Interaction Among Bovine Somatotropin, Insulin, and Gonadotropins on Steroid Production by Bovine Granulosa and Thecal Cells[1]

L. J. SPICER[2] and R. E. STEWART
Department of Animal Science, Oklahoma State University, Stillwater 74078

ABSTRACT

The objective of the present study was to determine the interactions among bST, insulin, and gonadotropins on steroid production by granulosa and thecal cells from bovine follicles. Basal production of estradiol by granulosa cells from small (1 to 5 mm) and large (≥8 mm) follicles (expressed as picograms of estradiol per 10^5 cells per 24 h) was not affected by 50 or 300 ng/ml of bST, but 300 ng/ml of bST inhibited estradiol production that was induced by FSH plus insulin in cells from small and large follicles. Progesterone production and proliferation by granulosa cells from large follicles were not affected by 3 to 100 ng/ml of bST. In cultures of thecal cells that exhibited a >3-fold increase in androstenedione production induced by LH, 3 to 30 ng/ml of bST further increased androstenedione production by 29 to 42%, but cell proliferation and progesterone production were unaffected by bST. In cultures of thecal cells that exhibited a <2-fold increase in androstenedione production induced by LH, 3 to 30 ng/ml of bST inhibited androstenedione production by 32 to 33% and inhibited cell proliferation by 9 to 13%, but progesterone was unaffected by bST. In summary, only pharmacologic doses of bST inhibited estradiol production by granulosa cells, but physiologic doses of bST altered androstenedione production by thecal cells, which indicated that bST might not have an important role in granulosa cell function but might play a role in thecal cell function in cattle.

(**Key words**: somatotropin, granulosa cells, estradiol production, ovarian follicles)

Abbreviation key: **FCS** = fetal calf serum.

INTRODUCTION

In addition to its known metabolic effects in cattle, bST has effects on ovarian function in vivo (7, 11, 12, 26, 27) and on the function of ovarian granulosa cells in vitro (13, 14, 24, 40). Specifically, bST increases granulosa cell proliferation, protein synthesis, and progesterone production by cells cultured from small (1 to 5 mm) bovine follicles (24). Whether bST differentially affects granulosa cell function in small and large follicles is unclear. Recent studies (13, 40) reported that bST inhibited incorporation of [^{3}H]thymidine by granulosa cells from large (>10 mm) follicles, but had no effect on incorporation of [^{3}H]thymidine by granulosa cells from small (<5 mm) or medium (5 to 10 mm) follicles of cattle. Also, reports of direct effects of bST on granulosa cell aromatase activity and on thecal cell androstenedione production in cattle have been few.

In vivo, bST increases during feed restriction of cattle (8, 39) and decreases as lactation progresses and energy balance becomes positive in dairy cows (33). Reproductive functions are reduced during feed restriction and negative energy balance (37, 39). Thus, increased bST secretion may be one mediator of the negative effects of feed restriction and negative energy balance on ovarian function. Long-term treatment of dairy cows with bST has been reported to have either no effect (5, 16) or negative effects on reproductive measures (10, 42). Some reports have indicated that bST might increase the incidence of twin births in dairy cows (5, 10), increase the superovulatory response of beef heifers to pregnant mare serum gonadotropin (12), and increase the number of corpora lutea in superovulated Holstein heifers (23). Also, bST treatment can decrease (26), increase (12, 27), or have no effect (11, 34) on estradiol concentrations in plasma of cattle. Therefore, the objective of our studies was to determine whether bST directly affects steroid production of granulosa and thecal cells obtained from small and large bovine antral follicles.

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[2]To whom reprint requests should be sent.

MATERIALS AND METHODS

Reagents and Hormones

Reagents were Dulbecco's modified Eagle's medium, Ham's F12, insulin (bovine; 25.7 U/mg), pronase E, collagenase, hyaluronidase, DNase, and fetal calf serum (**FCS**); all reagents were obtained from Sigma Chemical Co. (St. Louis, MO). Ovine FSH (F1913; FSH activity 15 × NIH-FSH-S1 U/mg) was obtained from Scripps Laboratories (San Diego, CA); testosterone was obtained from Steraloids Inc. (Wilton, NH); and bST (USDA-bGH-B1; growth hormone activity 1.9 × NIH-GH-B18 U/mg) and bovine LH (USDA-bLH-B5; LH activity 2.1 × NIH-LH-S1 U/mg; FSH activity <1.0% by weight) were obtained from the National Hormone and Pituitary Program (Baltimore, MD).

