Role of low circulating FSH concentrations in controlling the interval to emergence of the subsequent follicular wave in cattle

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The intervals between emergence of follicular waves 1 (first wave of an oestrous cycle) and 2, and between the associated FSH surges (surges 1 and 2), were studied in control ($n = 7$) and recombinant bovine (rb)FSH-treated ($n = 7$) heifers. The expected start of the deviation in follicle diameter between the two largest follicles of wave 1 was defined as the day on which the largest follicle reached 8.5 mm (day 0). In the control heifers, circulating concentrations of FSH decreased and oestradiol increased between day 0 and day 1.5 or day 2.0 in a reciprocal relationship. The opposite reciprocal relationship between an FSH increase and an oestradiol decrease occurred during the next 3 days. This temporal result is consistent with a negative systemic effect of oestradiol on FSH at this

time. rbFSH was administered in a dosage regimen that was expected to result in a similarity between FSH surge 2 in the rbFSH-treated group and surge 2 in the control group. On average, surge 2 and wave 2 occurred approximately 2 days earlier in the rbFSH-treated group than in the control group, and characteristics of the FSH surge and follicular wave were similar (no significant differences) between groups. These results support the hypothesis that low circulating FSH concentrations after the deviation in follicle diameter control the interval to emergence of the subsequent follicular wave. However, in one of seven rbFSH-treated heifers, the largest follicle from the apparent stimulation of rbFSH reached only 5.7 mm; therefore, the possibility of involvement of additional mechanisms cannot be dismissed.

Introduction

Two or three follicular waves develop during the bovine oestrous cycle (Ginther *et al.*, 1989). Each wave is preceded by a surge in circulating FSH (Adams *et al.*, 1992). The FSH surge and the resulting follicular wave that develop at the start of the oestrous cycle are termed surge 1 and wave 1, and are followed in about 10 days by surge 2 and wave 2. When the largest follicle of a wave reaches a mean diameter of 8.5 mm, the follicles begin to deviate in diameter into a single dominant follicle and several subordinate follicles (Ginther *et al.*, 2001a). The FSH concentrations continue to decrease for approximately 1 day after the start of deviation in follicle diameter. Surge 2 stimulates the emergence of follicles 4 mm in diameter from wave 2 about 10 days after emergence of wave 1 or 7 days after the start of deviation in follicle diameter of wave 1. The changes in the concentrations of FSH and other hormones during the interval between surges 1 and 2 have not been described adequately. Previous studies used reference points near the beginning of wave 1 (Badinga *et al.*, 1992; Sunderland *et al.*, 1996; Evans *et al.*, 1997) or normalized each wave and associated hormone concentrations to follicle emergence (Adams *et al.*, 1992) or deviation (Kulick *et al.*, 2001), thereby obscuring the mean pattern of the circulating hormone concentrations during the transition between surges.

The decrease in FSH concentrations after deviation in follicle diameter in surge 1 and maintenance of FSH at low concentrations after follicle deviation apparently assure continued FSH deprivation of the subordinate follicles (Ginther *et al.*, 2001a). In addition, the low FSH concentrations may delay the emergence of the next follicular wave until surge 2 develops appropriate FSH concentrations. The depression of circulating FSH during this time has been attributed to the secretion of oestradiol and inhibin by the dominant follicle of wave 1 (for a review, see Ginther *et al.*, 2001a). The dominant follicle may also secrete factors other than oestradiol and inhibin that suppress other follicles either directly (Armstrong and Webb, 1997) or through reduced circulating FSH. In this regard, a steroid-depleted fraction of follicular fluid suppressed follicle development in cattle even though the injected follicular fluid was > 95% free of inhibin (Law *et al.*, 1992) and suppressed both FSH and follicles despite the injection of an inhibin antiserum with the follicular fluid (Wood *et al.*, 1993). Administration of FSH during the transition between surges 1 and 2 can be expected to shorten the interval between waves 1 and 2, if low FSH concentrations account for the duration of the interval in control heifers. In one study, an FSH-rich pituitary extract administered 5 days after ovulation shortened the interval from treatment to the emergence of wave 2 (Bodensteiner *et al.*, 1996). However, the interpretation was obscured by the ovulation of the dominant follicle of wave 1 in most heifers. In another

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study, recombinant bovine FSH (rbFSH) was administered at days 5 and 6 after ovulation (Adams *et al.*, 1993), which is equivalent to days 2 and 3 after expected deviation in follicle diameter for wave 1. The day of emergence of wave 2 was not hastened by rbFSH treatment, indicating that the low FSH concentrations between waves did not account adequately for the duration of the interwave interval. The results of these studies indicate a need for further study of the mechanism that mediates the hiatus between the emergence of successive waves.

