Endocrine profiles and follicular development in early-weaned postpartum beef cows

K. F. Breuel¹, P. E. Lewis¹, E. K. Inskeep¹ and R. L. Butcher²

¹Division of Animal and Veterinary Sciences; and ²Department of Obstetrics and Gynecology, West Virginia University, Morgantown, WV 26505, USA

This study investigated whether treatment with progestagen, which improves fertility after early weaning in postpartum cows, altered concentrations of gonadotrophins or development and function of follicles. Patterns of luteinizing hormone (LH), oestradiol and follicle-stimulating hormone (FSH) and of follicular growth before first postpartum ovulation were compared in two experiments. At 17 to 25 days post partum, suckled anoestrous beef cows received an ear-implant containing 6 mg progestagen (norgestomet) for 9 days or served as untreated controls. Calves were weaned from all cows 7 days after initiation of treatment. Cows were observed for oestrous behaviour twice a day until 10 days after weaning. As expected, the proportion of anoestrous cows that formed a corpus luteum with a normal lifespan was greater \((P < 0.01)\) in cows treated with norgestomet (Expt 1, 17 of 24; Expt 2, 18 of 22) than in control cows (Expt 1, 2 of 16; Expt 2, 3 of 18). In general, patterns of secretion of LH and oestradiol and of final growth of the preovulatory follicle did not differ between control and norgestomet-treated cows. However, there was a transient rise in FSH in association with weaning in control cows that did not occur in norgestomet-treated cows. In addition, mean LH increased more rapidly and mean oestradiol was higher during the 3 days before the preovulatory surge of LH in the norgestomet-treated cows \((P < 0.01)\). These alterations in LH, FSH and follicular function, in the absence of any difference in rate of final growth of the preovulatory follicle, may mean that there was a lack of synchrony between follicular maturation and the LH surge in control cows which resulted in ovulation of a follicle that was not at an optimal stage of maturation. In comparison, treatment with norgestomet may partially synchronize follicular maturation and the LH surge.

Introduction

The first ovulation in cattle following parturition is usually associated with development of a corpus luteum that has a short lifespan (Menge et al., 1962; Foote and Hunter, 1964; Morrow et al., 1966; Humphrey et al., 1976; LaVoie et al., 1981; Manns et al., 1983). During the early postpartum period, short-lived corpora lutea form after weaning (Ramirez-Godinez et al., 1981, 1982a, b) or after administration of gonadotrophin-releasing hormone (GnRH) or human chorionic gonadotrophin (hCG) (Britt et al., 1974; Lishman et al., 1979; Carter et al., 1980; Kesler et al., 1980; Pratt et al., 1982). Fertility associated with the first postpartum ovulation, whether occurring spontaneously (Graves et al., 1968) or in response to weaning (Odde et al., 1980; Ramirez-Godinez et al., 1981), has been extremely low.

Several researchers (for review, see Lishman and Inskeep, 1991) have suggested that the short-lived corpus luteum may play a critical role in the transition from anoestrous to normal oestrous cyclicity by organizing endocrine and follicular events. Treatment with a progestagen, before induced ovulation, reduced the incidence of short-lived corpora lutea and increased fertility (Ramirez-Godinez et al., 1981). The mechanism(s) responsible for the failure of reproductive processes or by which treatment with progestagen corrects them remains uncertain, but probably involves changes in profiles of reproductive hormones. Alterations in preovulatory endocrine and follicular processes have been implicated as potential causes of subnormal luteal function and associated infertility in humans (diZerega and Hodgson, 1981), rats (Butcher, 1972), sheep (Coleman and Dailey, 1983; McLeod and Haresign, 1984) and cattle (Lishman et al., 1979; Odde et al., 1980; Pratt et al., 1982). The objective of these studies was to determine, in anoestrous postpartum beef cows, whether there are differences between cows treated with a progestagen (moderate fertility) and control cows (low fertility) in: (i) patterns of concentrations of LH, FSH or oestradiol during treatment with progestagen or before the first postpartum LH surge and (ii) size or growth pattern of the first postpartum ovulatory follicle.

Materials and Methods

Animals and treatments

Endocrine profiles and follicular development were examined in two experiments with postpartum beef cows of mixed breeding.
weaning, with a real-time B-mode linear array ultrasound (Pie Data 400, Pie Medical BV, Maastricht) fitted with a 5 MHz probe. This system allowed measurement of diameter of the follicle to the nearest 1 mm. These measurements were validated by removing ovaries from 31 cows after ultrasonography in earlier studies (Inskeep et al., 1988; Tortone et al., 1990). External diameter of the dissected largest follicles in those ovaries was always within 2 mm of, and averaged 1 mm larger than, the ultrasonic measurement of internal diameter. Upon onset of oestrous behaviour in each cow, ultrasonic imaging was performed twice a day in Expts 1 and 2 to determine the side and approximate time of ovulation (presence and then disappearance of a large follicle).

