Effect of Calf Isolation on Follicular Wave Dynamics, Gonadotropin and Metabolic Hormone Changes, and Interval to First Ovulation in Beef Cows Fed Either of Two Energy Levels Postpartum

K. Stagg, L.J. Spicer, J.M. Sreenan, J.F. Roche, and M.G. Diskin

Teagasc, (Agriculture and Food Development Authority), Athenry Research Centre, Athenry, Co. Galway, Ireland
Faculty of Veterinary Medicine, University College Dublin, Ballsbridge, Dublin 4, Ireland
Department of Animal Science, Oklahoma State University, Stillwater, Oklahoma 74078

ABSTRACT

The effects of postpartum energy intake, restricted suckling, and cow-calf isolation on concentrations of LH, FSH, growth hormone, and insulin-like growth factor-I (IGF-I) and on postpartum anestrous interval were determined by randomly allocating beef cows with a mean body condition score of 2.3 ± 0.1 to receive either 80 MJ metabolizable energy (low-energy diet [L]; n = 51) or 120 MJ metabolizable energy (high-energy diet [H]; n = 52) per cow per day from calving. At 30 days postpartum, cows within diet were randomized to 1) have continued access to their calves from birth to weaning (ad libitum suckling: ADLIB), 2) be suckled once-daily with their calves penned adjacent (restricted suckling, adjacent: RESADJ), 3) be isolated from all calves except for a once-daily suckling period (restricted suckling, isolated: RESISO). The mean postpartum interval was similar (p > 0.10) for L and H cows (62 and 63 days, respectively). RESADJ cows had a shorter (p < 0.05) postpartum interval than ADLIB cows, and RESISO cows had a shorter interval (p < 0.05) than RESADJ cows, with all effects independent (p > 0.10) of diet. FSH secretion pattern was not affected by diet, suckling treatment, sequential follicle wave number, or follicle wave retrospectively realigned to emergence of first ovulatory wave. Within 5 days of suckling restriction and calf isolation, the number of LH pulses increased from 0.18 to 0.48 pulses per hour (p < 0.05). Both mean LH and the mean number of LH pulses increased linearly (p < 0.01) during the six follicle waves up to the first ovulatory wave. From 80 days before, until the time of, first ovulation, growth hormone decreased (p < 0.05) while IGF-I increased (p < 0.05). Both mean LH and the follicle wave number were equally effective in permitting calf identification by its dam [8] and that elimination of both senses attenuate the negative effects of suckling on LH secretion.

INTRODUCTION

Prolonged postpartum anestrus is a major limitation to high reproductive efficiency in beef cows, with suckling and nutrition the major factors that influence the length of the postpartum anestrous interval (PPI). It is now clear that the suppressive influence of suckling is independent of neurosensory pathways within the teat or udder [1–5] and that maternal-offspring bonding is the essential component of the suckling-induced prolonged PPI in suckled beef cows [6, 7]. It has recently been shown that olfaction and vision are equally effective in permitting calf identification by its dam [8] and that elimination of both senses attenuate the negative effects of suckling on LH secretion. Many reviewers have concluded that prepartum nutrition, as reflected by body condition score (BCS) at calving, is a more important determinant of the PPI than postpartum nutrition [9, 10], with negative correlations reported between BCS at calving and the PPI in several studies [11–13]. In contrast, the effects of postpartum nutrition on the PPI are apparently inconsistent, with both no effect [13, 14] and significant effects [11, 12, 15] on PPI reported. This inconsistency may reflect interactions between pre- and postpartum nutrition, negative energy balance, body condition score (BCS), milk yield, and suckling as well as other environmental factors that influence the PPI. The relative importance of postpartum nutrition and suckling as determinants of PPI are not known. Nor is it clear whether a low plane of nutrition postpartum would abolish the beneficial effects of restricted suckling on shortening the PPI. Accordingly, the first objective of this experiment was to determine, for cows with a low to moderate BCS at calving, whether the ability of calf isolation to shorten the PPI in cows restricted to once-daily suckling is dependent on the level of energy fed in the postpartum period.

