by the initiation of a new follicular wave and development of the first dominant follicle postpartum. This new period of follicular dominance occurred in all cows examined despite average negative energy balance of -7.5 Mcal day⁻¹ during the first 3 weeks postpartum. It appears that initiation of follicular waves in the early postpartum cow is unperturbed by negative energy balance and occurs in response to the re-establishment of periodic FSH surges synchronized by the end of gestation. In milked dairy cows experiencing negative energy balance, LH, but not FSH, appears to be deficient after the first week postpartum (Lamming *et al.*, 1981; Beam and Butler, 1997). Therefore, FSH appears to be insensitive to metabolic energy status and signals the development of ovarian follicular waves at regular intervals following parturition in dairy cows. Because formation of a dominant follicle does not appear to be a limiting factor in the resumption of ovarian cycles postpartum, the function (that is steroidogenic capability) and fate of dominant follicles during negative energy balance becomes an important focus of investigation.

Patterns of dominant follicle fate

Although most dairy cows appear to develop dominant follicles during the second week postpartum, three patterns of follicular development based on the fate of the first-wave dominant follicle have been described (Savio *et al.*, 1990; Rajamahendran and Taylor, 1990; Beam and Butler, 1997): (1) ovulation of a dominant follicle during the first follicular wave after parturition; (2) development of a first-wave anovulatory dominant follicle followed by additional waves of follicular development before first ovulation; or (3) development of a first-wave dominant follicle that becomes cystic (Fig. 3). Pattern 1 (ovulatory) and pattern 3 (cystic) are characterized by development of oestrogen-active dominant follicles, whereas pattern 2 (anovulatory) is characterized by growth of dominant follicles that produce low peripheral concentrations of