Radioimmunoassays

Concentrations of progesterone in serum (100 µl) were determined as described by Butcher (1977), as modified for cattle by Sheffel et al. (1982). Sensitivity of the assay was 20 pg per tube, with intra- and interassay coefficients of variation (CV) of 6 and 13%, respectively. Concentrations of oestradiol in serum (1 ml) were determined as described by Butcher (1977). Sensitivity of the assay was 0.5 pg per tube and intra- and interassay CV were 7 and 5%, respectively. Concentrations of LH in serum (100 µl) were determined as described by Niswender et al. (1968). Sensitivity of the assay was 0.025 ng per tube, and intra- and inter-assay CV were 7 and 16%, respectively. Standard for LH was NIH-LH-B9. Concentrations of FSH in serum (150 µl) were determined as described by Garcia-Winder et al. (1986). Sensitivity of the assay was 0.05 ng per tube, and intra- and inter-assay CV were 8 and 6%, respectively. Standard for FSH was NIAMD-0FSH-RP1; both LH and FSH standards were obtained from the National Hormone and Pituitary Program of the NIH, Bethesda, MD.

Statistical analyses

Cows in each experiment were allotted arbitrarily to two groups of approximately equal numbers on the basis of interval from weaning to the LH surge (defined as the time of a rise in LH to ≥10 ng ml⁻¹, associated with onset of oestrus or a precipitous fall in oestradiol). Group 1 (n = 14 norgestomet-treated and 10 control in Expt 1 and 15 norgestomet-treated and 8 control in Expt 2) had an LH surge 0 to 4 days after weaning and Group 2 (n = 13 norgestomet-treated and 8 control in Expt 1 and 10 norgestomet-treated and 15 control in Expt 2) had an LH surge more than 4 days after weaning. In Expt 1, hormonal profiles were examined by analysis of variance for a split plot design (Steel and Torrie, 1980) with treatment, group and their interaction in the main plot and time of sampling and interactions with time in the subplot. The hormonal profiles (FSH, oestradiol and LH) were examined for three periods: (Period 1) implant insertion until weaning (days −7 to 0), (Period 2) weaning until implant removal (days 0 to 2) and (Period 3) 3 days before the LH surge. Intervals from weaning to the LH surge were examined for effects of treatment by analysis of variance. All of these analyses were conducted using general linear model procedures of the Statistical Analysis System (SAS, 1985). Proportions of cows with a corpus luteum

Blood samples

In Expt 1, blood samples were collected twice a day, via jugular venepuncture, beginning with initiation of treatment and continuing until ovulation or 10 days after weaning. In Expt 2, blood samples were collected, via jugular venepuncture, immediately before onset of treatment and then twice a day from 1.5 days before weaning until ovulation or 10 days after weaning. These samples were assayed for oestradiol and LH in both experiments and for FSH in Expt 1; selected samples were assayed for progesterone to verify that ovulation had not occurred before weaning (progesterone <1 ng ml⁻¹). After ovulation, samples were taken every third day until at least 14 days after oestrus and assayed for progesterone to determine lifespan of the induced corpus luteum (number of days progesterone was ≥1 ng ml⁻¹).

Ultrasonic imaging

In Expt 2, follicular development was monitored every other day, from 2 days before weaning until oestrus or 10 days after weaning, with a real-time B-mode linear array ultrasound (Pie Data 400, Pie Medical BV, Maastricht) fitted with a 5 MHz probe. This system allowed measurement of diameter of the follicle to the nearest 1 mm. These measurements were validated by removing ovaries from 31 cows after ultrasonography in earlier studies (Inskeep et al., 1988; Tortone et al., 1990). External diameter of the dissected largest follicles in those ovaries was always within 2 mm of, and averaged 1 mm larger than, the ultrasonic measurement of internal diameter. Upon onset of oestrous behaviour in each cow, ultrasonic imaging was performed twice a day in Expts 1 and 2 to determine the side and approximate time of ovulation (presence and then disappearance of a large follicle).

