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Plasma Concentrations of Growth Hormone, Growth Hormone Secretory Dynamics and Changes in Follicular Development During the Bovine EstrousCycle

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Abstract: An investigation was conducted to determine whether plasma concentrations of growth hormone (GH) and GH secretory profiles (pulse frequency and amplitude) were associated with ovarian follicular growth during the estrous cycle in the Brahman cow. To investigate these relationships, blood plasma was collected and transrectal ultrasonography of the ovaries performed on Brahman cows (n = 9) from d 1 following estrus (d 0) through the mid-luteal phase. Intensive blood sampling (10-min intervals for 6 h) was conducted on d 3 of the estrous cycle, d of the first observed 8 mm follicle and at 48 h after PGF2α injection. PGF2 α was administered at the appearance of the second 8 mm follicle post-estrus. Daily plasma samples were analyzed for progesterone (P4) and estradiol (E2), while samples collected daily and during intensive sampling were analyzed for GH by RIA. Plasma GH did not differ ($P > 0.10$) during the first 12 d of the estrous cycle, while plasma P4 increased ($P < 0.01$) and E2 decreased ($P < 0.01$). Overall GH mean, baseline GH, number of GH peaks and peak height also did not differ ($P > 0.10$) among intensive sampling periods. However, the amplitude of GH peaks tended ($P < 0.08$) to be greater at 48 h after PGF2α administration compared to d 3 of the estrous cycle or on the day of the first 8 mm follicle. Moreover, plasma GH on d 4 through d 8 were positively correlated (r-value ≥ 0.73 depending on day; $P < 0.02$) with numbers of large follicles on d 7 and 8. In summary, while mean daily plasma GH did not differ during the estrous cycle, correlations among GH, ovarian steroids and numbers of large follicles may support a role for GH in mediating ovarian follicular population dynamics, directly or indirectly, in the bovine.

Key words: Growth Hormone, Estrous Cycle, Ovary, Bovine, Brahman

Introduction

Evidence suggests that growth hormone (GH) plays a regulatory role in mediating ovarian function. Specifically in women, concentrations of GH and/or the number of GH secretory bursts have been found to be greater during the late follicular than during the early follicular (Faria *et al*., 1992; Hartman *et al*., 1993; Ovesen *et al*., 1998) and mid-luteal phases (Hartman *et al*., 1993) of the menstrual cycle. Moreover in both the human and bovine female, GH has been utilized to facilitate ovulation induction by gonadotropins (Gong *et al*., 1993; Homburg *et al*., 1988), and in the porcine female (Hsu *et al*., 1987) GH has been suggested to influence ovarian follicular development indirectly through stimulation of insulin-like growth factor-1 (IGF-1). Additional evidence that GH may influence ovarian follicular dynamics in cattle has been demonstrated following the administration of recombinant bovine GH (bST) in which an increase the number of antral follicles (2 to 5 mm) in both heifers (Gong *et al*., 1991) and cows (De la Sota *et al*., 1993) has been demonstrated. Collectively these, and other studies, suggest that GH may influence directly or indirectly ovarian follicular dynamics in the bovine female. Recently, Renner *et al*. (1995) reported that mean concentrations of GH as well as the number of GH pulses differed during various phases of the bovine estrous cycle. However, studies have not elucidated completely a relationship between endogenous GH secretory events and gonadal steroid production as they relate to follicular growth during the bovine estrous cycle *in vivo*. Therefore, the objectives of the present investigation were: (1) to determine whether circulating concentrations of GH may be associated with the growth of follicles during a portion of the bovine estrous cycle; and (2) to examine whether GH secretory characteristics change in relation to follicular

growth end-points and stage of the estrous cycle.

Materials and Methods

Animal Management and Experimental Design: Fourteen Brahman (*Bos indicus*) cows were selected from a group of 30 cows based on observations of natural estrus behavior. Of the 14 cows selected, 5 experienced anomalies associated with their estrous cycles during sampling (i.e., short or long estrous cycles) and were removed from sample collection when the anomaly occurred. Therefore, a total of 9 cows were utilized in the analysis. Cows were fed a soybean and corn ration in the evening (1600 to 1700 h) and samples collected in the morning (0700 to 0800 h) to alleviate any influence of feed intake on hormonal secretory profiles. Cows had ad libitum access to 'Coastal' bermudagrass hay, water and minerals throughout the investigation. All cows were in good body condition (5 to 6) and had a mean bodyweight of 430.3 ± 8.9 kg.