Cell Culture

Ovaries were obtained at a nearby commercial abattoir from beef and dairy cattle after slaughter. The ovaries were transported to the laboratory on ice (<120 min) and then processed as described previously (24). Briefly, granulosa cells from small (1 to 5 mm) and large (≥8 mm) follicles were collected by aspiration using a needle and syringe; cells then were washed twice in serum-free medium (24). Thecal cells were obtained from the ovaries in a similar manner as that described by Roberts and Skinner (31). Briefly, large follicles were dissected from the ovary, follicular fluid was aspirated, follicles were bisected, and each follicle wall was scraped and flushed with Ham's F-12 to remove any remaining granulosa cells. The theca interna layer was microdissected from the follicle wall and torn into small pieces. These pieces were digested for 1 h at 37°C in Ham's F-12 containing 1 mg/ml of collagenase, 1 mg/ml of hyaluronidase, 1 mg/ml of pronase E, and 0.01 mg/ml of DNase on a rocking platform shaker. Dispersion of thecal cells was aided by agitation of the fluid back and forth through a 10-ml polystyrene pipette at 15-min intervals. After incubation, undigested tissue was removed from the cell suspension by filtration through a syringe filter holder with a metal screen (149-μm mesh; Gelman Sciences, Ann Arbor, MI). The dispersed cells were centrifuged at 50 × *g* for 4 min, washed in serum-free medium, and recentrifuged at 50 × *g* for 4 min. Cells were resuspended in serum-free medium, and the number of viable cells was determined using the trypan blue exclusion method. Viable cells averaged 90 ± 3% of total thecal cells. Purity of the thecal cells from large follicles was assessed by measuring estradiol production induced by FSH, insulin, and both and then comparing estradiol production by thecal cells with that by granulosa cells. All treatments were applied for 1 d in the presence of 1 μg/ml of testosterone. As shown in Table 1, estradiol production by thecal cells was very low compared with that by granulosa cells from small follicles, and neither FSH nor insulin stimulated estradiol production as it did with granulosa cells; these results indicate that no significant granulosa cell contamination occurred in the thecal cell cultures.

TABLE 1. Evaluation of the purity of thecal cells by assessment of hormonally responsive aromatase activity.[1]

Cell type and treatment	Estradiol production (pg/10^5 cells per 24 h)	
	$\bar{X}$	SE
Granulosa[2]		
FSH (50 ng/ml)	50[a]	7
Insulin (1 μg/ml)	155[b]	8
FSH + Insulin	193[c]	7
Theca[3]		
FSH (50 ng/ml)	13[a]	2
Insulin (1 μg/ml)	19[a]	2
FSH + Insulin	19[a]	2

[a,b,c]Within cell type, means with different superscripts differ ($P < 0.05$).

[1]Cells were cultured for 2 d in 10% fetal calf serum, wells were washed, and then cells were cultured for 1 d in serum-free medium with the indicated hormones.

[2]Means from three replicate experiments of cells from small follicles.

[3]Means from six replicate experiments of cells from large follicles.

Medium was a 1:1 (vol/vol) mixture of Dulbecco's modified Eagle's medium and Ham's F12 containing 0.12 m*M* gentamicin and 38.5 m*M* sodium bicarbonate. Between 1 and 3 × 10^5 viable cells in 45 to 110 μl of medium were added to Falcon 24-well plates (Becton Dickinson and Co., Lincoln Park, NJ) containing 1 ml of medium. Cultures were kept at 38.5°C in a 5% CO_2 atmosphere. To obtain optimal attachment, cells were maintained in the presence of 10% FCS for the first 2 d of culture. After this time, cells were washed twice with 0.5 ml of serum-free medium, and incubations were continued in serum-free medium with or without added hormones; medium was changed every day. For experiments evaluating the effects of hormones on steroid production, hormonal treatments were applied for 2 d (i.e., from d 2 to 4 of culture) unless stated otherwise. The plating efficiencies (i.e., number of cells attached to the wells on d 1 divided by the number of viable cells added to

each well on d 0 multiplied by 100) of thecal and granulosa cells averaged 29 ± 2 and 24 ± 5%, respectively.