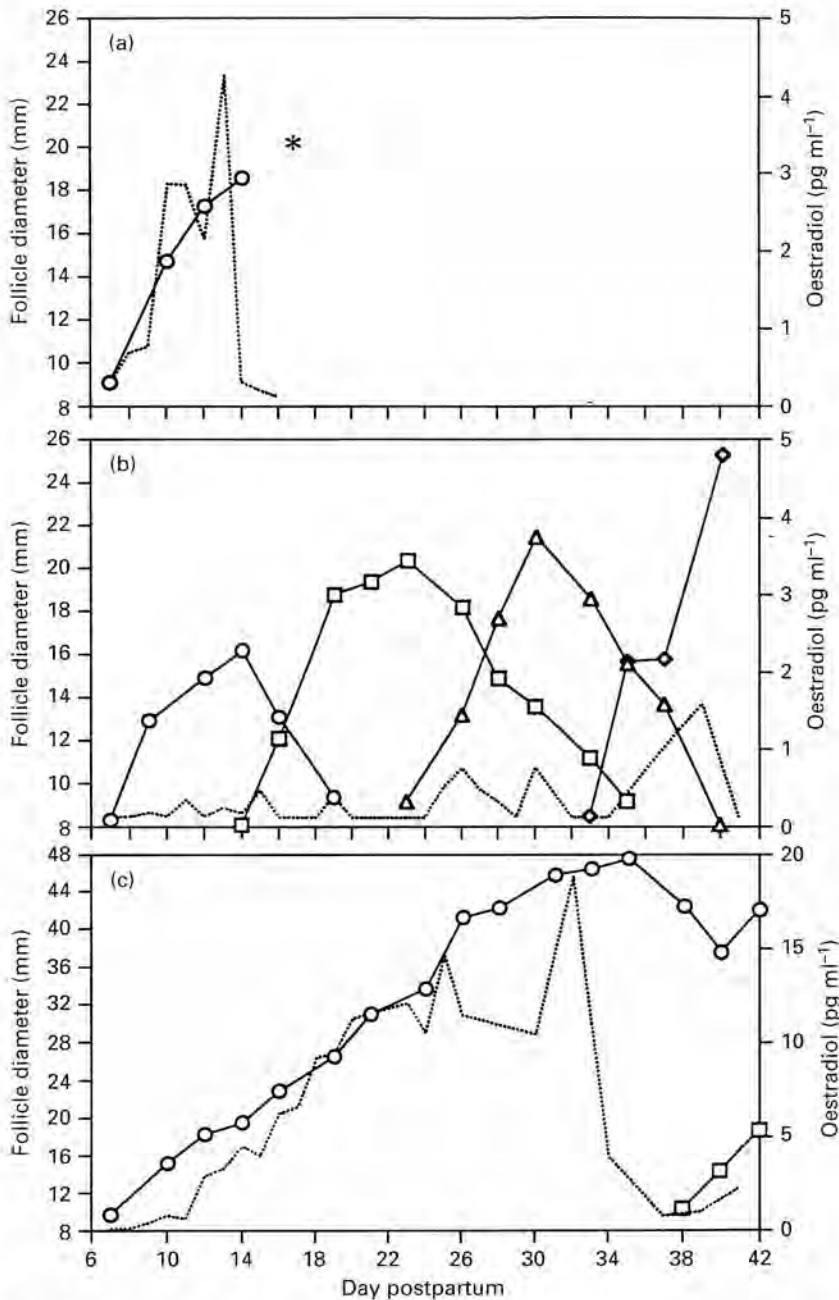


Fig. 3. Patterns of dominant follicle growth and oestradiol concentrations in three representative postpartum dairy cows that (a) ovulated the dominant follicle of the first follicular wave postpartum, (b) experienced multiple waves of anovulatory dominant follicle development and low plasma oestradiol, and (c) developed an oestrogen-active follicular cyst. *Denotes ovulation. Reproduced with permission from Beam and Butler (1997).

oestradiol and become atretic (Beam and Butler, 1997). The fate of the first-wave dominant follicle has a significant impact on the postpartum anovulatory interval (Beam and Butler, 1997); regression of the first-wave dominant follicle or formation of a follicular cyst results in a similarly prolonged interval to first ovulation (51 and 48 days, respectively) compared with that of cows that ovulate their first dominant follicle postpartum (20 days). Combined data from two studies examining postpartum follicular development in multiparous dairy cows ($n = 87$) show that 46% of cows ovulated the first dominant follicle postpartum; 31% experienced at least two waves of dominant follicle development before first ovulation, and the remaining 23% became cystic, with all but one cyst forming from the first follicular wave postpartum (Beam and Butler, 1997, 1998). The average duration of the anovulatory period in cows with anovulatory (noncystic) first-wave dominant follicles was 40 days, indicating that in most cows early ovulation failure is followed by additional waves of anovulatory follicle development before first ovulation is achieved. These data emphasize the importance of investigating the mechanisms regulating the emergence and function of the first dominant ovarian follicle after parturition, including possible metabolic differences between cows with different follicular fates and the effects of energy balance and specific metabolic hormones on follicular dynamics.

The effects of energetic stress on the HPO axis have been examined primarily at the hypothalamus and anterior pituitary, and the loss of pulsatile LH secretion has been shown to result from prolonged inadequate intake of dietary energy in postpartum beef cows (Perry *et al.*, 1991). Energy restriction in postpartum beef cows does not alter pituitary GnRH receptor density (Moss *et al.*, 1985). However, the results of studies examining pituitary responsiveness to GnRH in the postpartum cow remain equivocal: dietary energy restrictions have both decreased (Rutter and Randel, 1984) and increased (Whisnant *et al.*, 1985) responsiveness to GnRH.