The experimental design for sample collection and monitoring of ovarian follicular populations is depicted in Figure 1. Briefly, blood samples were collected daily following estrus (d 0) via tail venipuncture into tubes containing EDTA, centrifuged within 30 min after collection, and plasma stored at -20oC. Transrectal ultrasonography (Aloka 210 linear array transducer, 5 mHz rectal probe) was performed daily starting on d 1 of the estrous cycle to assess follicular development. Each ovary was scanned in more than one plane and at least two images per ovary recorded using a Sony UP 850 graphics printer. Follicular diameters were measured with sliding calipers to the nearest 0.1 mm on the printed images. Measurements were corrected for 10% distortion in area produced by the printer; which was previously determined in our laboratory and as reported by Quirk *et al.* (1986). Follicles were classified as small (≤ 5.0), medium (5.1 to 7.9 mm) or large (≥ 8 mm).

For intensive analysis of GH secretory activity, on d 3 of the estrous cycle and on the day in which the first 8 mm follicle appeared (Figure 1), cows were fitted with indwelling jugular catheters. Blood samples were obtained into collection tubes containing EDTA at 10-min intervals for 6 h. Blood samples were stored at 4oC for < 45 min prior to centrifugation, then the blood plasma harvested, and stored at -20oC until hormone analysis. At the observance of the second 8 mm follicle (Figure 1), cows received 25 mg of prostaglandin F2α (PGF2α; Lutalyse, Upjohn Co., Kalamazoo, MI) i.m. Forty-eight h after PGF2α administration, blood plasma samples were collected at 10-min intervals for 6 h (Figure 1) and processed to yield plasma as previously described.

Hormone Analysis: Daily plasma samples were analyzed for concentrations of progesterone (P4), estradiol (E2) and growth hormone (GH) by radioimmunoassay (RIA). Plasma from intensive blood collection periods (d 3 of the estrous cycle, day of observance of the first 8 mm follicle and 48 h after PGF2α administration) were analyzed solely for concentrations of GH.

Plasma concentrations of P4 were analyzed using a direct RIA procedure described by Williams (1989; Ab: #337 anti-progesterone-11-BSA serum, G.D. Niswender, CSU, Fort Collins, CO). The intra-assay coefficient of variation (CV) for P4 was 3.8%. Plasma concentrations of E2 were analyzed following extraction of samples (500 µl) with 5 ml diethyl ether. Extracts were then reconstituted with 500 µl of PBSG (phosphate buffered saline plus 0.1% gel) and assayed directly using a specific antisera (GDN-244; G.D. Niswender). All estimates were corrected for procedural losses with an extraction efficiency of 76.6%. The intra- and inter-assay CVs for E2 were 3.6 and 8.0%, respectively. Plasma concentrations of GH were analyzed using a double-antibody RIA procedure established in our laboratory as described by Welsh *et al.* (1987). Samples of 200 µl were assayed using a first antibody of rabbit-anti-goat GH (NIH-NHPP; AFP-CO123080) and a second antibody of sheep-anti-rabbit gamma globulin (#51-258; Antibodies Inc., Davis, CA) with 8% polyethylene glycol (PEG). The intra- and inter-assay CVs for GH were 7.1 and 11.4%, respectively.

Statistical Analysis: Continuous data, including daily plasma concentrations of P4, E2 and GH, as well as plasma concentrations of GH from intensive blood collection periods, were analyzed by ANOVA specific for repeated measures (StatView, 1992). Analysis of dependent variables containing single or calculated observations in time were performed by single factor ANOVA. These dependent variables included: number of small, medium or large follicles on designated days, and calculated GH secretory characteristics during intensive blood collection periods; which included overall GH mean, baseline concentrations of GH, number of GH peaks, amplitude of GH peaks and GH peak height. The overall GH mean was calculated by dividing the sum of the concentrations of GH

determined at 10-min intervals by the total number of samples collected during the sampling period (n = 36). Baseline concentrations of GH were determined by calculating a mean for the points below the overall GH mean minus 1 SD. The number of GH peaks was determined by selecting points above the overall GH mean plus 1 SD. Amplitude of GH peaks were calculated by subtracting the concentration of GH for each peak from the mean baseline concentration of GH. Peak height was determined by subtracting the concentration of the GH peak from the preceding corresponding GH point. Comparisons were made for these calculated variables among the intensive sampling periods (d 3, day of observance of the first 8 mm follicle and 48 h after PGF2α administration) for mean separation using the Bonnferroni-Dunn procedure providing a significant ($P < 0.05$) or, where specified, the tendency for a significant ($P < 0.10$) 'F'-ratio was observed.