Experiments 1 and 2 were designed to determine whether bST affected basal estradiol production or estradiol production induced by insulin, FSH, or both. Granulosa cells from small (Experiment 1) or large (Experiment 2) follicles were cultured for 2 d in 10% FCS and then were cultured in serum-free medium for an additional 2 d with testosterone (1 μg/ml), FSH (0 or 50 ng/ml), and insulin (0 or 1 μg/ml). During the last 2 d of culture, bST (0, 50 and 300 ng/ml) was also added to the medium. The doses of bST were selected based on a previous study (24) using bovine granulosa cells. The dose of FSH was selected based on preliminary studies that compared the effects of 1-d treatment with 0, 1, 2, 5, 50, or 100 ng/ml of ovine FSH on estradiol production by granulosa cells from small follicles treated with insulin in the presence of 1 μg/ml of testosterone; estradiol production averaged 192, 166, 189, 196, 265, and 280 ± 10 pg per 10^5 cells per 24 h, respectively (n = three experiments).

Experiment 3 was designed to determine whether bST affected granulosa cell production of progesterone induced by FSH plus insulin. Granulosa cells from large follicles were cultured for 2 d in 10% FCS and then in serum-free medium for an additional 2 d with FSH (0 or 50 ng/ml) and insulin (1 μg/ml). During the last 2 d of culture, bST (0, 3, 10, 30, or 100 ng/ml) was also added to the medium. The doses of bST were selected to represent the range of bST concentrations in the blood of untreated and treated cows (8, 11, 12, 26).

Experiment 4 was designed to determine whether bST affected thecal cell production of androstenedione or progesterone induced by LH plus insulin. Thecal cells from large follicles were cultured for 2 d in 10% FCS and then in serum-free medium for an additional 2 d with LH (0 or 100 ng/ml) and insulin (1 μg/ml). During the last 2 d of culture, bST (0, 3, 10, or 30 ng/ml) was also added to the medium. The doses of bST were selected as per Experiment 3. The dose of LH was selected based on preliminary studies that compared the effects of a 2-d treatment with 0, 3, 10, 30, or 100 ng/ml of bovine LH on androstenedione production by insulin-treated thecal cells from large follicles; androstenedione production averaged 15, 17, 25, 82, and 128 ± 6 pg per 10^5 cells per 24 h, respectively (n = 3 experiments).

Determination of Granulosa and Thecal Cell Numbers

Numbers of granulosa and thecal cells were determined at the termination of experiments using a Coulter counter (model Zm; Coulter Electronics, Hialeah, FL) as previously described (24). Briefly, cells were exposed to 0.5 ml of trypsin [0.25 % (wt/vol) in 0.15 *M* NaCl] for 20 min at 25°C, then scraped from each well, diluted in 0.15 *M* NaCl, and enumerated.

Assessment of Functional Aromatase Activity

Functional aromatase activity was assessed during the last 24 h of exposure of granulosa cells to 1 μg/ml of testosterone as previously described (35). After the last 24 h of incubation, concentrations of estradiol in medium were determined by radioimmunoassay, and cell numbers were determined. Estradiol production was expressed as picograms per 10^5 cells per 24 h.

Estradiol Radioimmunoassay

Concentrations of estradiol in culture medium collected on d 4 of culture were determined by radioimmunoassay as previously described (35, 36). The intrassay and interassay coefficients of variation were 9 and 10%, respectively. Assay sensitivity, defined as 90% of total binding, was 0.3 pg per tube.

Androstenedione Radioimmunoassay

Concentrations of androstenedione in culture medium collected on d 4 of culture were determined using solid-phase radioimmunoassay kits (ICN Biomedicals, Costa Mesa, CA). Serial dilutions (10, 20, 40, 60, and 100 μl) of culture medium displaced ^{125}I-labeled androstenedione from antiserum to produce a binding curve that was parallel to the standard curve. Intraassay and interassay coefficients of variation were 14 and 10%, respectively.

Progesterone Radioimmunoassay

Concentrations of progesterone in culture medium collected on d 4 of culture were determined with a radioimmunoassay as previously described (24). Intraassay and interassay coefficients of variation were 16 and 20%, respectively.