Radioimmunoassays

Concentrations of progesterone in serum (100 µl) were determined as described by Butcher (1977), as modified for cattle by Sheffel et al. (1982). Sensitivity of the assay was 20 pg per tube, with intra- and interassay coefficients of variation (CV) of 6 and 13%, respectively. Concentrations of oestradiol in serum (1 ml) were determined as described by Butcher (1977). Sensitivity of the assay was 0.5 pg per tube and intra- and interassay CV were 7 and 5%, respectively. Concentrations of LH in serum (100 µl) were determined as described by Niswender et al. (1968). Sensitivity of the assay was 0.025 ng per tube, and intra- and inter-assay CV were 7 and 16%, respectively. Standard for LH was NIH-LH-B9. Concentrations of FSH in serum (150 µl) were determined as described by Garcia-Winder et al. (1986). Sensitivity of the assay was 0.05 ng per tube, and intra- and inter-assay CV were 8 and 6%, respectively. Standard for FSH was NIAMD-0FSH-RP1; both LH and FSH standards were obtained from the National Hormone and Pituitary Program of the NIH, Bethesda, MD.

Statistical analyses

Cows in each experiment were allotted arbitrarily to two groups of approximately equal numbers on the basis of interval from weaning to the LH surge (defined as the time of a rise in LH to ≥10 ng ml⁻¹, associated with onset of oestrus or a precipitous fall in oestradiol). Group 1 (n = 14 norgestomet-treated and 10 control in Expt 1 and 15 norgestomet-treated and 8 control in Expt 2) had an LH surge 0 to 4 days after weaning and Group 2 (n = 13 norgestomet-treated and 8 control in Expt 1 and 10 norgestomet-treated and 15 control in Expt 2) had an LH surge more than 4 days after weaning. In Expt 1, hormonal profiles were examined by analysis of variance for a split plot design (Steel and Torrie, 1980) with treatment, group and their interaction in the main plot and time of sampling and interactions with time in the subplot. The hormonal profiles (FSH, oestradiol and LH) were examined for three periods: (Period 1) implant insertion until weaning (days −7 to 0), (Period 2) weaning until implant removal (days 0 to 2) and (Period 3) 3 days before the LH surge. Intervals from weaning to the LH surge were examined for effects of treatment by analysis of variance. All of these analyses were conducted using general linear model procedures of the Statistical Analysis System (SAS, 1985). Proportions of cows with a corpus luteum

Blood samples

In Expt 1, blood samples were collected twice a day, via jugular venepuncture, beginning with initiation of treatment and continuing until ovulation or 10 days after weaning. In Expt 2, blood samples were collected, via jugular venepuncture, immediately before onset of treatment and then twice a day from 1.5 days before weaning until ovulation or 10 days after weaning. These samples were assayed for oestradiol and LH in both experiments and for FSH in Expt 1; selected samples were assayed for progesterone to verify that ovulation had not occurred before weaning (progesterone <1 ng ml⁻¹). After ovulation, samples were taken every third day until at least 14 days after oestrus and assayed for progesterone to determine lifespan of the induced corpus luteum (number of days progesterone was ≥1 ng ml⁻¹).

Ultrasonic imaging

In Expt 2, follicular development was monitored every other day, from 2 days before weaning until oestrus or 10 days after weaning, with a real-time B-mode linear array ultrasound (Pie Data 400, Pie Medical BV, Maastricht) fitted with a 5 MHz probe. This system allowed measurement of diameter of the follicle to the nearest 1 mm. These measurements were validated by removing ovaries from 31 cows after ultrasonography in earlier studies (Inskeep et al., 1988; Tortone et al., 1990). External diameter of the dissected largest follicles in those ovaries was always within 2 mm of, and averaged 1 mm larger than, the ultrasonic measurement of internal diameter. Upon onset of oestrous behaviour in each cow, ultrasonic imaging was performed twice a day in Expts 1 and 2 to determine the side and approximate time of ovulation (presence and then disappearance of a large follicle).
of normal lifespan (progesterone > 1 ng ml\(^{-1}\) > 14 days after oestrus) were examined for effects of treatment and of high or low mean concentrations of oestradiol, LH or FSH for Periods 1, 2 or 3 by \(\chi^2\) analyses (Steel and Torrie, 1980).

In Expt 2, profiles of oestradiol during the 4 days before the LH surge were examined as described for Expt 1. The pattern of growth of the preovulatory follicle during the 5 days before the LH surge was examined as described for hormonal profiles in Expt 1. Rates of growth of preovulatory follicles during the 5 days before the LH surge were examined by comparing the linear regressions of follicular size on day within cow between treatments and groups. For graphic presentation, ultrasonographic measurements were plotted in relation to the time of the LH surge. Because measurements were taken only every other day, some cows provided data on days 0, 2, 4 and 6, whereas others provided data on days 1, 3 and 5. Means and standard errors were calculated for each day by estimating data for intermediate days for each cow as the average of follicular diameters on the day before and the day after the missing time point. These calculations smoothed the curve by removing variability among time points due to individual variation of follicular size among cows.

