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Summary

A study using twelve prepuberal Angus heifers was conducted to determine the effects that synthetic progesterone with or without gonadotropin-releasing hormone (GnRH) had on blood serum concentrations of progesterone, estradiol, and luteinizing hormone (LH) over a 48-hour period. Four heifers were given an implant containing the synthetic progestogen norgestomet for 9 days. Four other heifers were given a similar implant and also received an injection of GnRH after implant removal. The remaining four heifers served as controls and received no treatment. Serum progesterone and estradiol concentrations did not differ between treatments ($P>.8$). Heifers receiving norgestomet and GnRH had increased levels of serum luteinizing hormone (LH) during the 4.5 hours following the injection ($P<.0001$). Serum LH concentrations in heifers treated with norgestomet only did not differ from those of controls.

Key Words: Beef Heifers, Puberty, GnRH, Progesterone, LH

Introduction

Yearling heifers should be bred early in their first breeding season since 2-year-old cows have a longer postpartum interval than older females. Previous research has shown that administration of progesterone with or without gonadotropin releasing hormone (GnRH) reduces the age at puberty in beef heifers. This could be an effective management tool for producers that have difficulty in developing their replacement heifers to reach puberty prior to the start of their first breeding season. To be completely effective, the physiological mechanisms by which prepuberal treatment with progesterone or progesterone and GnRH cause earlier puberty must be understood. This knowledge could lead to a more efficient usage of these treatments. The following study was conducted to determine the effects a progesterone implant or a progesterone implant followed by an injection of GnRH would have on the hormonal profile in the prepuberal heifer.

Materials and Methods

Twelve purebred Angus heifers from the Cow-Calf Unit at South Dakota State University were selected from a group of 19 replacement heifers according to the following criteria: 1) no signs of estrus had been detected, and 2) the age of the heifer. Heifers had been observed once daily for signs of heat and were assumed to be prepuberal at the time of the study onset if estrus was not detected. The youngest heifers in the group which had not been observed in estrus were selected over older heifers, based on the theory that the older animals would more likely be pubertal. At an average age of 218 days and 628 lb in weight, heifers were randomly assigned to one of three treatments. Heifers in treatment one were controls and received no hormone treatment. Heifers in treatment two were given an implant[4] containing 6.0 mg of synthetic progestogen

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norgestomet for 9 days. Heifers in treatment three received the norgestomet implant and were also given 2000 μg GnRH[5] in a saline injection upon removal of the implant.

Heifers were fed a diet of corn silage prior to and at the beginning of the study. One day before removal of the norgestomet implant, the heifers were moved into the Animal Science Complex and were housed on concrete floors. Upon entering the building, heifers were provided ground alfalfa hay and water for ad libitum consumption until the termination of the study. Heifers were allowed 16 hours to adapt to their new environment before handling and onset of the serial bleeding. On day 9, all heifers were catheterized with a polyethylene jugular cannula. Catheters were not inserted into hormone treated heifers until implants were removed. A 10-ml blood sample was collected from each heifer after catheterization was completed. Heifers receiving GnRH were given the hormone in saline solution subcutaneously immediately after the first blood sample was collected via the catheter. Thereafter, 10-ml blood samples were collected from the catheters every .5 hour for the first 24 hours following catheterization and every 1 hour for the next 24 hours. Following each blood sample, 2 ml of heparinized saline solution (20 Units/ml) were injected into the catheter to prevent coagulation. Approximately 5 ml of blood were drawn through the catheter and discarded before each sample was collected in order to remove the injected heparin. Upon collection, blood samples were chilled and centrifuged. Serum was frozen in duplicate until assayed for progesterone, estradiol, and LH.

Blood serum progesterone concentrations were analyzed with a one-step radioimmunoassay procedure. Analysis was performed on the 0, 5, 10, 15, 20, 25, 30, 35, 40, and 45 hour samples from each heifer.

Blood serum estradiol-17ß concentrations were analyzed using a commercial estradiol assay. Original analysis was performed on the 0, 5, 10, 15, 20, 25, 30, 35, 40, and 45 hour samples from each heifer in one assay. Later analyses were performed on each hourly sample from 0 through 20 hours for each heifer. Blood serum LH concentrations were analyzed using a monoclonal double antibody radioimmunoassay. Analyses were performed on samples collected every other hour from 0 to 48 hours for all heifers. In addition, analysis was performed on all samples collected from 0 to 6 hours in heifers that received GnRH.

Progesterone, estradiol, and luteinizing hormone concentrations were analyzed using the GLM procedures of SAS (SAS, 1988). All data were analyzed as a split plot design to determine any hormonal differences between treatments, using individual heifer variance within treatment as the error term for the treatment effect. Repeated measures analyses were also performed to detect differences in hormone concentrations within treatments over time.