The aim of the present study was to test the hypothesis that low circulating FSH concentrations after deviation in follicle diameter control the interval to emergence of the subsequent follicular wave. The hypothesis was tested by administration of FSH in a dosage regimen that would produce an FSH surge similar to an endogenous FSH surge. Early emergence of wave 2 was taken as an indication that the hiatus between waves was attributable to the low FSH concentrations, without providing support for the existence of a direct follicle suppressor. In addition, the concentrations of FSH, LH and oestradiol during the interval between FSH surges 1 and 2 were characterized.

Materials and Methods

Animals and ultrasonography

The experiment encompassed an interovulatory interval. Holstein heifers aged between 24 and 36 months and weighing 490–680 kg were used. The feeding programme, the PGF_{2α} (Lutalyse; Pharmacia Co., Kalamazoo, MI) protocol for inducing luteolysis to schedule ovulation and the equipment and techniques for transrectal ultrasonography of ovaries and measurement of the follicles were as described by Ginther *et al.* (2000b). Scanning of follicles was carried out at 24 h intervals starting on the day of induced luteolysis at mid-dioestrus and continuing until the largest follicle (F1) of wave 1 reached 7.0 mm in diameter. Thereafter, scanning was carried out every 0.5 days until the largest follicle reached 12.0 mm in diameter and then every day until ovulation. All follicles that reached 4.0 mm in diameter were tracked from examination to examination until they regressed to < 4.0 mm in diameter as described by Ginther *et al.* (1989).

Collection of blood samples

Blood samples were collected by jugular venepuncture every day from the time at which the largest follicle was 4–7 mm in diameter and then every 0.5 days until ovulation. The samples were centrifuged at 500 *g* for 10 min and the plasma was decanted and stored at –20°C until assayed for FSH, LH and oestradiol.

Definitions

Follicle emergence was based on the day a follicle was first detected to be ≥ 4.0 mm in diameter. The day of wave

emergence was determined retrospectively as indicated by either the emergence of the first follicle of the wave, emergence of the future dominant follicle or emergence of the future largest subordinate follicle. The number of days involved in emergence (≥ 4.0 mm in diameter) of all follicles of the wave (base of wave) was determined by consecutive days with an emerging follicle, after allowing for 0.5 days without an emerging follicle. All follicles that were ≥ 4.0 mm in diameter were used to define the base of the waves. The number of follicles per wave was categorized as follicles with a diameter of ≤ 4.9 mm and ≥ 5.0 mm. The follicular waves were defined as major when F1 reached ≥ 10.0 mm in diameter and as minor when the F1 reached < 10.0 mm in diameter. Two follicles that reached ≥ 10.0 mm in diameter identified a wave with double dominant follicles. For such waves, F3 was used or the largest subordinate follicle, whereas F2 was used for waves with a single dominant follicle. The day on which F1 of wave 1 reached a mean of 8.5 mm in diameter was designated day 0 (expected start of deviation). As a result of the 0.5 day interval between scanning sessions, day 0 was determined as the day on which F1 first reached ≥ 8.2 mm in diameter to obtain a range of actual diameters with an average of approximately 8.5 mm (Ginther *et al.*, 2001a).

Treatment groups and endpoints

The heifers were selected at random into control (saline vehicle, *n* = 7) and rbFSH-treated (*n* = 7) groups. Heifers in the rbFSH group were given i.m. injections of recombinant DNA-derived bovine FSH (Granada Biosciences, Marquez, TX) reconstituted with physiological saline. The control heifers were given a volume of saline vehicle corresponding to the volume required for delivery of rbFSH. The treatments began on day 3 or 3 days after the beginning of the expected deviation in follicle diameter in follicular wave 1. The following dosage schedule for rbFSH was used: (i) day 3.0, 1.0 mg; (ii) day 3.5, 2.0 mg; (iii) day 4.0, 3.5 mg; (iv) day 4.5, 2.0 mg; and (v) day 5.0, 1.0 mg. rbFSH was used to permit evaluation of the effects of homologous FSH on the emergence of wave 2.