**Results**

Ninety-seven of the 115 cows assigned to treatment were anoestrous at weaning and 93 of these had an LH surge and ovulated within 10 days after weaning and were used in these studies. Occurrence of the LH surge was delayed (\(P < 0.05\)) in norgestomet-treated (5.2 ± 0.3 days) compared with control (4.1 ± 0.3 days) cows, averaged over the two experiments. The effect of norgestomet was mainly in group 1 (3.7 versus 2.3 days) rather than group 2 (6.6 versus 6.3 days; treatment × group, \(P < 0.05\)). Treatment with norgestomet increased (\(P < 0.05\)) the proportion of cows forming a corpus luteum with a normal lifespan (Expt 1, 17 of 24; Expt 2, 18 of 22) compared with controls (Expt 1, 2 of 16; Expt 2, 3 of 18). The ability of treatment with norgestomet to normalize the lifespan of the corpus luteum induced by weaning did not vary with group (19 of 24 versus 16 of 23 for groups 1 and 2, respectively).

**Experiment 1**

**Luteinizing hormone.** A transient decline in concentrations of LH was detected 12 h after implant insertion in norgestomet-treated cows (\(P < 0.01\); Fig. 2). Otherwise, patterns of secretion of LH were similar during periods 1 and 2 in control and norgestomet-treated cows (Fig. 2). The pattern of linear increase in LH (\(P < 0.01\)) with day during period 3 revealed a more rapid increase in norgestomet-treated than in control cows (\(P < 0.05\)).

Mean (±SEM) concentrations of LH were higher (\(P < 0.01\)) in cows in group 1 (1.11 ± 0.08, 1.62 ± 0.15, 1.85 ± 0.13 ng ml\(^{-1}\) for periods 1, 2 and 3, respectively) compared with those in group 2 (0.65 ± 0.08, 0.83 ± 0.14, 1.30 ± 0.14 ng ml\(^{-1}\), respectively). There was a group × treatment interaction in period 1 (\(P < 0.05\)) due largely to high LH in control cows in group 1 (1.26 ± 0.12 ng ml\(^{-1}\)) and low LH in control cows in group 2 (0.54 ± 0.13 ng ml\(^{-1}\)), while concentration in norgestomet-treated cows did not differ with group. Patterns of secretion of LH did not differ between groups except for a quadratic interaction with day in period 3 (\(P < 0.05\); Fig. 2) and there were no significant interactions of treatment and group.

**Oestradiol.** Mean concentrations of oestradiol during the 7 days before and the 2 days after weaning were similar in control and norgestomet-treated cows, but were increased (\(P < 0.01\)) by norgestomet treatment during period 3 (6.2 ± 0.3 versus 4.1 ± 0.4 pg ml\(^{-1}\)). A difference in pattern of oestradiol was detected in period 1 (treatment × day, linear; \(P < 0.01\); Fig. 3). This interaction was due largely to a drop in concentrations of oestradiol in norgestomet-treated cows 12–24 h after implant insertion followed by a gradual increase thereafter to relatively higher values than in control cows by day 0. Patterns of secretion of oestradiol did not differ with treatment during periods 2 and 3.

Mean concentrations of oestradiol were higher (\(P < 0.05\)) in cows in group 1 than in those in group 2 during periods 1 (2.6 ± 0.2 versus 2.0 ± 0.2 pg ml\(^{-1}\)) and 2 (5.2 ± 0.3 versus 2.4 ± 0.3 pg ml\(^{-1}\)) and tended to be higher in period 3 (5.6 ± 0.3 versus 4.7 ± 0.4 pg ml\(^{-1}\); \(P < 0.10\)). Concentrations of oestradiol increased in group 1 more than in group 2 during periods 1 and 2 (group × day, linear, \(P < 0.01\); Fig. 3). Patterns of oestradiol revealed an increase over the 3 days before the LH surge (day, linear \(P < 0.01\); day, quadratic \(P < 0.05\) which
Fig. 3. Profiles of oestradiol before (period 1) and after (period 2) weaning and before the LH surge (period 3) in (a) control (---O---, n = 18) and norgestomet-treated (––●––, n = 27) cows and (b) groups 1 (---O---, n = 24) and 2 (––●––, n = 21) in Expt 1. Error mean squares were 0.669, 1.352 and 2.153 for periods 1, 2 and 3, respectively.