Correlation coefficients (r values) were calculated using the procedures of StatView (1992) and Fisher's r to z transformations utilized to determine statistical significance. Correlations were made among follicular numbers, grouped by follicle sizes (small, medium and large), and hormone concentrations (GH, P4 and E2) relative to day of the estrous cycle (d 1 through 12), d of PGF2α administration, and at 24 and 48 h after PGF2α administration.

Results

Growth Hormone and Steroid Hormone Profiles Relative to Follicular Development from Days 1 through 12 of the Estrous cycle: Cows varied relative to the d of the estrous cycle when PGF2α was administered (i.e., on d 13 to d 20 of the estrous cycle depending on timing of the appearance of the second 8 mm follicle following estrus). Therefore, only the first 12 d of the estrous cycle were available for complete daily hormonal and ovarian follicular population analysis of all cows (n = 9). Plasma concentrations of GH did not differ ($P > 0.10$; Fig. 2, Panel A) during the first 12 d of the estrous cycle. In contrast, concentrations of P4 increased ($P < 0.0001$; Fig. 2, Panel B) and E2 decreased ($P < 0.005$; Fig. 2, Panel A) from d 1 to 12 of the estrous cycle (Fig. 2). Significant correlations were observed among serum concentrations of P4, E2 and GH between d 7 to 11. Specifically, concentrations of P4 were positively correlated with GH on d 7 ($r = 0.69$; $P < 0.03$), 8 ($r = 0.89$; $P < 0.01$) and 11 ($r = 0.72$; $P < 0.02$); while E2 was negatively correlated with GH on d 8 ($r = -.68$; $P < 0.05$), and tended to be negatively correlated with GH on d 9 ($r = -0.60$; $P < 0.08$).

With respect to ovarian follicular populations, the numbers of small (≤ 5.0 mm) follicles decreased ($P < 0.05$) following d 2 of the estrous cycle (Figure 2, Panel C), while medium-sized (5.1 - 7.9 mm) follicles and the total number of follicles present did not differ ($P > 0.10$) from one d to the next during the first 12 d of the estrous cycle. Numbers of large follicles (≥ 8.0 mm) changed over time ($P < 0.05$), increasing from d 4 through d 8, followed by a subsequent decline through d 11 (Figure 2, Panel C). No significant ($P > 0.10$) correlations between plasma concentrations of P4 and follicle numbers (i.e., small, medium or large follicles) were noted between d 1 through d 12 of the estrous cycle. Similarly, no correlations ($P > 0.10$) between numbers of small follicles and concentrations of E2 or GH were observed. In contrast, significant ($P < 0.05$) correlations among numbers of medium- and large-sized follicles were seen relative to plasma concentrations of E2 and GH. Specifically, between d 8 through d 10 of the estrous cycle, numbers of medium-sized follicles were negatively correlated with E2 (d 8: $r = -0.65$, $P < 0.06$; d 9: $r = -0.74$, $P < 0.01$; d 10: $r = -0.88$, $P < 0.01$). However, numbers of large-sized follicles were positively correlated with GH on d 8 ($r = 0.87$; $P < 0.01$), when the peak in numbers of large follicles occurred, and d 11 ($r = 0.70$; $P < 0.02$), when numbers of large follicles declined. Moreover, analysis of GH concentrations prior to the peak in numbers of large follicles (seen on d 8) showed positive correlations between concentrations of GH on d 4 through 8 and large-sized follicles on d 7 (range: $r = 0.73$ to .78; $P < 0.02$) and d 8 (range: $r = 0.79$ to 0.87; $P < 0.01$). These findings suggest that large growing follicles of the first follicular wave may have been positively affected by preceding plasma concentrations of GH.

Growth Hormone and Steroid Hormone Profiles Relative to Follicular Development Following PGF2α Administration: Following PGF2α administration, concentrations of GH and E2 did not differ ($P > 0.10$) between their respective 24 and 48 h time-points (Table 1). However, concentrations of E2 tended ($P < 0.07$) to be higher at 48 h after PGF2α injection than on the day of PGF2α administration. Luteolytic events following PGF2α were further characterized by concentrations of P4 which were lower ($P < 0.05$) at 24 and 48 h (see Table

Table 1: Plasma concentrations (mean ± SEM) of growth hormone (GH), progesterone (P4) and estradiol (E2), and the percentage (%) of small, medium and large ovarian follicles present at 0, 24 and 48 h following PGF2α administration.