The re-establishment of a pulsatile LH secretion pattern conducive to preovulatory follicular development and function is recognized as a key event in the return of ovarian cyclicity by the postpartum dairy cow experiencing negative energy balance (Lamming *et al.*, 1981; Malven, 1984; Canfield and Butler, 1991). As shown in Fig. 4, the frequency of LH pulses is significantly lower during the first follicular wave postpartum (days 8–12) in cows that develop an anovulatory dominant follicle compared with cows that develop an ovulatory dominant follicle (Beam and Butler, 1994). Peters *et al.* (1985) treated dairy cows between days 3 and 8 postpartum with 2.5 μg GnRH intravenously at intervals of 2 h for 48 h, and reported the induction of an episodic pattern of LH release and a sustained rise in plasma oestradiol. However, a preovulatory-type LH surge was exhibited in only one of nine animals. When the same GnRH treatment protocol was applied to anovulatory dairy cows later than day 10 postpartum, the accelerated pulsatile LH pattern was followed by a preovulatory-type LH surge, ovulation and normal luteal function (Lamming *et al.*, 1982). Thus, endocrine responses in the postpartum cow are highly dependent on the stage postpartum. Differences in the severity of either induced or spontaneous negative energy balance is also likely to be an important variable. Overall, it appears that postparturient recovery of pituitary LH content and responsiveness to GnRH is complete by day 10 postpartum in dairy cows (Lamming *et al.*, 1982; Malven, 1984), and available evidence across species suggests a predominantly hypothalamic locus for the primary effect of decreased energy intake (Schillo, 1992). In the early postpartum dairy cow the reduced activity of the GnRH pulse generator is expressed as reduced pulsatile LH support of follicular steroidogenesis necessary for the induction of an LH surge and ovulation. However, a seemingly low LH pulse frequency (2 pulses per 6 h) is apparently adequate to sustain the morphological development of dominant ovarian follicles by the second week postpartum. This observation is consistent with the growth and differentiation of competent dominant follicles during the mid-luteal phase of the oestrous cycle in cattle when LH pulse frequency is low (Rahe *et al.*, 1980; Driancourt *et al.*, 1991).

Searching for a Signal

Metabolic and hormonal cues

It is reasonable to predict that factors signalling the HPO axis of an energy deficit are part of the metabolic and endocrine milieu characteristic of negative energy balance and early lactation.

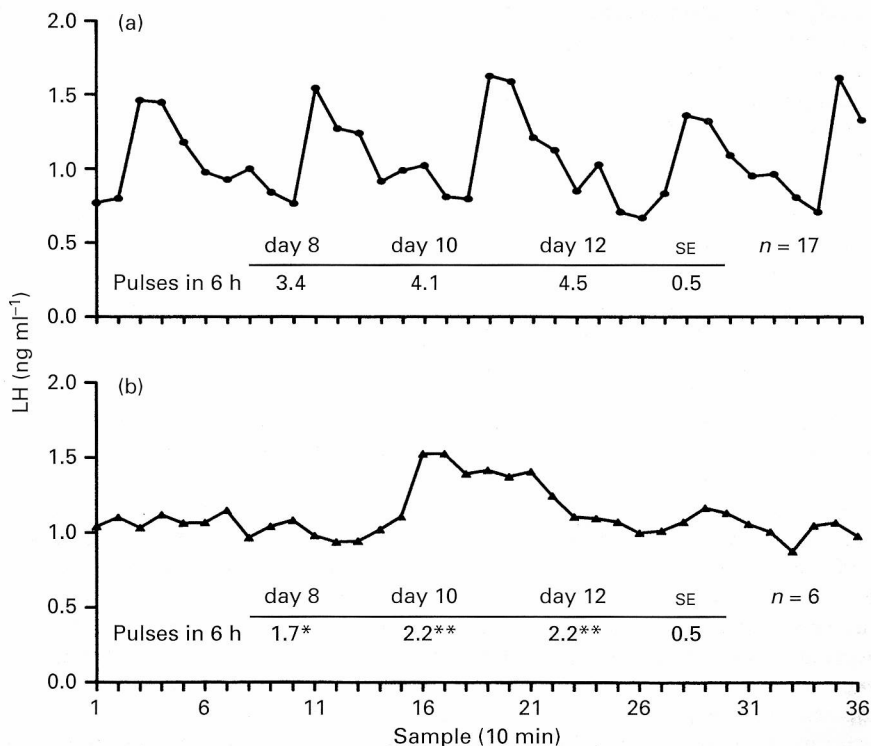


Fig. 4. Representative LH profiles on day 12 postpartum during development of either an ovulatory (a) or anovulatory (b) dominant follicle in the first follicular wave postpartum. Mean number of LH pulses per 6 h on day 8, 10 and 12 postpartum for each group is shown as an inset. Pulse frequency was significantly different (** $P < 0.01$, * $P < 0.05$) within day between groups. Data from Beam and Butler (1994).