The main cause of prolonged postpartum anestrus in suckled cows is not the failure of dominant follicles to develop but rather their failure to ovulate [16, 17]. The roles of gonadotropins and other endocrine regulators of follicular function such as growth hormone (GH) and insulin-like growth factor-I (IGF-I) during the early postpartum anestrous period are not clear. Ultrasonography now makes it possible to compare changes in follicle wave status with endocrine and metabolic parameters in order to delineate the endocrine cause of prolonged anestrus in suckled beef cows. Thus, the second objective was to measure the concentrations of FSH and LH during successive follicle waves leading to first ovulation, and also to determine whether changes in GH and IGF-I concentrations are associated with the resumption of ovulation.

MATERIALS AND METHODS

Animals and Treatments

Hereford × Friesian and Angus × Friesian beef cows (n = 103) that had calved normally were used in the experiment. Cows and calves were weighed, and BCS was assessed at 2, 30, and 60 days after calving and again at
weaning. Body condition score was assessed [18] on a scale of 0–5, on which 0 is severe emaciation and 5 is obese. The cows used in the study had a low-to-moderate BCS (mean ± SEM = 2.3 ± 0.1) at calving when the experiment started.

At calving the cows were randomly assigned, within genotype and parity, to either a low-energy diet (L) providing 80 MJ metabolizable energy per cow per day (this is close to 100% of the recommendation [19] for a 460-kg beef cow producing about 8 kg of milk and with no change in live weight), or to a high-energy diet (H) providing 120 MJ metabolizable energy per cow per day. The diets comprised 60% grass silage and 40% concentrates (barley, soya bean, mineral, and vitamins; 16% crude protein). Within each treatment, cows were housed and fed in groups of not more than 5 cows according to calving date and with an adjoining creep area provided for calves. Competition for feed was minimized by having a small number of cows in each pen.

At 30 days postpartum, cows were randomly allotted, within diet, to one of 3 suckling frequency treatments: 1) continuous access of calves to cows from birth to weaning (ad libitum suckling: ADLIB), 2) once-daily suckling in which the calves were penned adjacent to, and had audio and visual contact with, their dams (restricted suckling, adjacent: RESADJ), and 3) once-daily suckling in which the cows were isolated in a separate building about 60 meters away from their calves and from other cows and calves (restricted suckling, isolated: RESISO); while these cows were out of sight of all calves, they were still within the audio range of their own and other calves. The cows on the restricted-suckling treatments were allowed access to their calves from 0800 to 0830 h daily. Cows remained indoors on these treatments until 2 days after their second postpartum ovulation, when they were turned out to pasture, and thereafter all calves had full access to their dams until weaning at 205 days.

Ovarian Ultrasonography

The ovaries of each cow were scanned daily from Day 5 postpartum or as soon as uterine involution allowed ultrasonic visualization of each ovary, until each cow had a second ovulation, as described previously [17]. An Aloka SSD-500V (Aloka, Japan) ultrasound scanner equipped with a 7.5-MHz transducer was used to monitor individual follicles on a daily basis [20]. The definitions of the follicle parameters used were those outlined previously [17]. Day of ovulation was defined as the day on which a large dominant follicle, present on either ovary the previous day, had disappeared.

Blood Sampling and Hormone Assays

A total of 70 cows were blood-sampled twice daily by jugular venipuncture from Day 10 postpartum to 1 day after the second ovulation had occurred. A random sample of 30 cows were blood-sampled from indwelling jugular catheters at 15-min intervals for 10 h at emergence and during the selection and dominant phases of successive follicle waves from Day 29 postpartum; this continued at a minimum interval of 3–4 days until the first ovulation had occurred. After collection, blood samples were immediately placed in “iced” water and centrifuged at 1000 × g at 4°C; the plasma stored at −20°C until required for assay.