Statistical Analyses

Experimental data are presented as the least squares means (± SE) of measurements from triplicate culture wells from two or more experiments. Each experiment was performed two or more times with different pools of granulosa and thecal cells collected from 20 to 60 ovaries ($\bar{X}$ ± SE, 32 ± 3) for each

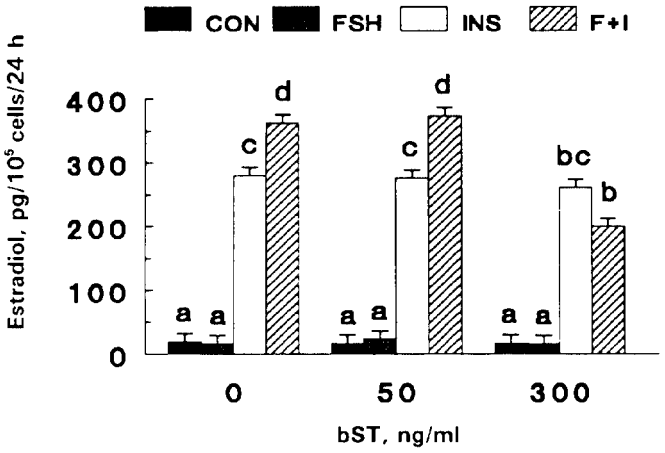

Figure 1. Effects of bST on estradiol production by granulosa cells collected from small follicles (Experiment 1). Granulosa cells were cultured for 2 d in the presence of 10% fetal calf serum and then treated in serum-free media with 1 μg/ml of testosterone, 0 or 50 ng/ml of FSH, and 0 or 1 μg/ml of insulin with or without the various doses of bST for an additional 2 d. Values are means from four separate experiments. Means without a common letter (a, b, c, d) differ ($P < 0.05$). CON = Control, INS = insulin, and F + I = FSH plus insulin.

pool. Main effects and interactions of dependent variables (e.g., steroid production) were assessed using the general linear models procedure of SAS (32). Main effects were bST dose, insulin, FSH or LH, experiment, and the various interactions. Each well was a replicate, and each experiment contained three replicates per treatment. When steroid production was expressed as nanograms or picograms per 10^5 cells per 24 h, cell numbers at the termination of the experiment were used for this calculation. Specific differences in steroid production between treatments were determined using Fisher's protected least significant difference procedure (29).

RESULTS

Experiment 1

In cultures of granulosa cells from small follicles, 2-d treatment with 50 and 300 ng/ml of bST had no effect ($P > 0.10$) on basal estradiol production or on estradiol production induced by insulin; insulin alone increased ($P < 0.05$) estradiol production 15-fold (Figure 1). However, 300 ng/ml of bST inhibited ($P < 0.05$) estradiol production induced by FSH plus insulin by 55%; 50 ng/ml of bST had no effect on estradiol production (Figure 1). Also, 2-d treatment with FSH had no effect on estradiol production by granulosa cells from small follicles in the absence of insulin but increased ($P < 0.05$) estradiol production in the presence of insulin (Figure 1).

Experiment 2

In cultures of granulosa cells from large follicles, 2-d treatment with 50 and 300 ng/ml of bST had no effect ($P > 0.10$) on basal estradiol production or on estradiol production induced by insulin; insulin alone increased ($P < 0.05$) estradiol production 1.7-fold (Figure 2). However, bST inhibited ($P < 0.05$) estradiol production induced by FSH plus insulin such that the increase induced by FSH in the presence of insulin was attenuated by 50 ng/ml of bST and completely blocked by 300 ng/ml of bST (Figure 2). Also, 2-d treatment with FSH had no effect ($P > 0.10$) on basal estradiol production, but FSH increased ($P < 0.05$) insulin-induced estradiol production by granulosa cells from large follicles (Figure 2).