Therefore, studies investigating potential metabolic signals for ovarian activity have focused primarily on blood metabolites and metabolic hormones known to fluctuate during altered metabolic states. Increased plasma non-esterified fatty acids (NEFA) have been suggested as a potential cue of energy balance status on initiation of first ovulation (Canfield and Butler, 1990; Canfield and Butler, 1991). However, plasma NEFA and glucose are similar during the first 2–3 weeks postpartum in dairy cows developing either an ovulatory or anovulatory first-wave dominant follicle postpartum (Beam and Butler, 1997, 1998). It is generally accepted that any mechanism coupling metabolic status with ovarian function will ultimately involve a hormonal component. Metabolic data for postpartum dairy cows from a previous study (Beam and Butler, 1997), including measures of energy balance and the metabolic hormones insulin and GH during the first 2 weeks postpartum, were analysed by step-up logistic regression analysis in which ovulation or anovulation of the first postpartum dominant follicle served as the binary dependent variable (Table 1). Logistic regression analysis of these variables produced a significant model that correctly classified 86.5% of the experimental animals (32 of 37 cows) as having either an ovulatory or anovulatory first-wave dominant follicle. The plasma insulin:GH ratio during week 1 postpartum and the day of the energy balance nadir served as the most significant contributors to the model. The insulin:GH ratio was increased due to both higher insulin and lower GH concentrations during only the first week postpartum in cows possessing dominant follicles that ovulated (Beam and Butler, 1997). These results indicate that hormonal differences in the immediate postpartum period may influence follicular function during the first follicular wave after parturition. Insulin has been shown

Table 1. Step-up logistic regression analysis of metabolic variables during the first 2 weeks postpartum in lactating dairy cows with an ovulatory or anovulatory dominant follicle^{a,b}

Variable	Chi-square $\beta = 0$	Probability $\beta = 0$	Last R-Square
Insulin:GH ratio Week 1	4.95	0.0261	0.1339
Day of EB Nadir	4.88	0.0271	0.1324
Mean insulin Week 1	3.85	0.0496	0.1075
Change in EB Day 1–14	3.74	0.0530	0.1047

^aModel r-square = 0.4552, $P < .0001$, model Chi-square = 26.73; ovulation or anovulation of the first-wave dominant follicle postpartum served as the binary dependent variable. The model correctly classified 17/19 ovulators and 15/18 nonovulators (86.5% correct).

^bAnalysis of data from Beam and Butler (1997). Analysis variables for the first 2 weeks postpartum included plasma insulin, GH, insulin-like growth factor I (IGF-I), insulin:GH ratio, net energy intake, mean energy balance (EB), day of the energy balance nadir and change in energy balance.

to stimulate follicular cells *in vitro* in a variety of species including cattle (Spicer *et al.* 1993) and small increases postpartum could have important effects during the very early stages of follicular development. Furthermore, an increased insulin:GH ratio following parturition may be conducive to greater hepatic IGF-I production (McGuire *et al.*, 1995), resulting in increased amounts of this growth factor earlier postpartum. Indeed, in cows developing oestrogen-active, ovulatory dominant follicles during the first follicular wave postpartum, circulating insulin-like growth factor I (IGF-I) is significantly higher during the first 2 weeks postpartum compared with that in cows developing oestrogen-inactive dominant follicles that regress (Beam and Butler, 1997, 1998). Concentrations of circulating IGF-I are in turn positively correlated with IGF-I concentrations in follicular fluid of large bovine follicles (Echternkamp *et al.*, 1990). Similar to insulin, IGF-I is known to affect follicular cell function *in vitro*; stimulation of steroidogenesis and proliferation in both thecal (Spicer and Stewart, 1996) and granulosa cells (Spicer *et al.*, 1993) are well documented. In bovine thecal cells, IGF-I increases the number of LH-binding sites and enhances LH-induced production of progesterone and androstenedione *in vitro* (Spicer and Stewart, 1996; Stewart *et al.*, 1996). Both oestradiol and FSH increase the number of IGF-I receptors in granulosa cells, which have been shown to be greater in number in large bovine follicles compared with small follicles (Spicer *et al.*, 1994), and may thus form a self-amplifying system of IGF-I stimulation in the growing and differentiating dominant follicle postpartum. In dairy cows, there is an apparent relationship between the steroidogenic activity of the first dominant follicle postpartum and circulating concentrations of IGF-I (Fig. 5).