Variable	Dayof PGF2α Administration	Hours after PGF2α Adminstration	
		24	48
Hormone:			
GH (ng/ml)	40.3 ± 7.8	37.9 ± 5.2	38.2 ± 3.7
P4 (ng/ml)	4.1 ± 1.0a	0.86 ± .29b	0.44 ± .18b
E2 (pg/ml)	14.2 ± 2.9d	18.8 ± 1.1de	19.9 ± 1.7e
Percentage of Follicles2:			
Small	50.0 ± .11a	42.4 ± .07b	27.8 ± .11c
Medium	33.2 ± .14a	29.4 ± .08b	22.2 ± .12c
Large	16.8 ± .04a	28.2 ± .11b	50.0 ± .14c

Superscripts differ within row: abc, $P < 0.05$; de, $P < 0.07$.
1PGF2αa (25 mg i.m.) was administered on the d on which the 2nd 8 mm follicle following estrus was observed (range: d 12 to d 20 of the estrous cycle).
2Percentage (%) of follicles present on both ovaries relative to follicular size - small: 2 to 5 mm, medium: 5.1 to 7.9 mm, large: ≥ 8 mm.

Table 2: Growth hormone (GH) secretory characteristics (mean ± SEM) on d 3 of the estrous cycle, d of the first 8 mm follicle following estrus and 48 h following the administration of PGF2α.

GH Characteristic	Day 31	1st 8 mm Follicle2	48 h post-PGF2α3
Overall GH mean (ng/ml)	26.8 ± 2.3	22.3 ± 2.3	22.6 ± 1.3
Baseline GH (ng/ml)	15.3 ± 2.5	13.4 ± 2.4	9.4 ± 2.1
# of GH Peaks / 6 h	2.8 ±0.2	3.1 ±0.2	2.6 ±0.4
GH Peak Amplitude (ng/ml)	26.2 ± 3.9a	23.1 ± 3.8a	47.7 ± 13.0b
GH Peak Height (ng/ml)	41.5 ± 4.1	36.4 ± 3.1	57.1 ± 11.9
GH Std. Deviation (ng/ml)4	7.7 ± 1.2	8.0 ± 1.4	11.5 ± 2.7

Superscripts differ within row: ab, $P < 0.08$.
1Day 3 of the estrous cycle; 2Day of the first observed 8 mm follicle following estrus; 3At observance of the second 8 mm follicle following estrus, 25 mg PGF2α was administered and intensive sampling for GH characteristics conducted 48 h later.
4GH Std. Deviation = a measure of GH variability in which the standard deviation around the 36 samples collected at 10-min intervals were calculated for GH and compared among the three intensive sampling periods.

1) than on the d of PGF2α administration. At 24 and 48 h after PGF2α administration, plasma concentrations of P4 were negatively correlated with E2 ($r = -0.76$, $P < 0.01$ and $r = -0.82$, $P < 0.01$, respectively). However, significant correlations between concentrations of GH and E2 or P4 were not observed ($P > 0.10$) following PGF2α. Relative to follicular events following the administration of PGF2α, numbers of small- and medium-sized follicles decreased ($P < 0.05$) from the d of PGF2a administration to 48 h post-PGF2α (Table 1). In contrast, numbers of large follicles increased ($P < 0.05$) from the d of PGF2α administration to 48 h after PGF2α (Table 1). While plasma concentrations of P4 were not found to be correlated with follicular populations after PGF2α administration, concentrations of GH at 24 h after PGF2α were positively correlated with numbers of large follicles ($r = 0.68$; $P < 0.04$), and concentrations of E2 at 24 and 48 h ($r = 0.61$, $P < 0.08$ and $r = 0.72$, $P < 0.02$, respectively) were positively correlated with numbers of medium-sized follicles.