Experiment 3

In granulosa cells from large follicles, none of the doses of bST affected ($P > 0.10$) progesterone production induced by FSH plus insulin (Figure 3). In the absence of bST, FSH had no effect ($P > 0.10$) on progesterone production by granulosa cells from large follicles cultured in the presence of insulin. However, 100 ng/ml of bST reduced ($P < 0.05$) granulosa cell

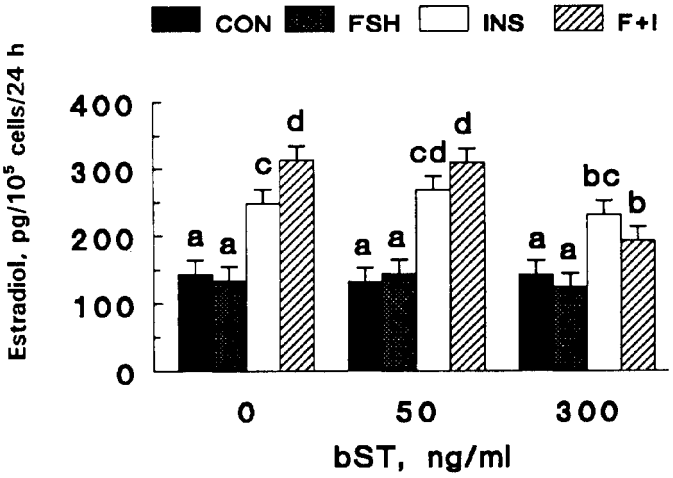

Figure 2. Effects of bST on estradiol production by granulosa cells collected from large follicles (Experiment 2). Granulosa cells were cultured for 2 d in the presence of 10% fetal calf serum and then treated in serum-free media with 1 μg/ml of testosterone, 0 or 50 ng/ml of FSH, and 0 or 1 μg/ml of insulin with or without the various doses of bST for an additional 2 d. Values are means of three separate experiments. Means without a common letter (a, b, c, d) differ ($P < 0.05$). CON = Control, INS = insulin, and F + I = FSH plus insulin.

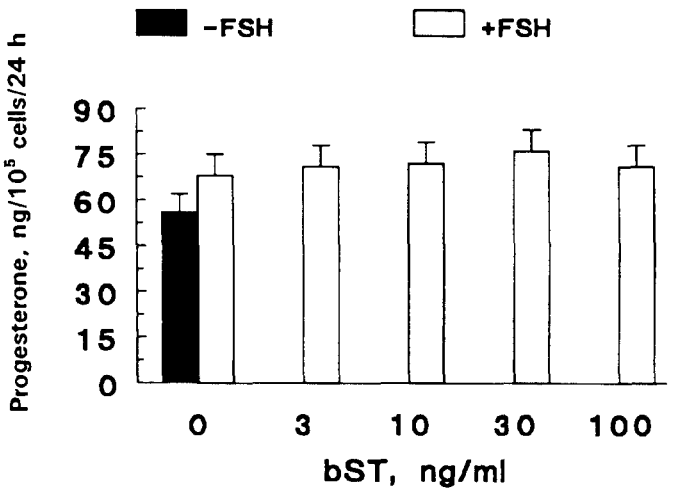

Figure 3. Effects of bST on progesterone production by granulosa cells collected from large follicles (Experiment 3). Granulosa cells were cultured for 2 d in the presence of 10% fetal calf serum and then treated in serum-free media with 1 μg/ml of insulin and 0 or 50 ng/ml of FSH with or without the various doses of bST for an additional 2 d. Progesterone production in the absence of FSH and bST but in the presence of insulin is depicted by the dark bar. Values are means of three separate experiments.

numbers by 24%; at 3, 10, and 30 ng/ml, bST had no effect on cell numbers (Table 2).

Experiment 4

In thecal cells that exhibited >3-fold increase in androstenedione production induced by LH, 3, 10, and 30 ng/ml of bST further stimulated (29 to 42%; $P < 0.05$) androstenedione production (Figure 4A), but progesterone production (Figure 4B) and cell proliferation (Table 2) were unaffected ($P > 0.10$) by bST. In thecal cells that exhibited <2-fold increase in androstenedione production induced by LH, 3, 10, and 30 ng/ml of bST inhibited (32 to 33%; $P < 0.05$) androstenedione production induced by LH plus insulin (Figure 5A). In comparison, none of the doses of bST affected ($P > 0.10$) progesterone production induced by LH plus insulin (Figure 5B). In the absence of LH, bST had no effect ($P > 0.10$) on androstenedione or progesterone production. Also, 3, 10, and 30 ng/ml of bST reduced ($P < 0.05$) thecal cell numbers by 9 to 13% (Table 2).