FSH, LH and oestradiol concentrations for day –1 to day 6 in the control heifers were used to characterize the temporal hormonal relationships for the intersurge interval and including the last portion of surge 1 and the first portion of surge 2. The days used were intended to represent the interval from the expected start of deviation in follicle diameter in wave 1 to emergence of wave 2. The data for the diameters of F1 and F2 and for FSH concentrations extending from the pretreatment ovulation to treatment after ovulation were used to compare groups by normalizing to the beginning of each major and minor wave, as determined from emergence of F1. The diameters of the F1 and F2, and FSH concentrations were compared between groups by normalizing the three endpoints to the day of emergence of the dominant follicle of wave 2. The induced

FSH surge 2 in the rbFSH-treated group and surge 2 in the controls were normalized to the first apparent increase in the FSH means, and the two surges were compared for the following 5.5 days. An interval of 5.5 days was used because the mean FSH concentrations had returned to mean values that were equivalent to the onset of the surges in both groups. Discrete endpoints for comparing surge 2 and wave 2 between the control and rbFSH-treated groups were characteristics of the FSH surge (peak or maximal concentration, interval from day 3 to peak), day of emergence of follicles, maximum diameter of dominant follicle, and characteristics of the follicular wave (width of base, numbers of follicles).

Hormone assays

Plasma concentrations of FSH and LH were determined using radioimmunoassay; details on the modifications and validations for use in this laboratory for FSH (Adams *et al.*, 1992) and LH (Ginther *et al.*, 2000b) have been reported. The crossreactivity of the rbFSH preparation with reference to the bFSH standard (AFP-5318C) used in the assay was < 1%. Mean assay sensitivity was 0.01 ng ml⁻¹ for FSH and 0.2 ng ml⁻¹ for LH. The intra-assay coefficients of variation were 4.1% for FSH and 5.4% for LH.

Oestradiol was measured in plasma samples using a commercially available radioimmunoassay kit (Ultra Sensitive Estradiol assay, DSL-4800; Diagnostic Systems Laboratory, Webster TX) that has been validated for analysis of bovine samples (Turzillo and Fortune, 1990). Assay details as used in this laboratory are described by Kulick *et al.* (1999). Mean assay sensitivity was 0.35 pg ml⁻¹. The mean intra- and interassay coefficients of variations were 6.8% and 7.3%, respectively.

Statistical analyses

The sequential follicle and hormone data were normally distributed, and data transformation was not used. Sequential data were analysed by MIXED procedures with a repeated statement and a first order autoregressive structure to account for autocorrelation between sequential measurements. The analyses considered the circulating hormone concentrations and diameters of F1 and F2 in the control heifers on days -1 to 6, differences in FSH concentrations between control and rbFSH-treated groups on days 0-10, comparison of surge 2 between the two groups with the data normalized to the day preceding the first apparent increase in concentrations, and comparison of diameters of F1 and F2 of wave 2 between the two groups by normalizing to the day of emergence of F1. Significant main effects and interactions were examined further by paired and unpaired *t* tests. Discrete endpoints were compared between groups by unpaired *t* tests. The data are presented as the mean $\pm$ SEM. A probability of $P < 0.05$ indicated that a difference was significant and probabilities between $P > 0.05$ and $P < 0.1$ indicated that a difference approached significance.

Fig. 1. Means $\pm$ SEM for (a) diameters of the dominant follicle (F1) of wave 1 and circulating concentrations of (b) FSH and (c) oestradiol in the control group ($n = 7$), extending from the later portion of FSH surge 1 to the early portion of surge 2. Significant effects of day were found for F1 ($P < 0.0001$), FSH ($P < 0.0005$) and oestradiol ($P < 0.0003$). The vertical broken line shows the maximal oestradiol concentration.

Results

Mean diameters of the largest follicle, and circulating concentrations of FSH and oestradiol for day -0.5 to day 6.0 (day 0 = beginning of expected deviation) in the control group and the results of the statistical analyses are shown (Fig. 1). Concentrations of FSH decreased ($P < 0.01$) between day 0 and day 1.5, increased ($P < 0.01$) between day 1.5 and day 3.0, decreased (approached significance, $P < 0.09$) between day 3.0 and day 4.5, and then increased again ($P < 0.01$). Concentrations of oestradiol increased

Fig. 2. Means $\pm$ SEM ($n = 7$) for circulating FSH concentrations of surge 2 in control (●) and recombinant bovine (rb)FSH-treated (○) heifers ($n = 7$ per group). Days of rbFSH treatment and mean day of emergence (Emer) of the dominant follicle of wave 2 for each group are indicated.