Patterns of energy balance

The recovery of pulsatile LH secretion patterns leading to the enhancement of follicular function has been associated with changing patterns of energy balance over time in the postpartum dairy cow. In a study of energy balance and dietary lipid effects on postpartum follicular development in high producing dairy cows (Beam and Butler, 1997), mean daily energy balance did not have a significant relationship with follicular function. However, improvement in energy balance from its most negative level (nadir) enhanced follicular competence, because dominant follicles during the first follicular wave postpartum exhibited greater steroidogenic output and ovulatory success when development occurred after the day of the energy balance nadir (75% ovulated) compared with before the day of the energy balance nadir (24% ovulated). Furthermore, in cows that experienced consecutive waves of follicular development before first ovulation (type 2 of Fig. 3), dominant

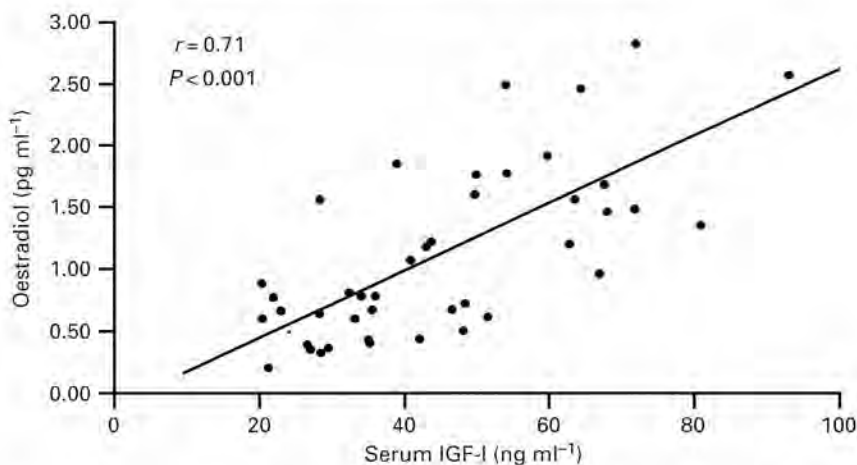


Fig. 5. Linear regression of mean plasma concentrations of oestradiol during the development of the first dominant follicle postpartum (day 8–14) on mean serum concentrations of insulin-like growth factor I (IGF-I) during days 1–21 postpartum in dairy cows ($n = 42$). Analysis of data from Beam and Butler (1998).

follicle diameter and plasma oestradiol concentrations were increased after the energy balance nadir compared with the follicular wave occurring before the energy balance nadir (Fig. 6). Examination of the pooled data from two recent studies by our laboratory (Beam and Butler, 1997; 1998) shows a significant difference ($P < 0.01$) in the day of the energy balance nadir between cows that developed either an ovulatory (6.9 ± 1.3 days, $n = 40$) or anovulatory (15.5 ± 1.6 days, $n = 27$) dominant follicle during the first follicular wave postpartum. Enhancement of follicular steroidogenesis and diameter after the energy balance nadir is consistent with effects of increased LH pulse frequency on follicular function (Glencross, 1987), and with previously reported increases in LH pulse frequency following the day of the energy balance nadir (Canfield and Butler, 1991). The specific signal after the energy balance nadir leading to increased LH pulse frequency remains unknown, but does not involve endogenous opioid peptides because naloxone administration to postpartum dairy cows does not affect any variable of LH secretion (Canfield and Butler, 1991).

Energy Balance and Postpartum Follicular Dynamics

Few studies have examined directly the relationship between energy balance and follicular dynamics during the early postpartum period in dairy cows. Lucy *et al.* (1991) examined follicular dynamics in dairy cows by ultrasonography and reported an effect of energy balance on different populations of ovarian follicles postpartum. The number of class 1 (3–5 mm) and class 2 (6–9 mm) follicles decreased, and the number of class 3 (10–15 mm) follicles increased with more positive energy balance before day 25 postpartum. The authors suggested that as cows improve in energy balance, the movement of smaller follicles into larger size classes is enhanced. Comparisons between lactating and nonlactating cows have also shown differences in follicular recruitment, with nonlactating cows having a greater number of class 1 (3–5 mm), class 2 (6–9 mm) and class 3 (10–15 mm) follicles during the first follicular wave of a synchronized oestrous cycle (De La Sota *et al.*, 1993). During the first follicular wave postpartum (days 8–14) in cows receiving three levels of dietary fat, the number of class 1 and class 2 follicles was not correlated with energy balance during either the first or second week postpartum, regardless of diet (Beam and Butler, 1997). Likewise, the number of class 1 and class 2 follicles on day 8 postpartum was not correlated with energy balance