Fig. 4. Profiles of follicle-stimulating hormone (FSH) before (period 1) and after (period 2) weaning and before the luteinizing hormone (LH) surge (period 3) in (a) control (---O---, n = 18) and norgestomet-treated (––●––, n = 27) cows and (b) groups 1 (---O---, n = 24) and 2 (––●––, n = 21) in Expt 1. Error mean squares were 0.0361, 0.0179 and 0.0094 for periods 1, 2 and 3, respectively.

deviated only slightly from being parallel for the two groups (group × day, quadratic, P = 0.05; Fig. 3).

**Follicle-stimulating hormone.** Mean concentrations and patterns (Fig. 4) of FSH during the 7 days before weaning were similar in control and norgestomet-treated cows. Mean concentrations of FSH during this period did not differ among cows in groups 1 and 2, although an interaction of group and day (linear, P < 0.01) was detected, mainly because of an increase in FSH in group 2 just before weaning.

Mean concentrations of FSH during the 2 days after weaning were higher (P < 0.05) in group 2 than in group 1 (0.79 ± 0.04 versus 0.66 ± 0.04 ng ml⁻¹). There was a trend for a treatment by group interaction (P < 0.10), due largely to the high FSH in control cows in group 2 (0.89 ± 0.07 ng ml⁻¹), since mean concentrations of FSH were similar among control and norgestomet-treated cows in group 1 and norgestomet-treated cows in group 2 (0.65 ± 0.07 to 0.70 ± 0.05 ng ml⁻¹). Concentrations of FSH peaked and decreased rapidly in control cows just after weaning but changed little in norgestomet-treated cows during this period (treatment × day, linear, P < 0.01; Fig. 4). Patterns of secretion of FSH did not differ between groups (Fig. 4).

During period 3, mean concentrations of FSH were similar in control and norgestomet-treated cows, but patterns differed (treatment × day, linear, P < 0.05; Fig. 4). Concentrations of FSH in control cows were slightly higher initially and declined more rapidly as the LH surge approached compared with those in norgestomet-treated cows. Mean FSH was higher for cows in group 1 than in group 2 (0.66 ± 0.03 versus 0.58 ± 0.03; P < 0.05) and the pattern in group 1 was characterized by an initial rise and a more rapid decline than in group 2 (group × day, P < 0.05; Fig. 4).

**Relationship of lifespan of corpora lutea to hormone concentrations.** Overall (control and norgestomet-treated) subsequent lifespan of the corpus luteum was not related to mean concentrations of LH, oestradiol and FSH during any period studied. However, within norgestomet-treated cows, lifespan of the corpus luteum was normal in a higher (P < 0.05) proportion of cows with high mean concentrations of LH during each of the 3 periods (11 of 12) than of cows with low mean concentrations of LH (6 of 12). Similarly, a greater (P < 0.05) proportion of norgestomet-treated cows that had high mean concentrations of oestradiol during the 2 days after weaning formed a corpus luteum with a normal lifespan (11 of 12) than of those with low mean concentrations of oestradiol (6 of 12).

**Experiment 2**

Mean concentrations of oestradiol over the 4 days before the LH surge were affected by treatment, group and the interaction between treatment and group (P < 0.01). Mean concentrations
Fig. 5. Profiles of oestradiol during the 4 days before the luteinizing hormone (LH) surge in control cows (— — —) in groups 1 (○, n = 9) and 2 (●, n = 15) and norgestomet-treated cows (— — — —) in groups 1 (□, n = 15) and 2 (■, n = 10) in Expt 2. Error mean square was 1.437.

Fig. 6. Patterns of growth of the preovulatory follicle during the 5 days before the LH surge in (a) control (— — — ○ — ○ —, n = 15) and norgestomet-treated (— — — — —, n = 18) cows and (b) groups 1 (— — — ○ — ○ — —, n = 20) and 2 (— — — — — —, n = 13) in Expt 2. Mean ± SEM is estimated for each time point.

In norgestomet-treated cows in group 1 averaged 9.5 ± 0.5 pg ml⁻¹ compared with 4.9 to 5.9 ± 0.5 pg ml⁻¹ in the other three subgroups. Similarly, patterns of oestradiol were affected by group and the interaction between treatment and group (P < 0.01; Fig. 5); an earlier rise in oestradiol in the norgestomet-treated cows in group 1 accounted for most of the difference.