Growth Hormone Secretory Events During the Bovine Estrous Cycle: To examine whether GH secretory characteristics differed during the estrous cycle relative to circulating steroid hormone concentrations and follicular status, serial blood samples were collected on d 3 of the estrous cycle, day of the first 8 mm follicle and 48 h after the administration of PGF2α (Fig. 1). Mean plasma GH, baseline GH, number of GH peaks, and GH peak height did not differ ($P >$

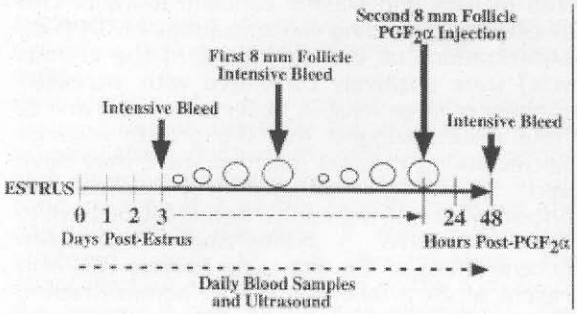

Fig. 1: Schematic representation of experimental design. Blood samples were collected daily following estrus until the administration of PGF2α; which was given on the day when the second 8 mm follicle appeared. Daily observations of ovarian follicular population dynamics were conducted using ultrasonography, and the numbers and sizes of follicles on both ovaries recorded. Intensive blood collection periods were conducted on d 3 of the estrous cycle, on the day on which the first 8 mm follicle was observed, and at 48 h following the administration of PGF2α. During these periods, blood plasma was collected at 10-min intervals for 6 h.

0.10) among the intensive blood collection periods (Table 2). However, the amplitude of the GH peaks tended ($P < 0.09$) to be greater at 48 h after PGF2α administration compared to d 3 of the estrous cycle or at the appearance of the first 8 mm follicle (Table 2). Further analysis of the 48 h time-point revealed that the d on which PGF2α was given (i.e., d of detection of a second 8 mm follicle) revealed no differences ($P > 0.10$) in GH secretory characteristics between cows in which the follicle appeared on d 12 to d 15 (n = 4) versus d 16 to d 20 (n = 5). Similarly, cows also did not differ ($P > 0.10$) in their GH secretory characteristics on the d of the first 8 mm follicle between cows with 8 mm follicles on d 4 through d 6 (n = 3) versus d 7 through d 9 (n = 6). As an additional measure of GH secretory variability, we further assessed whether statistical differences were present around the standard deviation (SD) of concentrations of GH relative to intensive blood collection periods; as previously described by Renner *et al.* (1995). Unlike Renner *et al.* (1995) who found that the GH SD was greater on d 12 than on d 3 of the estrous cycle or at 24 and 72 h after PGF2α administration, analysis of the SD of GH concentrations among

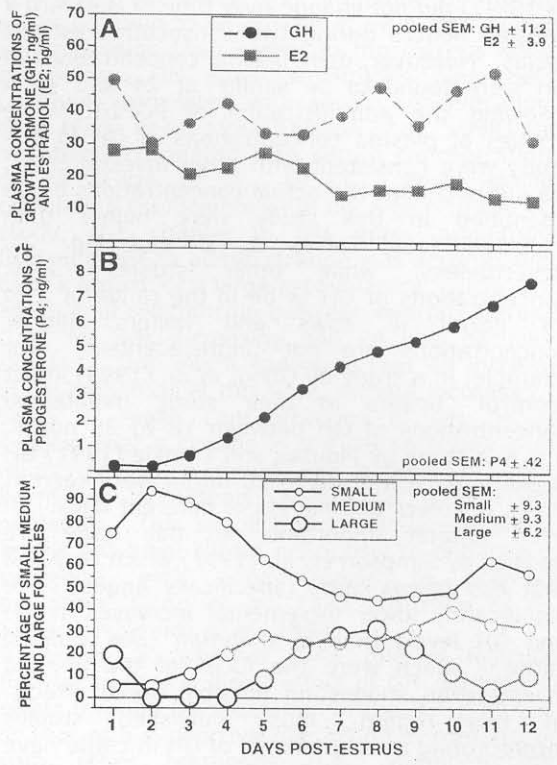

Fig. 2: Plasma concentrations of growth hormone (GH; Panel A), estradiol (E2; Panel A) and progesterone (P4; Panel B) in Brahman cows during the first 12 d of the estrous cycle, and the percentage (%) of small, medium and large follicles (Panel C) present on both ovaries (least squares means and pooled 12-d SEM).

the 10-min intervals for each of the three intensive sampling periods in this study revealed no differences ($P > 0.10$; Table 2). However, it should be noted that the variability among cows within the intensive sampling periods in this study was higher than the variability reported by Renner *et al.* (1995). Breed type or differences between intensive sampling protocols may have contributed to the disparity between these two studies in using GH SD as a measure of changes in GH secretory variability.