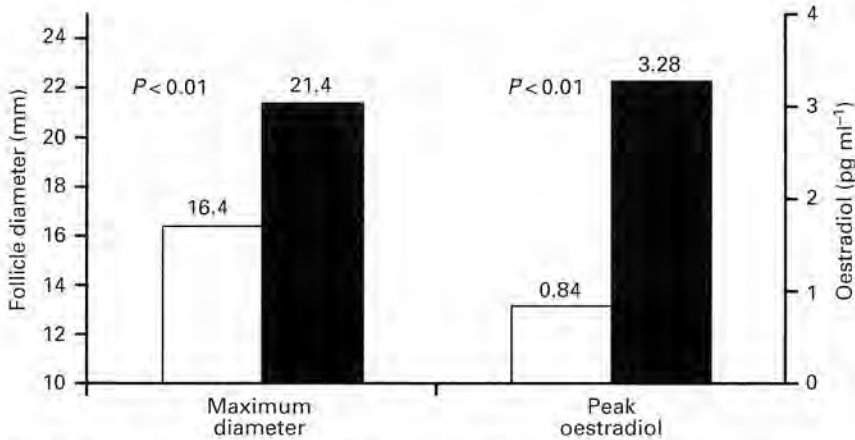


Fig. 6. Mean maximum diameter of dominant follicle and peak oestradiol concentration during consecutive follicular waves occurring immediately before (□) and after (■) the energy balance nadir in postpartum dairy cows ($n = 11$). Both maximum follicle diameter and peak oestradiol concentrations were greater in follicular waves developing after the energy balance nadir (Beam and Butler, 1997).

during the first week postpartum in dairy cows fed prilled fatty-acids at 0 or 2.9% of ration DM (Beam and Butler, 1998). Furthermore, the interaction between energy balance and follicle size classes reported by Lucy *et al.* (1991) was not significant when cows receiving calcium salts of long-chain fatty acids were removed from the analysis, and no effect of energy balance on follicle populations was noted after day 25 postpartum. A clear relationship between energy balance and follicular recruitment remains to be demonstrated during the very early stages of lactation.

The development of a dominant follicle postpartum in dairy cows is tolerant to periods of energy deficiency as demonstrated by the selection and growth of a follicle over 15 mm in diameter during the second week postpartum despite negative energy balance (Beam and Butler, 1997, 1998). However, results of several studies indicate that the ultimate diameter and oestrogen production of dominant follicles are influenced by metabolic factors. In prepubertal heifers (Bergfeld *et al.*, 1994), postpartum suckled beef cows (Perry *et al.*, 1991) and cyclic lactating dairy cows (Lucy *et al.*, 1992c), growth of dominant follicles is reduced during dietary energy restriction. As noted previously, dominant follicle diameter and plasma oestradiol increased after energy balance improved from its most negative level in early postpartum dairy cows (Beam and Butler, 1997). Comparisons between lactating and nonlactating dairy cows have revealed differences in dominant follicle development, and smaller dominant follicles are observed in nonlactating compared with lactating cows during the first follicular wave of a synchronized oestrous cycle (De La Sota *et al.*, 1993). In the same study, plasma oestradiol concentrations during the preovulatory period were several-fold higher in nonlactating compared with lactating cows. In a study conducted by Beam and Butler (1994), cows were either not milked (DRY), milked twice per day (2×) or three times per day (3×) following parturition. This resulted in different energy balance and body weight loss among groups during the first 4 weeks postpartum. Although peak plasma oestradiol was similar among groups, maximum diameter of the dominant preovulatory follicle from the first follicular wave postpartum was larger in both 3× (23.9 ± 1.2 mm) and 2× cows (21.0 ± 0.9 mm) compared with DRY cows (16.5 ± 0.9 mm). Therefore, lactational status or large differences in energy balance do not prevent the formation of follicular waves but apparently alter the growth and ultimate diameter of dominant follicles. Collectively, results of studies comparing lactating and nonlactating dairy cows indicate that dominant follicles of lactating cows in negative energy balance have a lower oestradiol output per unit of gross follicular size compared with nonlactating cows in positive energy balance. Differences in metabolic hormones such as insulin or IGF-I could be involved in the large difference in