Fig. 3. Means $\pm$ SEM ($n = 7$) for circulating FSH concentrations of surge 2 in (a) control (—●—) and (b) recombinant bovine (rb)FSH-treated (---○---) groups ($n = 7$ per group) and diameters of the two largest follicles (F1, F2) of wave 2 (---■---). Data for all endpoints are normalized to the mean day of emergence of the future dominant follicle for each group. Days of rbFSH treatment are shown.

($P < 0.02$) between day -0.5 and day 2.0 and then decreased ($P < 0.02$). Concentrations of LH did not change significantly between day -0.5 and day 6.0 (not shown).

The number of major follicular waves per interovulatory interval was two or three in the control heifers and two to four in the rbFSH-treated heifers, but the mean number did not differ between the control group (2.6 ± 0.2) and the rbFSH-treated group (3.0 ± 0.3). Each major wave of the interovulatory interval was associated with a distinct FSH surge (not shown). A minor follicular wave occurred in seven of the 14 heifers (no difference between groups). The minor waves emerged on average 5.7 days before the ovulation at the end of the interovulatory interval. The maximum diameter of F1 was 5.0–8.7 mm. The follicles started to regress before ovulation.

Surge 2 reached a peak (Fig. 2; $P < 0.0001$) and wave 2 emerged (Fig. 3; $P < 0.0004$) about 2 days earlier in the rbFSH-treated group than in the control group (Table 1). For surge 2, there were significant ($P < 0.0001$) differences among the average number of days for the rbFSH-treated group and the control group for 5.5 days after the first apparent mean FSH increase; the difference between the two groups and the interaction were not significant (Fig. 2). The mean FSH concentration at the peak of the surge was not different between groups (Table 1). The characteristics of wave 2 were not different between groups, except that the base of the wave was wider in the rbFSH-treated group (Table 1). The temporal relationships between emergence of wave 2 and the peak of surge 2 and the growth profiles for F1 and F2 were not different between groups (Fig. 3). The number of dominant follicles per wave was similar for the control (1.3 ± 0.2) and rbFSH-treated heifers (1.4 ± 0.2). There were no differences between groups in maximum

Table 1. Characteristics (mean $\pm$ SEM) of FSH for surge 2 and follicle endpoints for wave 2 in control and recombinant bovine (rb)FSH-treated heifers^a

Endpoint	Controls (n = 7)	rbFSH-treated (n = 7)	Group effect
FSH surge			
Peak FSH concentration (ng ml ⁻¹)	0.40 $\pm$ 0.05	0.38 $\pm$ 0.04	NS
Interval day 3 to peak (days)	7.4 $\pm$ 0.2	5.1 $\pm$ 0.1	<i>P</i> < 0.001
Day of emergence			
First follicle of wave	6.8 $\pm$ 0.4	4.7 $\pm$ 0.1	<i>P</i> < 0.0001
Future dominant follicle	6.9 $\pm$ 0.3	5.0 $\pm$ 0.3	<i>P</i> < 0.0004
Future largest subordinate follicle	7.3 $\pm$ 0.3	5.4 $\pm$ 0.3	<i>P</i> < 0.0003
Maximum size of dominant follicle			
Diameter (mm)	15.5 $\pm$ 1.0	15.2 $\pm$ 1.1	NS
Number of days from emergence	8.6 $\pm$ 1.0	10.2 $\pm$ 1.1	NS
Wave characteristics			
Width of base (number of days) ^b	1.3 $\pm$ 0.2	2.6 $\pm$ 0.6	<i>P</i> < 0.03
Number of follicles			
4.0–4.9 mm	1.7 $\pm$ 0.6	2.9 $\pm$ 0.7	NS
$\geq$ 5.0 mm	5.0 $\pm$ 1.0	4.9 $\pm$ 0.6	NS

^arbFSH treatment to simulate an FSH surge was carried out on days 3–5. Day 0 is the day on which the largest follicle of wave 1 reached $\geq$ 8.5 mm (expected beginning of follicle deviation).

^bNumber of days for emergence of all follicles $\geq$ 4.0 mm of wave 2.