Mean diameters of the prevoluntary follicles (mm) over the 5 days before the surge of LH differed owing to treatment (12.9 ± 0.4 versus 12.4 ± 0.4 for norgestomet-treated and control, respectively; P < 0.05), group (13.8 ± 0.4 versus 11.2 ± 0.4 for groups 1 and 2, respectively; P < 0.005) and day (P < 0.001), but there was no interaction of treatment and group (Fig. 6). Neither the mean diameter at the time of the LH surge (14.9 ± 0.6 mm) nor the pattern of final growth of the prevoluntary follicle differed between treatments or groups. The linear increase in diameter during the 5 days before the LH surge (0.8 mm day⁻¹) did not differ with treatment or group (Fig. 6).

Discussion

In these studies, patterns of concentrations of LH, oestradiol and FSH, and final growth of the prevoluntary follicle were examined in control and norgestomet-treated postpartum beef cows before and during the induction of oestrus and ovulation by weaning their calves.

There was a transient decrease in concentrations of LH and oestradiol from 12 to 24 h after insertion of a norgestomet implant. This transient decline in LH was probably associated with an initially greater release of norgestomet from the implant (Chien et al., 1982). The decrease in oestradiol was probably caused by the fall in LH, but a direct effect of norgestomet on secretion of oestradiol or on regression and replacement of a follicle has not been ruled out.

During the 7 days from insertion of the norgestomet implant to weaning (period 1), there were no differences in mean concentrations of LH, oestradiol or FSH between norgestomet-treated and control cows. The gradual increases in LH before weaning have been seen by others in anestrous beef cows and are believed to be due to increased frequency of LH pulses with time post partum (Williams and Ray, 1980; Peters et al., 1981; Humphrey et al., 1983). Garcia-Winder et al. (1986) reported that norgestomet had no effect on mean concentration of LH, but they found an increase in pulse frequency of LH on day 6 after insertion of a norgestomet implant, compared with control cows. Increases in mean concentrations of oestradiol in serum (Sheffel et al., 1982; Johnson et al., 1992), and in follicular content of oestradiol, as well as larger follicular size (Garcia-Winder et al., 1987; Johnson et al., 1991) were found after day 6 in postpartum cows implanted with norgestomet. Garcia-Winder et al. (1986) proposed that the increase in frequency of LH pulses was responsible for the increase in serum oestradiol, although the greater number of LH receptors in the largest follicle of norgestomet-treated cows (Inskeep et al., 1988) probably contributes to the increase in serum oestradiol toward the end of the 9-day implant period.

While concentrations of FSH did not differ between norgestomet-treated and control cows before weaning, a decline in FSH in both norgestomet-treated and control cows occurred during the first 5 days of the implant period. We
suggest that this decline in FSH was due to negative feedback from a wave of follicular growth; perhaps new waves of follicular development were established by parturition and were synchronized among animals in this study by selection of cows with very similar intervals from parturition to initiation of treatment (22 ± 1 days). The tendency for FSH to rise by day of weaning in control cows is consistent with regression of this wave of follicles, with duration of follicular waves (Murphy et al., 1990) and with wave-like patterns of FSH in cycling (Bryner et al., 1990) and anoestrous cows (Schams et al., 1978; Webb et al., 1980). The suppression of FSH during the remainder of the experimental period in norgestomet-treated cows could represent persistence of a follicle in response to treatment. In cycling cows with a norgestomet implant, the largest follicle persisted after luteal regression (Savio et al., 1990; Rajamahendran and Taylor, 1991), which was attributed to an increase in pulse frequency of LH (Savio et al., 1990). Treatment of cows with sufficient progesterone to give luteal phase concentrations in serum lowered frequency of pulses of LH (Roberson et al., 1989) and produced turnover of follicular waves (Sirois and Fortune, 1990), whereas low doses increased frequency of pulses of LH and caused the largest follicle to persist. An implant containing 6 mg of norgestomet probably acts similarly to subluteal phase concentrations of progesterone (Roberson et al., 1989), thus increasing frequency of pulses of LH (Garcia-Winder et al., 1986) and causing the largest follicle to persist while blocking the LH surge and ovulation.

On the basis of patterns of FSH, there appeared to be synchrony of follicular development among animals. However, heterogeneity in hormonal profiles among cows was seen when cows were assigned arbitrarily to two nearly equal groups, based upon time from weaning to the LH surge. As expected, cows that ovulated first had higher concentrations of LH and oestradiol, and lower concentrations of FSH, than those in which ovulation occurred later. These profiles are consistent with a more advanced follicular development in some cows.