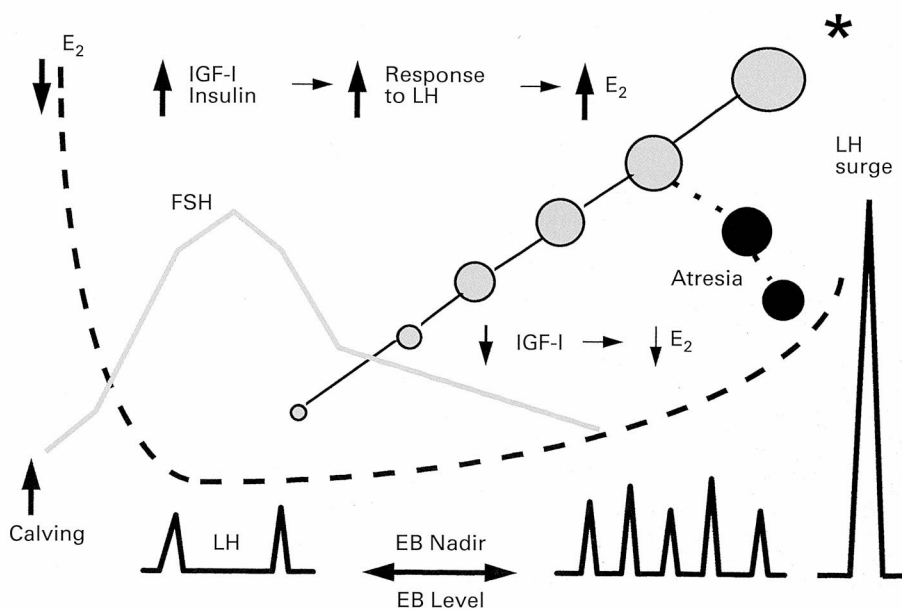


Fig. 7. Schematic representation of a basic model describing dominant follicle development (circles) and function in relation to changing metabolic and reproductive hormones, and energy balance (EB), during the first follicular wave postpartum in dairy cows. The first-wave follicle either ovulates (*) or undergoes atresia (dark circles). LH pulse frequency is modulated by the day of the EB nadir and, to a lesser extent, the level of EB. The large upward arrows indicate increased insulin-like growth factor I (IGF-I) and insulin leading to improved responsiveness to LH and greater oestradiol (E_2) production by the dominant follicle.

preovulatory follicular diameter between lactating and nonlactating dairy cows. For example, insulin treatment increased follicular diameter and concentrations of oestradiol in follicular fluid of superovulated cows (Simpson *et al.*, 1994). Other studies have implicated a role of the GH:IGF-I axis in follicular growth and turnover in cyclic cattle. In lactating dairy cows (days 60–100 postpartum) bovine somatotrophin (bST) increased the rate of dominant follicle turnover, preovulatory follicular growth and oestrogen secretion during a synchronized oestrous cycle compared with cows treated with saline (De La Sota *et al.*, 1993). Treatment of lactating dairy cows with recombinant bST during follicular waves of the oestrous cycle has also increased the size of the subordinate (or second largest) ovarian follicle (Lucy *et al.*, 1993). Moreover, an association has been reported between twinning rate in cattle and concentrations of both peripheral and follicular fluid IGF-I (Echternkamp *et al.*, 1990). Perhaps the most convincing evidence for the role of peripheral IGF-I in follicular function *in vivo* is data collected during the oestrous cycle in cattle that have a growth hormone receptor deficiency (GHRD; Chase *et al.*, 1998). In these cattle, GH is chronically increased and IGF-I is low in the peripheral circulation. The dominant follicle of the first follicular wave of the oestrous cycle stops growing at approximately 9 mm in diameter, when expression of LH receptors on granulosa cells is known to begin and to contribute to final maturation of the dominant follicle (Xu *et al.*, 1995). According to the authors, the timing of follicular regression in cows with GHRD was an indication of a failure in gonadotrophic support, and it was speculated that low circulating IGF-I led to inadequate LH receptor function or expression within granulosa cells of the first-wave dominant follicle. A similar scenario may be occurring in the early postpartum cow experiencing negative energy balance. As noted previously, serum IGF-I is low in dairy cows that develop anovulatory dominant follicles during the first follicular wave postpartum, and differences in IGF-I between