NS: not significant.

diameter or in the day on which F1 of wave 1 reached a maximum diameter (controls: day 6.3 $\pm$ 0.6, 16.5 $\pm$ 0.7 mm; treated: day 7.0 $\pm$ 0.6, 17.5 $\pm$ 0.6 mm) or in diameter profile of F2 (not shown).

The FSH surge in two of seven rbFSH-treated heifers was multi-modal. The first portion of the surge was included in the means and occurred on the days of the induced surge 2 in the other rbFSH-treated heifers. In one heifer with a bimodal surge, a separate wave with a dominant follicle emerged with each portion of the surge. In the second heifer, the emergence of follicles encompassed a base of 7 days with a dominant follicle emerging at the end of the first portion of the surge and another in association with the second portion (Fig. 4). The diameter of F1 temporally associated with the first portion of the surge reached only 5.7 mm.

Discussion

The concentrations of FSH in the control heifers continued to decrease for 1.5 days after the day of the expected start of deviation of follicle diameter during wave 1 in agreement with studies by Ginther *et al.* (1999, 2000b). An apparent minor FSH surge occurred on average between day 1.5 and day 4.5, as indicated by a significant increase in FSH concentration between day 1.5 and day 3.0, and a decrease that approached significance between day 3.0 and day 4.5.

A confirmatory study will be needed because of the unexpected occurrence and a probability for a decrease that only approached significance.

The increasing concentrations of oestradiol beginning approximately on the day of expected deviation in follicle diameter are in agreement with previous studies and have been attributed to the developing dominant follicle (Ginther *et al.*, 2000a,b). The decrease in oestradiol that begins 2 days after the start of follicle deviation in wave 1 seems consistent with reports of decreases that start 4 days after oestrus (Badinga *et al.*, 1992; Sunderland *et al.*, 1996). Maximal oestradiol concentration occurred when the dominant follicle reached a mean of 12.5 $\pm$ 0.7 mm in diameter. Thus, the decrease in oestradiol occurred during the later portion of the growth phase and early plateau phase of the dominant follicle of wave 1 and reached low concentrations before the peak of FSH surge 2 or the emergence of wave 2 on day 5. The apparent temporal relationship between oestradiol and FSH involved low concentrations of oestradiol, implying considerable sensitivity of cattle to oestradiol. In this regard, a single injection of 0.02 mg oestradiol resulted in a significant reduction in the FSH increase that followed ablation of F1 at the expected start of deviation of follicle diameter (Ginther *et al.*, 2000a). The maximum circulating concentration that resulted from the 0.02 mg injection was 3.6-fold higher than the assay sensitivity, which is close to the 3.2-fold difference in the present study.

Fig. 4. FSH concentrations (—○—) and follicle diameters for individual follicles in a recombinant bovine (rb)FSH-treated heifer with a multi-modal FSH profile, probably representing rbFSH at first and endogenous FSH later. Follicles emerged throughout the prolonged surge.

The increase in oestradiol and the continuing decrease in FSH occurred approximately on the same days. The decrease in oestradiol was associated temporally with the increase in FSH. These temporal results are consistent with a reported conclusion of a functional reciprocal relationship between the two hormones (Ginther *et al.*, 2000a). The indications that FSH concentrations were reduced by low circulating concentrations of oestradiol do not clarify the relative role of other follicular factors, especially inhibin (Bleach *et al.*, 2001).

The recombinant DNA technology that was used to prepare the rbFSH has been described, and the biological activity of the rbFSH preparation was parallel to that of a 1988 USDA bFSH standard based on *in vitro* and *in vivo* bioassays (Chappel *et al.*, 1988). The low immunological crossreactivity of the rbFSH preparation was unexpected and has not been reported in other studies in which the same rbFSH product was used. The reason for the low crossreactivity is unknown, but speculatively may reflect, at least in part, the use of highly purified bFSH assay standard in the present study compared with the standard used in characterizing the rbFSH product. In this regard, the anti-ovine FSH and the bovine FSH reference standard used in the present study have been validated for assay of bovine FSH in our laboratory (Adams *et al.*, 1992). The mean dose of rbFSH that was used in the present study is similar to the doses of the same product used by others in studies in cattle for basic follicle research (Adams *et al.*, 1993; Mihm *et al.*, 1997; Rivera and Fortune, 2001). Furthermore, the total 2 day quantity for the five treatments at 12 h intervals (9.5 mg) was close to the quantity that induced a positive but minimal superovulatory response (Looney *et al.*, 1988;

Wilson *et al.*, 1989). Unlike in previous studies, the rbFSH was given in progressively increasing and decreasing quantities with the expectation of simulating a wave-stimulating FSH surge. This goal was accomplished, as indicated by the non-significant differences between the control and rbFSH-treated groups in maximal FSH concentration during the surge and in concentrations for 5.5 days after the first apparent increase in the means.