Results and Discussion

Serum progesterone concentrations were not affected by treatment ($P=.82$) or time x treatment interaction ($P=.99$). However, progesterone levels did differ over time ($P<.0001$). This difference was due to a higher progesterone concentration at the first sampling time than at all other times. Average progesterone concentrations of each treatment group are listed in Table 1. Mean baseline concentrations for the control and norgestomet groups over the sampling period were .02 ± .09 ng/ml and .03 ± .08 ng/ml, respectively. Norgestomet plus GnRH treated heifers had a mean baseline progesterone concentration of .03 ± .09 ng/ml for the 48-hour sampling period.

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Table 1. Average serum progesterone concentrations over a 48-hour period in prepuberal heifers treated with norgestomet or norgestomet + GnRH

Treatment	Serum progesterone, ng/ml
Controls	.02 ± .01[a]
Norgestomet	.03 ± .01
Norgestomet + GnRH	.03 ± .01

[a]Means ± standard error.

Table 2. Average serum estradiol concentrations over a 48-hour period in prepuberal heifers treated with norgestomet or norgestomet + GnRH

Treatment	Serum estradiol, pg/ml
Controls	4.21 ± .29[a]
Norgestomet	3.80 ± .31
Norgestomet + GnRH	4.25 ± .36

[a]Means ± standard error.

Serum estradiol was not affected by treatment (P=.92) or time x treatment interaction (P=.57). However, serum estradiol was affected by time (P<.0001) and individual heifer nested within treatment (P<.0001). The three youngest heifers had the highest baseline estradiol levels found in the 12 animals. Each of these heifers had been assigned to a different treatment group. Average estradiol concentrations of each treatment group are listed in Table 2. Baseline levels of estradiol were 4.21 ± 2.99 pg/ml for control heifers, 3.80 ± 3.19 pg/ml for norgestomet treated heifers, and 4.25 ± 3.66 pg/ml for the norgestomet plus GnRH treated heifers. Serum estradiol in control heifers fluctuated during the sampling period. Norgestomet treated heifers did not show any differences from controls in estradiol concentrations, although they had somewhat lower levels between 17 and 20 hours after onset of collection. Norgestomet plus GnRH treated heifers also had similar estradiol concentrations to controls but appeared to have elevated levels between 3 to 5 hours and at 7 hours following onset of the serial bleeding. Mean values at these times ranged from 6.05 ± 4.75 pg/ml to 8.21 ± 3.62 pg/ml.

Serum luteinizing hormone was affected by individual heifer nested within treatment (P<.0001), time x treatment interaction (P<.0001), time (P<.0001), and treatment (P=.0384). The heifer effect was due to one norgestomet treated heifer having a higher baseline LH level than the other three heifers in that treatment group and also to a large variability in the amount of LH released in response to GnRH in the norgestomet plus GnRH treated heifers. The time x treatment interaction was due to the elevated levels of serum LH found in the norgestomet plus GnRH treated heifers between 0 and 6 hours after onset of collection. Control heifers and norgestomet treated heifers did not display any LH elevations. Baseline LH levels for the norgestomet plus GnRH treated heifers were calculated as the average of the values not included in the peak. Mean LH baseline values for each treatment group are shown in Table 3. Baseline LH concentrations were 1.58 ± .52 ng/ml, 1.80 ± .43 ng/ml, and 1.71 ± .27 ng/ml for the control, norgestomet, and norgestomet plus GnRH treated groups, respectively. Figure 1 illustrates the average LH concentrations for each treatment group for the 48-hour sampling period. The peak height in norgestomet plus GnRH treated heifers averaged 11.62 ± 5.33 ng/ml and ranged from 6.12 to 18.50 ng/ml. The oldest heifer in this group had the lowest LH peak, while the two youngest heifers had the highest LH peaks. This may be due to the age of the heifers, since the approach of puberty has been associated with the establishment of lower and more stable levels of LH. The peak height occurred 2 hours following injection of GnRH in three of the heifers and 2.5 hours following the injection in the fourth heifer. Duration of the LH peak varied from 3 to 5 hours. Elevations in luteinizing hormone concentrations were first observed in samples collected .5 hour after the GnRH injection in two heifers and within 1 hour in the remaining two heifers. Following the LH peak, all norgestomet plus GnRH treated heifers showed decreased serum LH for 1 to 2 hours before returning to baseline levels. The decrease in LH levels following the peak in norgestomet

Table 3. Average serum luteinizing hormone concentrations over a 48-hour period in prepuberal heifers treated with norgestomet or norgestomet + GnRH

Treatment	Serum LH, ng/ml
Controls	1.58 ± .05[a]
Norgestomet	1.80 ± .09
Norgestomet + GnRH	1.71 ± .14

[a]Means ± standard error.

plus GnRH treated heifers may have been due to a depletion of pituitary stores and(or) down regulation of LH secretion.