FSH concentrations were determined in blood samples taken twice daily from Day 10 postpartum until first ovulation in 25 cows that were randomly chosen from the 100 cows in the experiment. FSH was assayed by RIA [21], using the NIDDK-anti-oFSH antibody (AFP-1C 5288113), ovine tracer (USDA oFSH 12), and a bovine FSH standard preparation (USDA B1 bFSH), all supplied by the National Hormone and Pituitary Program. The sensitivity of the assay was 3 ng/ml, and the intra- and interassay coefficients of variation for two serum samples containing 12.2 and 42.3 ng/ml of FSH were, respectively, 13.8% and 9.2% (n = 12) and 12.3% and 7.6% (n = 6).

Plasma LH concentrations were determined by RIA [22] with the following modifications for bovine plasma: 200-μl aliquots of plasma or standard (NIAMDD-oLH-24, NIH, USA), 100 μl of monoclonal antibody (518B7 anti-LH at 1:150 000 dilution; supplied by Dr. Jan Roser, University of California, Davis), and 100 μl oLH-I-125 radioligand (∼10 000 cpm/tube) were added to 11 × 64-mm polystyrene test tubes on Day 1. Tubes were vortexed and incubated at room temperature for 24 h. On Day 2, 50 μl of donkey-anti mouse second antibody (SAC-CELL, A-SAC 4; IDS, Tyne and Wear, UK) was added to each tube, and tubes were then incubated at room temperature for 30 min. Distilled water (200 μl) was then added, and the tubes were centrifuged for 5 min at 700 × g; the supernatant was aspirated, and the pellet was counted for 1 min in the gamma-counter. Sensitivity of the assay was 0.4 ng/ml, and the mean intra- and interassay coefficients of variation for three controls containing 2.2, 4.8, and 10.2 ng/ml LH were 12.6%, 8.3%, and 6.8% (n = 6) and 11.5%, 8.7%, and 8.2% (n = 10), respectively.

Plasma IGF-I and GH were determined in samples taken at weekly intervals from 10 days postpartum up to the first postpartum ovulation. Plasma IGF-I was quantified by RIA after an acid-ethanol extraction procedure [23]. Intra- and interassay coefficients of variation for six assays were 12.3% and 13.9%, respectively. Sensitivity of the assays averaged 4.2 ng/ml. Plasma concentrations of GH were quantified by RIA [24] with NIH-GH-B17 for standards; the intra- and interassay coefficients of variation (n = 3 assays) were 8% and 12%, respectively.

Statistical Analysis

The effects of diet and suckling treatments on cow and calf performance were determined by analysis of variance using PROC GLM [25]. The model included the effects of diet, suckling treatment, diet × suckling treatment interaction, parity (primiparous and multiparous), and calf sire. To normalize error distribution and to stabilize treatment variances, days from calving to first and second ovulation were log-transformed. The effects of diets and suckling treatments were then determined after analysis of variance using PROC GLM [25]. The model included the effects of diet, suckling treatment, diet × suckling treatment interaction, and parity (primiparous and multiparous).

The mean concentration of LH, number of pulses, pulse amplitude, and pulse length were determined using the PC-pulsar program [26], modified for the IBM-PC by J. F. Gitzelman and V. D. Ramirez (University of Illinois, Urbana). The G parameters used were 2.80, 2.50, 2.30, 1.80, and 1.50 for G(1), G(2), G(3), G(4), and G (5), respectively. Initially, the number of LH pulses on Day 29 (the day before suckling restriction and isolation) and on Day 34 were compared using ANOVA appropriate for repeated measures. The LH data were then normalized relative to the ovulatory follicle wave. The LH data for each 15-min sample, number of LH pulses per hour, and pulse amplitude and pulse length val-
ues were analyzed using ANOVA appropriate for a repeated-measures design with animal effects nested within diet × suckling treatments. Orthogonal polynomial procedures were used to partition linear, quadratic, and cubic effects. The model included diet and suckling treatments, follicle wave relative to ovulatory follicle wave, and follicle stage within each wave (emergence, selection, and dominance). FSH followed a wave-like pattern, with peak concentration occurring on the day before the emergence of a new follicle wave. These data were, therefore, first normalized relative to emergence of a new follicle wave and then analyzed using ANOVA appropriate for repeated-measures design with animal effects nested within diet × suckling treatments. The model included the effects of diet and suckling treatments and follicle wave relative to ovulatory follicle wave.