DISCUSSION

Results of the present study show that 1) pharmacologic, but not physiologic, doses of bST inhibited estradiol production by granulosa cells of small and large follicles, 2) that bST has no effect on progesterone production by granulosa cells or thecal cells of large follicles, and 3) that bST at physiologic doses could either stimulate or inhibit androstenedione production by thecal cells of large follicles depending on whether thecal cells responded to LH.

Results of the present study suggested that bST inhibited estradiol production only at pharmacologic doses and in the presence of both FSH and insulin. For granulosa cells from both small and large follicles, bST blocked the increase in estradiol production that had been induced by FSH. In addition, granulosa cells of small and large antral follicles exhibited similar sensitivity to the inhibitory effect of bST. The mechanism by which bST decreased FSH-induced estradiol secretion by granulosa cells from small and large follicles remains to be elucidated. Also shown in the present study, FSH increased estradiol production by granulosa cells from small follicles after 1 and 2 d of treatment. These results are in contrast to work by Gong et al. (14), who found that 42 h of treatment with ≥50 ng/ml of recombinant bST (with no medium change) stimulated, but FSH treatment did not affect, basal estradiol production by bovine granulosa cells from large (>10 mm) follicles. In this same study (14), bST alone or FSH alone had no effect on estradiol production by granulosa cells from small (<5 mm) and medium (5 to 10 mm) follicles. Because bST decreased incorporation of [^{3}H]thymidine (13) and because this decrease was used to adjust estradiol production in studies of Gong et al. (14), the bST-induced increases in estradiol production by bovine granulosa cells in that study (14) likely were

TABLE 2. Effect of a 2-d bST treatment on proliferation of granulosa cells (Experiment 3) and thecal cells (Experiment 4) from large bovine follicles.[1]

Dose of bST	Granulosa (Experiment 3)[2]	Theca (Experiment 4)[3]	
		Responders[4]	Nonresponders[5]
(ng/ml)	(×10^{-5})		
0	1.96[a]	3.35	3.73[a]
3	1.88[a]	3.27	3.38[b]
10	1.88[a]	3.21	3.31[b]
30	1.87[a]	3.34	3.24[b]
100	1.48[b]	...	...
Pooled SE	0.11	0.09	0.11

[a,b]Means within a column without a common superscript differ ($P < 0.05$).

[1]Values are means from three separate replicate experiments for granulosa cells and four separate replicate experiments for thecal cells.

[2]All cells were treated with insulin and FSH.

[3]All cells were treated with insulin and LH.

[4]Responders: n = 2 experiments in which androstenedione production was increased by LH >3-fold.

[5]Nonresponders: n = 2 experiments in which androstenedione production was increased by LH <2-fold.

due indirectly to a decrease in incorporation of [^{3}H]thymidine. Other studies (28) have shown that 1 to 1000 ng/ml of porcine somatotropin have no effect on estradiol production by porcine granulosa cells. In contrast, studies with human granulosa cells indicated that ≥50 ng/ml of human somatotropin, which has activity similar to prolactin, had no effect on basal estradiol production but increased estradiol production that was induced by IGF-I (3) and FSH (4). In studies with cultured rat granulosa cells, 3-d treatment with ovine somatotropin (≥30 ng/ml) had either no effect on (21) or stimulated (19) estradiol production (not corrected for final cell numbers) that was stimulated by gonadotropin in the absence of insulin. Also, 12 h of treatment with bST (10 to 200 ng/ml) stimulated estradiol secretion in perfused ovaries of rabbits (1, 41). Reasons for the discrepancies among studies on the effect of somatotropin on estradiol production by granulosa cells are unclear, but such disparities may reflect variations in the culture system utilized (e.g., frequency of medium change), species differences, variations in the differentiated state of granulosa cells, or variations in the duration of somatotropin treatment. We observed that a 1-d treatment with either 300 ng/ml of bST or 50 ng/ml of FSH had no effect on cell