cows with either anovulatory or ovulatory dominant follicles precede the observed differences in follicular oestradiol production (Beam and Butler, 1998). Changes in systemic concentrations of IGF-I and IGF-binding proteins affect their concentrations in follicular fluid and follicular development in heifers (Cohick *et al.*, 1996), and IGF-I is known to contribute to induction of LH receptors in granulosa cells and to increase the sensitivity of follicular cells to LH stimulation (Spicer and Echternkamp, 1995). Lucy *et al.* (1992c) have shown a positive relationship between the ratio of oestrogen to progesterone in follicular fluid and plasma IGF-I in lactating Holstein cows. In the early postpartum cow, low concentrations of circulating IGF-I may contribute to reduced follicular responsiveness to a given level of gonadotrophic support, low oestradiol synthesis, and anovulation of dominant ovarian follicles.

A Working Model

On the basis of the preceding discussion, the following model is proposed for postpartum follicular development leading to first ovulation in lactating dairy cows experiencing negative energy balance. Major aspects of the model are represented schematically in Fig. 7. The release of negative feedback effects on FSH secretion by the clearance of high gestational oestrogens allows for an increase in mean plasma concentrations of FSH during days 3–7 postpartum. The increased FSH initiates a wave of follicular development during days 6–8 postpartum characterized by the recruitment of a pool of small (< 9 mm) follicles, from which usually one follicle is selected to become dominant. The dominant follicle emerges and continues to grow throughout the second week postpartum. Both the initiation of the follicular wave, including follicular recruitment, and emergence of a dominant follicle are largely insensitive to the severity of early postpartum negative energy balance. The steroidogenic activity, as measured in peripheral plasma, and ovulatory competence of this first dominant follicle are related to when postpartum energy balance begins to improve from its most negative level and the associated increase in LH pulse frequency. The level or severity of negative energy balance has a lesser modulating role in LH pulse frequency, but may influence the overall hormonal milieu including concentrations of circulating insulin and IGF-I conducive to enhanced follicular cell responsiveness to the ongoing pulsatile LH secretion pattern. If the day of the energy balance nadir occurs before, or soon after, the emergence of the dominant follicle, a concurrent increase in LH pulse frequency, responding to metabolic signals at the hypothalamus, would promote differentiation of the dominant follicle and increased oestradiol production leading to the induction of an LH surge and first ovulation. A delayed energy balance nadir, even in situations of relatively modest energy deficits, would probably result in low LH pulse frequency, low production of oestradiol, ovulation failure and atresia of the dominant follicle. Reduced concentrations of IGF-I, and perhaps insulin, contribute to reduced follicular responsiveness to gonadotrophic stimulation and to low oestradiol production by the dominant follicle, exacerbating the effects of low pulsatile LH secretion. The fate of the first-wave dominant follicle affects the duration of the postpartum anovulatory interval, with either the development of an anovulatory or cystic dominant follicle resulting in a greater number of days to first ovulation. Prolonged anovulatory intervals in turn impact negatively on first-service conception rates and overall reproductive performance.

Conclusion

The metabolic demands on modern dairy cattle impact negatively on the physiology regulating reproductive organs postpartum. In addition to the level of negative energy balance, changes in energy balance over time, such as improvement from the energy balance nadir, appear to be modulating postpartum ovarian activity. Together with patterns of LH secretion, evidence is strong for a role of the GH:IGF-I system in promoting follicular function postpartum and for IGF-I serving as a metabolic modulator of ovarian activity during negative energy balance. Challenges for the future include: greater delineation of the role of circulating IGF-I, as well as insulin, in follicular

function during negative energy balance; the determination of the importance of ovulatory follicle size to subsequent fertility in lactating cows; and the development of nutritional strategies to increase postpartum energy intake and foster improvements in energy balance with attendant increase in circulating metabolic hormones beneficial to final follicular differentiation and ovulation at an early stage postpartum.

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