After weaning (period 2), concentrations of LH and oestradiol had an increasing, and FSH had a declining, profile with time. Weaning is believed to increase pituitary responsiveness to GnRH, thus increasing frequency of pulsatile release of LH (Walters et al., 1982a, b; Shively and Williams, 1989), which stimulates increased secretion of oestradiol (Walters et al., 1982c). The greater concentrations of oestradiol and lower concentrations of FSH with no difference in LH in norgestomet-treated compared with control cows are consistent with a more responsive follicle in norgestomet-treated cows, which agrees with the greater number of LH receptors in the largest follicle after exposure to progesterone (Inskeep et al., 1988; Braden et al., 1989). The decline in FSH is probably due to negative feedback from these more active follicles, through increased oestradiol and inhibin (not measured in this study, but increased with greater follicular development in ewes; Campbell et al., 1990).

High concentrations of LH in cows ovulating by 4 days after weaning probably reflect stimulation of pituitary sensitivity to GnRH by greater secretion of oestradiol before weaning. The marked increase in oestradiol and decline of FSH could reflect more advanced development of a follicle and its negative feedback on FSH in those cows that ovulated within 4 days after weaning. Cows that ovulated later did not exhibit increases in either LH or oestradiol during the 2 days after weaning, but had a marked decline in FSH. This decline in FSH could occur because removal of suckling increased sensitivity of the pituitary to negative feedback on FSH or because of a decreased ratio of oestradiol to inhibin (not measured) secreted by follicles not prepared for ovulation.

The increasing profiles of LH and oestradiol and declining profile of FSH before the surge of LH (period 3) were similar to profiles and concentrations at this time in cycling cows (Hansel and Convey, 1983; Bryner et al., 1990) and at first and second postpartum oestrus (Ramirez-Godinez et al., 1982b). The increases in LH and oestradiol in norgestomet-treated cows and in cows ovulating by 4 days after weaning are consistent with a more advanced follicular development (as discussed above for periods 1 and 2). Presence of the norgestomet implant for 2 days after weaning delayed ovulation compared with controls by an average of 1.4 days in group 1 cows. As seen in Fig. 5, cows in this subgroup had increased concentrations and a plateau of oestradiol before ovulation and were responsible for both the higher concentrations of oestradiol and larger follicle in norgestomet-treated cows. Increased size and sensitivity of follicles was discussed above in relation to changes in periods 1 and 2.

Ovulatory response of anoestrous cows to exogenous GnRH has been shown to depend on size and maturity of follicles and concentrations of oestradiol (Kesler et al., 1977; Lishman et al., 1979; Zalied et al., 1980; Smith et al., 1983). Rates of follicular growth did not differ among treatments or times of ovulation. Murphy et al. (1990) found similar rates of follicular growth at the first and second oestrus post partum, whereas Perry et al. (1991) reported a decreased rate of growth before first compared with second ovulation. The ovulatory follicle was larger in cows ovulating by 4 days after weaning (group 1) than in those ovulating later (group 2) and was slightly larger during the 5 days before and LH surge in norgestomet-treated than in control cows. In postpartum cows suckling calves, diameter of the largest follicle was greater in norgestomet-treated than in control cows (Garcia-Winder et al., 1987; Inskeep et al., 1988). Garcia-Winder et al. (1987) found that treatment with norgestomet increased the difference in size between the largest and second largest follicles. According to Johnson et al. (1991), this divergence began 6 to 8 days after treatment with norgestomet was initiated. After treatment of cycling cows with norgestomet and regression of the corpus luteum with prostaglandin F20, Savio et al. (1990) observed that the dominant follicle present at the time of treatment persisted for 15 days and ovulated after implant removal in five of eight cows. They attributed the persistence to an increased pulse frequency of LH.

The lack of an overall association of duration of luteal phase with concentrations of hormones is in agreement with the conclusion of Sheffel et al. (1982) that in cows that formed a corpus luteum after injection of hCG, duration of induced luteal phase was not related to concentration of oestradiol, except as a function of pretreatment with norgestomet. In this study, the proportion of norgestomet-treated cows that formed a corpus luteum with a normal lifespan was related to concentrations of both LH and oestradiol. However, despite the fact that norgestomet-treated cows that ovulated early in response to weaning had higher LH and oestradiol, they did not form a corpus luteum with normal lifespan any more often (19 of 24) than those with an increased interval from weaning to the LH surge (16 of 23). Thus the
effectiveness of norgestomet was not lost if ovulation did not occur within 4 days after weaning.