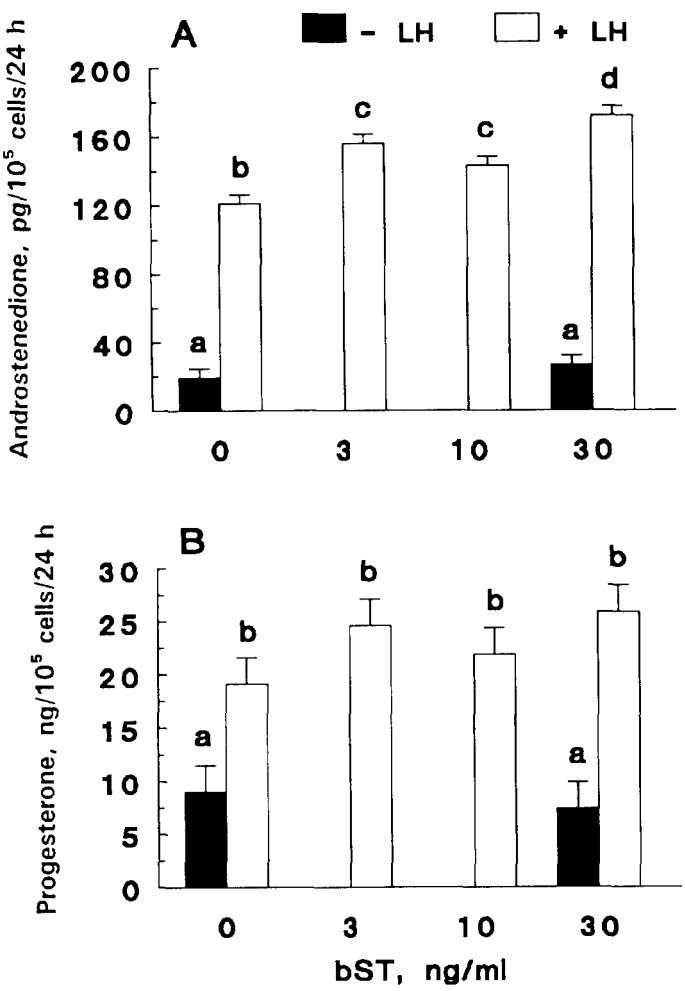

Figure 4. Effects of bST on androstenedione (A) and progesterone (B) production by thecal cells collected from large follicles that responded well to LH (Experiment 4). Thecal cells were cultured for 2 d in the presence of 10% fetal calf serum and then treated in serum-free media with 1 μg/ml of insulin and 0 or 100 ng/ml of LH with or without the various doses of bST for an additional 2 d. Values are means of two separate experiments. Within a panel, means without a common letter (a, b, c, d) differ ($P < 0.05$).