The IGF-I and GH data on Day 25 and Day 35 postpartum were analyzed using repeated-measures ANOVA. The model included the effect of diet and suckling treatment. All of the IGF-I data were pooled, normalized relative to day of first postpartum ovulation, and then analyzed using repeated-measures ANOVA. Orthogonal polynomial procedures were used to partition linear and quadratic effects.

RESULTS

Cow and Calf Performance

Cow live weights and BCS at Day 2 after calving were 465 ± 10.1 kg and 2.4 ± 0.10, and 468 ± 8.7 kg and 2.3 ± 0.09, for L and H cows, respectively. There was no interaction (p > 0.05) between plane of nutrition and suckling treatment for any of the parameters measured. Plane of nutrition did not affect cow live weight (H: 442 ± 9.7 kg; L: 428 ± 10.9 kg; p = 0.19) or BCS (H: 2.3 ± 0.09; L: 2.2 ± 0.10; p = 0.27) at Day 60 postcalving. Neither feeding level (L: 279 ± 6.1 kg; H: 283 ± 4.1 kg; p > 0.05) nor suckling treatment (ADLIB: 282 ± 5.9 kg; RESADJ: 283 ± 5.7 kg; RESISO: 279 ± 6.5 kg; p > 0.05) affected weaning weight.

Interval to First Postpartum Ovulation

Three cows had ovulated by Day 30 postpartum and were removed from the experiment. There was no interaction (p > 0.05) between diet and suckling treatment on PPI (Table 1). Diet did not affect (p = 0.76) PPI. However, restricting the suckling frequency shortened (p < 0.0001) PPI. Furthermore, the RESISO cows had a shorter (p = 0.02) PPI than the RESADJ cows. Within 10 days of commencement of the suckling treatments, 50% of the cows in the RESISO group had ovulated (Fig. 1) compared with 20% in the RESADJ and 3% in the ADLIB groups (p < 0.05). Shorter (≤16 days) than typical estrous cycles occurred in 85% of cows after first ovulation irrespective of treatment; 12% of cycles were of normal duration (18–24 days), and 3% were longer (>24 days) than normal. Neither suckling treatments nor diet affected (p > 0.10) the interval between first and second ovulation.

Concentrations of FSH

Plasma concentrations of FSH were not affected (p > 0.05) by postpartum diet, restricted suckling, follicle wave number, or follicle wave relative to ovulatory wave. Increases in plasma concentrations of FSH were observed before follicle wave emergence, with peak concentrations observed on the day before emergence and followed by a decrease on the day of emergence. Nadir concentrations were observed two days after emergence (Fig. 2).

Concentrations of LH

At Day 29 postpartum, one day before initiation of suckling treatments, the mean number of LH pulses was 0.18 ± 0.03 pulses per hour. However, on Day 34 postpartum, the mean number of LH pulses was greater (p < 0.05) in the RESISO (0.48 ± 0.06) than in the other suckling treatment groups (ADLIB; 0.21 ± 0.04, RESADJ; 0.23 ± 0.05 pulses per hour), whose LH pulse frequency was not dif-

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**TABLE 1. Effect of plane of postpartum nutrition and restricted suckling, with or without calf isolation, on the interval (back-transformed means) from calving to first ovulation (PPI).**

<table>
<thead>
<tr>
<th>Diets*</th>
<th>Suckling treatment</th>
<th>ADLIB</th>
<th>RESADJ</th>
<th>RESISO</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>80 MJ ME/cow (L)</td>
<td></td>
<td>81</td>
<td>58</td>
<td>52</td>
<td>62</td>
</tr>
<tr>
<td>120 MJ ME/cow (H)</td>
<td></td>
<td>78</td>
<td>65</td>
<td>51</td>
<td>63</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>79a</td>
<td>62b</td>
<td>51c</td>
<td>62</td>
</tr>
</tbody>
</table>

* ME, metabolizable energy.

a,b,c Value within a row with different superscripts is different (p < 0.02 at least).
ferent (p > 0.05) at this time. The effect of stage of follicle wave growth on concentrations of LH is presented in Table 2. Mean concentration and pulse frequency of LH increased linearly (p < 0.01) from the 6th follicle wave before the emergence of the first ovulatory wave postpartum (Fig. 3). Pulse amplitude was similar (p > 0.05) throughout the period, whereas the duration of each pulse changed quadratically (p < 0.05) as first ovulation approached.