The control heifers had two or three major follicular waves for an interovulatory interval, consistent with previous studies in several laboratories (Ginther *et al.*, 1996). Although rbFSH treatment did not increase the mean number of major waves, two of seven rbFSH-treated heifers had four follicular waves. In contrast, none of 54 control heifers in this and previous studies (Ginther *et al.*, 1989; Kulick *et al.*, 2001) had four waves. An increase in the number of waves can be expected when the emergence of one of the waves is hastened, as shown by cauterization of the dominant follicle of wave 1 (Ko *et al.*, 1991). Each major wave was associated with a prominent FSH surge, as reported by Adams *et al.* (1992).

A finding that has not been reported previously in cattle is the occurrence of minor waves in both control and rbFSH-treated heifers. Such waves occurred in 50% of heifers toward the end of the interovulatory interval. These waves did not represent the beginning of a major wave because the follicles regressed before the end of the interovulatory interval. Emergence of the minor waves seemed to be associated with an FSH surge in five of seven heifers; however, a significant change in FSH concentrations was not detected when concentrations were normalized to the day of wave emergence. Therefore, clarification

of the relationship between minor waves and FSH increases requires further study. Minor waves with associated FSH surges have been reported in mares (Ginther and Bergfelt, 1992).

In two rbFSH-treated heifers with an apparent multi-modal FSH surge 2, the first portion of the surge occurred on the days of the induced surge in the other treated heifers and apparently represented the injected rbFSH. The second portion apparently resulted from endogenous FSH but this could not be determined directly. Surge 2, including the first portion of the multi-modal surge in the two heifers and the associated follicular wave 2, occurred an average of 2 days earlier in the treated group than in the controls. The characteristics of wave 2, including maximum diameter of the largest follicle, were similar between the two groups. However, an exception was that the base of the follicular wave lasted for about 1 more day in the rbFSH-treated group.

The early induction of a follicular wave when rbFSH was administered during the low FSH concentrations between surges 1 and 2 supported the hypothesis that the hiatus in emergence of follicles between waves in the control heifers was attributable to the low FSH concentrations. This result did not provide support for the presence of a direct follicle suppressor in delaying the emergence of wave 2. It is not known why rbFSH did not hasten the emergence of wave 2 in the study by Adams *et al.* (1993), in which 2 mg per injection of the same product was administered at 12 h intervals for 2 days (total quantity, 10 mg). Progressively increasing and decreasing doses were not used to simulate an FSH surge, as in the present study. Several reservations must accompany the interpretation that the results of the present study are not consistent with the presence of a direct follicle-to-follicle suppressor. First, a potential suppressor may be released only when FSH concentrations are low, and its release could have been prevented or overridden by the exogenous FSH. Second, a suppressor may have been present soon after deviation in follicle diameter or before the start of FSH treatment. Third, in the present study a wave with a dominant follicle did not develop in one of the treated heifers during the first portion of a multi-modal surge; the first portion is likely to represent rbFSH. The largest follicle that emerged during the first portion of the multi-modal profile reached 5.7 mm, which may have been inadequate to stimulate a return of FSH to baseline (Ginther *et al.*, 2001a). The inhibition of follicle growth during the early or induced portion of the surge could have resulted from the presence of a follicle suppressor in this heifer. Although this occurred in only one of seven heifers, it encourages caution in the overall interpretation.

In conclusion, during the interval between FSH surges 1 and 2 in the control heifers, concentrations of FSH decreased or increased as oestradiol increased or decreased, respectively, in a reciprocal relationship. Treatment of heifers with rbFSH 3–5 days after expected deviation of follicle diameter in wave 1 resulted in a peak in surge 2 and the emergence of wave 2 that was 2 days earlier than in control heifers. The results from the present study indicate that the hiatus in

follicle growth between waves is attributable to low FSH concentrations.

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