Herdmates of the heifers in this study that had entered puberty were approximately 50 days older than the selected heifers. Therefore, it is probable that the heifers used in this serial bleeding had not reached sufficient age to respond to hormonal treatment and enter puberty at the time of treatment. Conversely, it is also possible that the 48-hour study period would have been too brief to observe any physiological changes that may have occurred.

It is unknown why progesterone concentrations were higher in the first blood sample than in all others collected. However, the highest levels of progesterone found in the first blood sample collected are typical of progesterone levels reported in prepuberal heifers. Although not significant because of the high variability among the heifers, there did appear to be an increased production of estradiol in the several hours following GnRH administration in the norgestomet plus GnRH treated heifers in this study. The estradiol increase appeared at approximately the same time as the LH peak. The removal of the norgestomet implant followed by a GnRH injection in the prepuberal heifers may have initiated follicular production of estradiol without completely inducing puberty within the following 48 hours.

Prepuberal progestogen administration in the norgestomet only treated heifers did not alter the LH profile in comparison to the controls in this study. Therefore, assuming the heifers in this study were capable of reaching puberty at the this time, the mechanism by which prepuberal norgestomet treatment induces early puberty in heifers does not appear to be due to an alteration in the release of LH in the 48 hours following implant removal. All of the norgestomet plus GnRH treated heifers had an increase in serum LH following treatment. Although none of these heifers attained the amplitude found during the preovulatory surge in the bovine, they did approach these levels. Therefore, the mechanism that prepuberal progesterone and GnRH treatment causes earlier puberty in beef heifers to occur may be due to a change in LH release from the pituitary.

In summary, administration of a 9-day norgestomet implant with or without GnRH in prepuberal heifers at an average of 218 days of age and 628 lb in weight did not change serum progesterone or estradiol concentrations. However, progesterone and estradiol did vary over time. Prepuberal heifers given both norgestomet and GnRH displayed an LH peak approaching preovulatory levels. This peak lasted 3 to 5 hours with the peak height occurring 2 to 2.5 hours following the GnRH injection. No elevations of LH were present in control or norgestomet treated heifers.

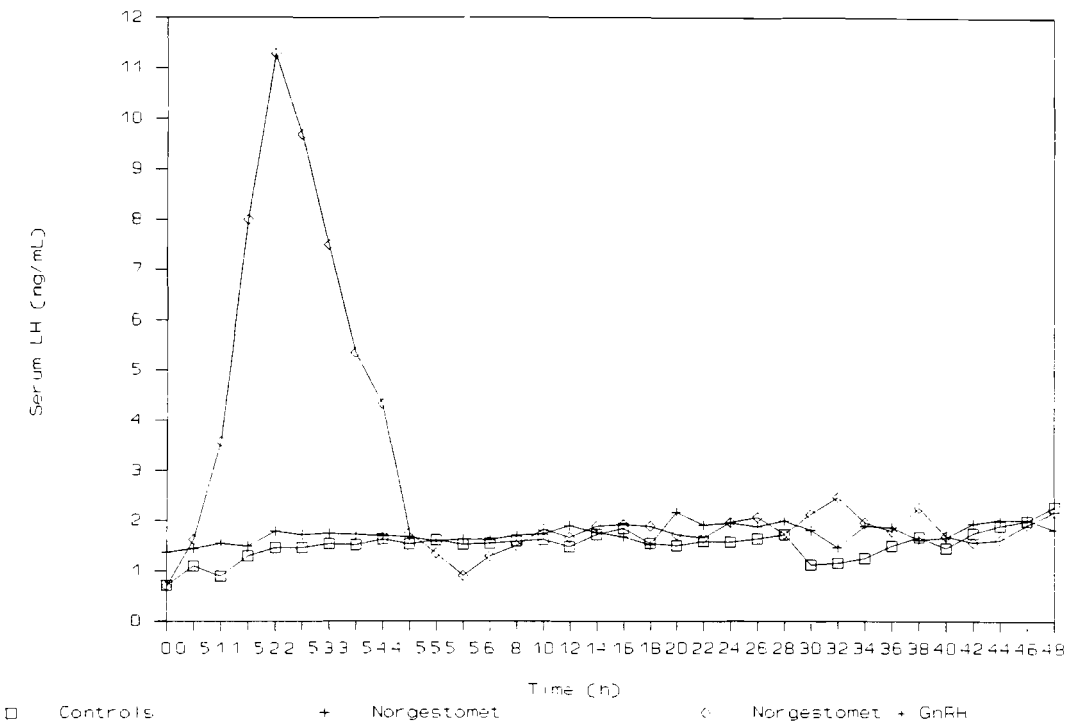

Figure 1. Average luteinizing hormone concentrations (ng/ml) in prepuberal heifers following treatment with norgestomet or norgestomet and GnRH.