Discussion

Daily concentrations of GH in this study were similar during the first 12 d of the estrous cycle in the Brahman cow. This finding is consistent with those reported by Gong *et al.* (1991) who also observed that concentrations of GH (as well

as IGF-1) did not change over time in Hereford x Friesian heifers during two consecutive estrous cycles. Moreover, daily plasma concentrations of GH were found to be similar at 24 and 48 h following the administration of PGF2α. While profiles of plasma concentrations of GH in this study were consistent with other investigations, we did note that the actual concentrations of GH quantified in this study were higher than expected (see Tables 1, 2 and Fig. 2). Nevertheless, while other studies report concentrations of GH to be in the range of 2 to 15 ng/ml in cows and heifers, higher concentrations are not unprecedented. For example, in a study by Gong, *et al.* (1993) intact "control" heifers in their study maintained concentrations of GH between 18 to 25 ng/ml, and in a study by Plouzek and Trenkle (1991) GH peaks in excess of 20 to 30 ng/ml were seen at times in steers and heifers of different ages. Of even greater importance to this issue are findings by Simpson et. al. (1997) which revealed that *Bos taurus* cattle (specifically Angus) have significantly lower incremental increases in GH and GH levels than in Brahman (*Bos indicus*) cattle - which were the focus of the present investigation suggesting possible breed effects. In this regard, most published studies incorporating measurements of GH in cattle have been conducted on *Bos taurus* breeds. With respect to ovarian steroids (E2 and P4), changes occurring for these hormones were typical of those seen during the estrous cycle of normal Brahman cows, as well as following PGF2α administration. In short, daily profiles of plasma concentrations of GH and ovarian steroids (E2 and P4) were consistent with those reported previously in the bovine.

A major aim of the present investigation was to not simply chronicle daily hormonal concentrations of GH and ovarian steroids, but to relate any changes in GH secretory characteristics to follicular events. As evidence of a potential role for GH in mediating ovarian follicular dynamics in the bovine, the administration of exogenous somatotropin (bST) has been reported to increase the number of antral follicles on the ovary (De la Sota *et al.*, 1993; Gong *et al.*, 1992), while GH-receptor deficient cattle have been shown to have fewer numbers of antral follicles (Chase *et al.*, 1998). In the present investigation, correlations between endogenous circulating concentrations of GH and numbers of small (i.e., antral) follicles were not observed. However, positive correlations were noted between numbers of medium- (5.1 to 7.9 mm) and large- (≥ 8mm) sized follicles and plasma concentrations of GH. Specifically, preceding concentrations of GH (i.e., concentrations on d 4 through 8 of the estrous cycle) were positively correlated with increased numbers of large follicles observed on d 7 and 8. These results suggest that the growth of large follicles during the first follicular wave may have been positively affected by circulating concentrations of GH earlier in the estrous cycle. This positive relationship between concentrations of GH and large follicles was also present at 24 h following PGF2α administration. Such a relationship between GH and increased numbers of large follicles (i.e., increases in follicle size following GH exposure) and follicular function is not unprecedented. For example in the perfused rabbit ovary, GH treatment in combination with hCG increases follicular diameter as well as intra-follicular E2 and IGF-1 content (Yoshimura *et al.*, 1994). Similarly, recombinant human GH administered to rats increases the number of large follicles on the ovary (Ozawa *et al.*, 1996), while bST administered to postpartum cows enhances the intra-follicular E2 and IGF-1 content of large follicles (Angrade *et al.*, 1996). While findings in this study suggest a strong relationship between numbers of large follicles and circulating plasma concentrations of GH, Lucy *et al* (1993) have previously reported that most follicles, and the stroma of the bovine ovary, express little mRNA for the somatotropin receptor. However, the GH receptor has been localized (by RT-PCR) within bovine cumulus oocyte complexes *in vitro* where GH receptor mRNA was found in cumulus cells, mural granulosa cells and oocytes (Izadyar *et al.*, 1997). While the abundance, localization and significance of the GH receptor in bovine follicles requires additional study, GH receptors are clearly present in the bovine CL and other reproductive tissues of the bovine including the endometrium (Heap *et al.*, 1996; Yuan *et al.*, 1996). Despite evidence of a possible direct role for GH in stimulating follicular function, studies suggest that GH (at least in the bovine) may play a more indirect, supportive role in mediating follicular events through regulation of an extra- and/or intra-ovarian IGF/IGF binding protein system. As measurements of circulating or intra-follicular concentrations of IGFs were not conducted in the present study, it is unclear whether the relationship between GH and follicular growth (particularly with respect to the presence of large follicles) might be attributed to GH-dependent influences on the IGF system. Nevertheless, additional support for GH's actions (whether direct or indirect) in the development

of large follicles in the bovine is further evidenced by our investigation of GH secretory dynamics during various stages of the estrous cycle.