Concentrations of GH and IGF-I

Overall mean plasma concentrations of GH were similar (p > 0.05) on Day 25 (14.0 ± 1.55) and on Day 35 (15.05 ± 1.70) but were greater (p < 0.05) in cows on the L (18.2 ± 2.31) than on the H (11.4 ± 1.80) diets. Plasma concentrations of GH were unaffected by suckling treatment on Day 35 (ADLIB 13.7 ± 1.7; RESADJ 13.8 ± 1.7 and RESISO; p > 0.05). Mean plasma concentrations of GH were consistently greater (p < 0.001) in cows on the L than on the H diet throughout the anovulatory period from Day 75 before, up to first ovulation (Fig. 4a). There was no effect (p > 0.05) of diet on concentrations of IGF-I before the initiation of suckling treatment, and the concentrations were not different (p > 0.05) on Day 25. On Day 35 postpartum, 5 days after initiation of the suckling treatments, concentrations of IGF-I were similar (p > 0.05) in the RESISO (31.3 ± 4.50) and RESISO (31.2 ± 4.40) groups and were greater in both of these groups than in the ADLIB (19.4 ± 3.1) group. Mean plasma concentrations of IGF-I increased linearly (p < 0.001) from 75 days before, to the day of, ovulation (Fig. 4b). Overall mean (L and H pooled) plasma concentrations of GH decreased linearly (p < 0.01) from 75 days before, to the day of, ovulation (Fig. 4b). As plasma concentrations of GH decreased, plasma concentrations of IGF-I increased (r = −0.45; p < 0.001).

TABLE 2. Effect of stage of anovulatory dominant follicle wave on concentrations of LH secretion (mean ± SE) in anestrous beef cows blood-sampled every 15 min for a 10-h period at intervals of 3–4 days.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Emergence</th>
<th>Selection</th>
<th>Dominance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean LH (ng/ml)</td>
<td>1.18 ± 0.163a</td>
<td>1.20 ± 0.194b</td>
<td>1.37 ± 0.156b</td>
</tr>
<tr>
<td>Mean no. LH pulses/h</td>
<td>0.23 ± 0.036a</td>
<td>0.23 ± 0.063a</td>
<td>0.27 ± 0.036a</td>
</tr>
<tr>
<td>Pulse duration (min)</td>
<td>34.3 ± 4.6a</td>
<td>39.1 ± 6.22a</td>
<td>36.5 ± 4.26a</td>
</tr>
<tr>
<td>Pulse amplitude (ng/ml)</td>
<td>1.00 ± 0.190a</td>
<td>1.23 ± 0.243ab</td>
<td>1.41 ± 0.175b</td>
</tr>
</tbody>
</table>

a,b,c Value within a row with different superscripts is different (p < 0.05).