The lifespan of the induced corpus luteum was not associated with concentrations of FSH before or after weaning (Expt 1). Garcia-Winder et al. (1986) observed lower concentrations of FSH on day 6 of treatment in norgestomet-treated cows that had a high incidence of short-lived corpora lutea than in those having normal corpora lutea. They proposed that a threshold value of FSH may be necessary for treatment with norgestomet to be effective. However, administration of FSH to anestrous cows before induction of ovulation failed to increase the incidence of normal corpora lutea (Lishman et al., 1979).

In conclusion, patterns of LH and oestradiol during treatment and weaning and rate of growth of the preovulatory follicle were similar between control and norgestomet-treated cows. However, there was a transient increase in FSH in association with weaning in control cows. In addition, mean LH increased more rapidly and oestradiol was higher in the norgestomet-treated cows during the 3 days immediately before the preovulatory surge of LH. These results may mean that control cows there was a lack of synchrony between the preovulatory follicle and the LH surge that resulted in ovulation of a pre- or post-mature follicle. In comparison, treatment with norgestomet may have partially synchronized follicular maturation and the LH surge, resulting in ovulation of a follicle that was more nearly at its peak of function.

This study was supported by USDA grants 86-CRRC-1-2138 and 89-37240-4714 and Hatch project 321 (NE-161) of the West Virginia Agricultural and Forestry Experiment Station and is published with approval of the Station Director as Scientific Paper No. 2306. The authors thank D. Kirkpatrick-Keller for assays of proteins and steroids; E. Henderson, Ceva Laboratories (now Sanofi Animal Health) for norgestomet implants; G. D. Niswender, Colorado State University, for antisera to LH, L. E. Reichart, Jr, Albany Medical College, for purified LH; NIAIDDD and the National Hormone and Pituitary Agency for reagents for assays for FSH; and E. C. Townsend for assistance with statistical analyses.

References


Butcher RL (1977) Changes in gonadotropins and steroids associated with unilateral ovarioectomy in rats Endocrinology 101 830–840

Campbell BK, Mann GE, McNiely AS and Baird DT (1990) The pattern of ovarian inhibit, estradiol and androstenedione secretion during the estrous cycle of the ewe Endocrinology 127 227–235


Biomedical Assessments Vol 14, pp 413–463 Ed. YW Chien. Marcel Dekker, New York


DiZerega GS and Hodgen GD (1981) Luteal phase dysfunction infertility: a sequel to aberrant folliculogenesis Fertility and Sterility 35 489–499


Graves WE, Lauderdale JW, Hauser ER and Casida LE (1968) Relationship of postpartum interval to pituitary gonadotropin, ovarian follicular development and fertility in beef cows University of Wisconsin College Agriculture Life Science Research Bulletin 270 23–26

Hansel W and Convey EM (1983) Physiology of the estrous cycle Journal of Animal Science 57 (Supplement 2) 604–624

Humphrey WD, Koritnik DR, Kalenbach CC, Dunn TG and Niswender GD (1976) Progestrone and LH in postpartum suckled beef cows Journal of Animal Science 43 (Supplement 1) 290


Johnson SK, Del Vecchio RP, Townsend EC and Inskeep EK (1992) Role of proglandin F2α in follicular development and subsequent luteal life span in early postpartum beef cows Domestic Animal Endocrinology 9 49–56


Murphy MG, Boland MP and Roche JF (1990) Pattern of follicular growth and resumption of ovarian activity in postpartum beef suckler cows Journal of Reproduction and Fertility 90 523–533


Ramirez-Godinez JA, Kiracofe GH, Carnahan DL, Spire MF, Beeman KB, Stevenson JS and Schalles RR (1982a) Evidence for ovulation and fertilization in beef cows with short estrous cycles Theriogenology 17 409–414


Shively TE and Williams GL (1989) Patterns of tonic luteinizing hormone release and ovulation frequency in suckled anestrous beef cows following varying intervals of temporary weaning Domestic Animal Endocrinology 6 379–387

Sirosi J and Fortune JE (1990) Lengthening the bovine estrous cycle with low levels of exogenous progesterone: a model for studying ovarian follicular dynamics Endocrinology 127 916–925


Walters DL, Short RE, Convey EM, Staigmiller RB, Dunn TG and Kaltenbach CC (1982b) Pituitary and ovarian function in postpartum beef cows. II. Endocrine changes prior to ovulation in suckled and non-suckled postpartum cows compared to cycling cows Biology of Reproduction 26 647–654