Examination of GH secretory characteristics during different phases of the estrous cycle was accomplished relative to follicular end-points, with intensive sampling for concentrations of GH conducted on d 3 of the estrous cycle, on the day on which the first 8 mm follicle was observed, and at 48 h following the administration of PGF2α (given on the d on which the second 8 mm follicle was observed). Our findings showed that the overall plasma GH mean and baseline concentrations of GH did not differ among the intensive blood collection periods. Similarly, Gong *et al* (1991) also found that while bST-treated heifers had higher circulating concentrations of GH than controls, no differences were seen in basal concentrations of GH within the control and bST-treated groups between the mid-luteal (d 12) and follicular phases (d 19) of the estrous cycle. Moreover, no differences in basal GH secretion rate between the early follicular and periovulatory phases have been reported in the human female (Oveson *et al.*, 1998). In contrast in a study by Renner *et al.* (1995), higher serum concentrations of GH in heifers on d 3 were observed compared to concentrations of GH at 24 and 72 h after PGF2α administration. The disparity between the study by Renner *et al* (1995) and the present investigation, relative to differences in concentrations of GH on d 3 versus those observed after PGF2α administration, may have been due to possible breed effects (Simpson *et al.*, 1997; discussed previously) or differences observed in GH secretory variability. Renner *et al.* (1995) further reported increases in the number of GH pulses on d 12 compared to d 3 and at 24 and 72 h after PGF2α administration. While a companion intensive GH analysis time-point to the d 12 intensive bleed by Renner *et al.* (1995) is not present in our investigation, the number of GH peaks (or pulses as it were) did not differ relative to stage of the estrous cycle in our study. However, increased numbers of GH secretory bursts and elevated pulsatile GH production rates have been observed in humans during the periovulatory phase compared to the early follicular phase (Oveson *et al.*, 1998). It is interesting to note that neither the study by Renner *et al* (1995) or the present investigation observed increases in the number of GH pulses between 24 and 72 h after PGF2α administration in the bovine.

While the number of GH pulses and GH peak height did not differ, the amplitude of the GH peaks did tend to be greater at 48 h after PGF2α administration than earlier in the estrous cycle. In women, GH pulse amplitudes have been positively correlated with serum concentrations of E2 (Faria *et al.*, 1992; Oveson *et al.*, 1998) and negatively correlated with serum concentrations of P4 (Faria *et al.*, 1992). While similar correlation coefficients were not significant in our study, the change in GH pulse amplitudes following PGF2α administration was accompanied by the growth of a large preovulatory follicle, and an inverse relationship between ovarian steroids (i.e., E2 was increasing and P4 was declining rapidly). One might speculate that an increase in GH amplitude at this time, without increases in basal or mean concentrations of GH, would suggest that a change in the GH secretory code might act to augment the actions of other hormones in support of follicular growth and ovulation. This suggestion is not unwarranted, as GH has been shown previously to enhance or synergize with gonadotropins to promote ovulation (Gong *et al.*, 1993; Lanzone *et al.*, 1996; Yoshimura *et al.*, 1994).

In summary, whether GH's role in follicular development is a direct effect of GH, an indirect effect through GH mediation of the IGF pathway, or that of a supportive role acting in conjunction with other hormones (i.e., gonadotropins) remains to be elucidated completely. Nevertheless, in the present investigation we report positive correlation coefficients between endogenous circulating concentrations of GH and numbers of large follicles during portions of the bovine estrous cycle. Moreover, we also report that while most GH secretory characteristics did not differ relative to steroid hormone status or follicular activity, GH pulse amplitudes tended to be higher following PGF2α administration (i.e., around the time of estrus). Collectively, these findings suggest that endogenous circulating concentrations of GH may have a positive effect on either the recruitment or subsequent development of large ovarian follicles in the bovine.

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