DISCUSSION

This study shows that in beef cows restricted to once-daily suckling for 20–30 min, complete calf isolation advances the interval to first postpartum ovulation, and it confirms other data [1–8] indicating that breaking the maternal-calf bond shortens this interval compared with the interval associated with ad libitum calf access/suckling (suckling effect). The beneficial effects of restricted suckling and calf isolation on shortening the PPI in suckled beef cows was not dependent on the level of nutrient intake (maintenance or ~40% above) during the early postpartum period. In this experiment, it was not possible to determine the relative contributions of suckling or cow-calf bonding, but the overall data suggest that maternal bonding and suckling are key components prolonging PPI. The necessity of the cow’s own calf to suckle in order to delay first ovulation suggests that the maternal-calf bond is the more important of the two cues originating from continuous suckling [27]. In the present study, the RESISO cows, although out of sight of their own and other calves and probably out of their olfactory range, were within the audio range of their own calves. This suggests that visual and/or olfactory recognition of their own calves are the key components in maintenance of the suckling effect and therefore, in delaying first ovulation in beef cows. This confirms results of recent studies [8] showing that blind, olfactory-intact cows appear to recognize their own calves by smell and that anosmic sighted cows recognized their own calves by sight. However, when both senses were impaired, cows appeared to lack adequate sensory input to identify their own from unrelated calves, and in this case the time of first ovulation was similar to that in weaned cows. The magnitude of the reduction (28 days) in the PPI observed in the RESISO group is similar to the 20-day reduction recorded in a study [28] that employed a model similar to the one in the present study, though in that study the ad libitum cows had a shorter PPI.

FIG. 3. Mean LH pulse frequency (pulses/h) (a), mean concentration of LH (ng/ml) (b), mean pulse amplitude (ng/ml) (c), and mean pulse duration (min) (d) during the postpartum period in suckled beef cows.
(61 days) than the 79 days recorded for cows in the present study.

The energy levels provided after calving (80 or 120 MJ/day) did not affect the PPI in cows calving at a BCS of 2.3. This agrees with other published data [13] showing no difference in the PPI between cows fed 55 and 145 MJ metabolizable energy/cow per day. Other authors have reported effects of postpartum energy intake on the length of the PPI, but this effect may be confined to cows with low BCS at calving [11, 12]. The threshold BCS below which postpartum energy intake influences the PPI has not yet been determined. In a separate study with cows that had a mean BCS of 2.26 at parturition [13], which is similar to the mean BCS of 2.3 in the current study, there was no effect of energy intake postpartum on PPI. The results presented are in line with the conclusions from previous studies that prepartum feeding level, as reflected in BCS at calving, is a more important determinant of the PPI than postpartum feeding level [11–13].

The specific changes in the gonadotropic hormones FSH, in regulating follicle wave emergence, and LH, in determining the fate of the dominant follicle, are important in determining the time of first postpartum ovulation. The present study shows that there are recurrent increases in the plasma concentrations of FSH from 1 to 4 days before the emergence of each new nonovulatory follicle wave in anestrous postpartum suckled beef cows, with the highest concentrations occurring on the day immediately before new wave emergence. These changes in FSH are similar to those that occur before a new wave during the estrous cycle [29, 30] or before puberty in heifers [31, 32]. The concentrations of FSH were affected neither by wave number before first ovulation nor by the postpartum anestrous interval, irrespective of its duration. Also, concentrations of FSH were not affected by nutrition or maternal-calf bonding. Thus, it appears that concentrations and pattern of FSH during the postpartum anestrous period are not limiting factors that lead to atresia rather than to ovulation of dominant follicles.

In the present study, unlike many earlier reports, LH data were also normalized relative to the first postpartum ovulatory follicle wave rather than relative to the PPI. This allows more exact physiological comparisons of hormone profiles relative to the stage of follicle wave as impending ovulation approaches. Mean LH concentration and pulse frequency increased linearly, pulse duration decreased, and there was no change in pulse amplitude, with each succeeding anovulatory follicle wave preceding the ovulatory wave. It was evident that 4 days after reducing the suckling frequency and breaking the cow-calf bond, there was more than a 2-fold increase in the frequency of LH pulses, and 50% of these cows ovulated within a further 6 days. In contrast, there was no increase in the frequency of LH pulses in cows that were left adjacent to their own calves, even when the suckling frequency was reduced. This suggests that in the postpartum anestrous period, maternal bonding rather than suckling per se is more important in regulation of the GnRH release, and hence LH pulse frequency. A similar rise in LH pulse frequency was seen 24–48 h after temporary weaning in other studies [5, 33, 34], though there is evidence [35] that this increase may be attenuated by the return of the calves to their dams. In the current study, the acute increase in LH pulse frequency occurred even though cows were suckled once each day. The acute increase in LH pulse frequency and resumption of ovulation following removal of the suckling effect shows that the majority of suckled beef cows, provided they are not seriously compromised nutritionally, are capable of ovulation by 30–40 days postpartum but are prevented from doing so by the cow-calf-bonding effect(s) at this time.

It is now well established that the metabolic endocrine axis, including GH and IGF-I, can affect reproductive function. In agreement with other studies in cattle [24, 36, 37], blood concentrations of GH in this study were inversely related to nutrient intake. This agrees with the role of GH in fat mobilization of cows that are in negative energy balance in the early postpartum period [38]. However, unlike IGF-I (see later discussion), there was no evidence that plasma concentrations of GH were affected by restricting the suckling frequency, suggesting that changes in GH are not important in mediating the endocrine events leading to early resumption of ovulation in once-a-day suckled postpartum beef cows.

Concentrations of IGF-I were low in all cows at Day 25 postpartum and remained low in the ADLIB cows up to Day 35 postpartum. However, in both the RESADJ and RESISO groups, in which cows were induced to ovulate by removal of the suckling effect, concentrations of IGF-I were increased within 5 days of introduction of restricted suckling, with or without calf isolation. Similarly, when the plasma IGF-I data were pooled and analyzed relative to day of ovulation, it was clear that the concentrations increased linearly up to time of first postpartum ovulation. A similar pattern of elevated (+40%) concentrations of IGF-I during the first 2 wk after calving has been observed in dairy cows that ovulated the first postpartum dominant follicle compared to cows in which the first dominant follicle failed to ovulate [39]. Peripheral concentrations of IGF-I are positively associated with body condition and nutrient intake [40, 41], and decreased concentrations of IGF-I are associated with delayed puberty [42] and increased postpartum anestrous intervals [43–45] in beef cattle. Increased concentrations of IGF-I could directly stimulate proliferation or steroidogenic capacity of thecal [46, 47] and/or granulosa cells [48, 49]. It has also been indicated that IGF-I may...
directly affect pituitary [50, 51] and hypothalamic function [52]. However, the potential sites of action of IGF-I in affecting reproductive function are not clear from the results presented here. Neither is it clear what regulates the increased IGF-I rise after initiation of calf isolation and once-daily suckling. Maintenance and increased bioavailability of IGF-I play an important role in selection of the dominant follicle in cattle [53–55]; however, measurement of plasma IGF-I is of limited value because of the diverse components that produce IGF-I, the presence of IGF binding proteins, and the relative role of locally produced IGF-I and its putative endocrine actions in regulating follicular fate. Notwithstanding these limitations, there was clear divergence between declining GH and increasing peripheral IGF-I concentration during the postpartum period leading up to first ovulation. This uncoupling between GH and IGF-I concentrations in postpartum anestrous cows, previously observed in undernourished cattle [56, 57], has an important role in determining when or which dominant follicle will ovulate. Clearly also, factors other than GH are important in the regulation of peripheral IGF-I because once-a-day suckling increased IGF-I without affecting GH. This could point to a role for estrogen from the dominant follicle exposed to increased LH pulse frequency in partial regulation of IGF-I.

In conclusion, this study demonstrates that the suckling effect in beef cows is the major factor affecting the duration of the PPI, and it suggests that the maternal bond is more important than suckling in regulating LH pulse frequency, the key endocrine factor determining whether or not a dominant follicle ovulates. Removal of the suckling effect results in a rapid increase in LH pulse frequency, a response not dependent on the level of postpartum nutrition, at least within the nutritional limits of this study. There were sequential transient increases in FSH concentrations throughout the anestrous period, and each increase was associated with emergence of each new follicle wave. Mean concentrations of FSH did not vary with follicle wave number, suggesting that FSH, unlike LH, is not a major factor determining resolution of ovulation in postpartum beef cows. Concentration of IGF-I increased before resumption of ovulation, but further studies are required to determine its specific role in the resumption of ovulation. The restricted suckling and cow-calf isolation strategy used in this experiment is simple, noninvasive, and easy to apply, and it is a good model for studying the endocrine regulation of onset of ovulation in postpartum suckled cows.

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