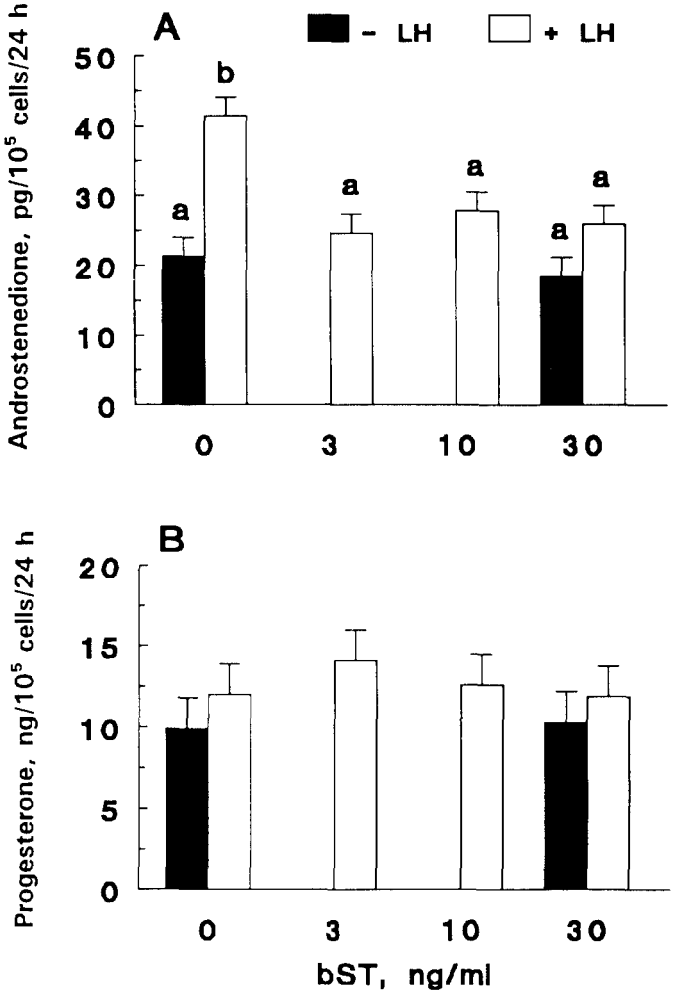

Figure 5. Effects of bST on androstenedione (A) and progesterone (B) production by thecal cells collected from large follicles that responded poorly to LH (Experiment 4). Thecal cells were cultured for 2 d in the presence of 10% fetal calf serum and then treated in serum-free media with 1 μg/ml of insulin and 0 or 100 ng/ml of LH with or without the various doses of bST for an additional 2 d. Values are means of two separate experiments. Within a panel, means without a common letter (a, b) differ ($P < 0.05$).

numbers or estradiol production by bovine granulosa cells from large follicles (data not shown); however, a 1-d treatment with FSH increased estradiol production by bovine granulosa cells from small follicles (Table 1). Moreover, the 40 to 55% inhibition in estradiol production in the present study was accompanied by small (15 to 23%) decreases in cell numbers (data not shown), indicating that the inhibitory effect of bST was not due to an indirect effect on cell growth.

Results of the present study indicated that bST had no effect on progesterone production by granulosa or thecal cells of large bovine follicles stimulated by gonadotropin. Previously, Langhout et al. (24) and others (22) have observed that ≥100 ng/ml of somatotropin enhanced progesterone production induced by insulin but that basal progesterone production was not affected by somatotropin. Similarly, bST had no effect on basal progesterone production by bovine granulosa cells from small (<5 mm) and medium (5 to 10 mm) follicles (14) or on basal progesterone secretion by perfused rabbit ovaries (41). Also, human somatotropin had no effect on basal progesterone production but increased progesterone production by human granulosa cells that had been stimulated by FSH (4). However, Gong et al. (14) found that recombinant bST stimulated basal progesterone production and FSH-induced progesterone production by bovine granulosa cells from large (>10 mm) follicles. Similarly, ovine somatotropin consistently augmented progesterone production induced by gonadotropins in cultured granulosa cells of rats (2, 19, 21) and pigs (18, 22). Reasons for the discrepancies between these studies were likely due to differences in the culture system utilized, stage of differentiation of the granulosa cells, and the presence or absence of gonadotropin and other components in the culture medium.

Results of the present study also showed that bST at physiologic concentrations had a direct stimulatory effect on androstenedione production by bovine thecal cells if thecal cells responded well to LH, but bST inhibited androstenedione production if thecal cells responded poorly to LH. This new finding indicated that bST might have indirectly affected estradiol production by influencing aromatizable estrogen precursors. Why a differential response of thecal cells to LH and bST treatment in vitro was observed is uncertain. Perhaps the stage of development of the follicles when thecal cells were collected influenced the response of thecal cells to LH and bST. For example, thecal cells from atretic follicles may be unresponsive to LH, but thecal cells from growing follicles may respond to LH. In support of this suggestion, Ireland and Roche (20) found that follicles showing estrogen activity (i.e., presumably healthy, growing follicles) had a greater number of thecal LH:human chorionic gonadotropin binding sites than did follicles not showing estrogen activity (i.e., presumably atretic follicles). Thus, depending on whether the theca interna is responsive to LH and whether aromatase activity is elevated within the follicle, bST may or may not alter estradiol secretion. Such interactions might explain why the effect of bST on estradiol secretion in vivo has been inconsistent (11, 12, 26, 27, 34).

In addition to its inhibitory effect on steroid production, bST also inhibited proliferation of granulosa and thecal cells from large follicles. The effect of bST was greater on granulosa cells (25% reduction with 100 ng/ml) than on thecal cells (9 to 13% reduction with ≥3 ng/ml). Previously, Langhout et al. (24) observed that a 2-d treatment with ≥30 ng/ml of bST increased insulin-induced proliferation of granulosa cells from small bovine follicles but had no effect when the insulin was excluded from the medium. Others (40) have reported that a 1-d treatment with 30 ng/ml of bST in serum-free medium, whether or not FSH was present, had no effect on incorporation of basal [^{3}H]thymidine, a measure of DNA synthesis, by bovine granulosa cells that were collected from small (<5 mm), medium (5 to 10 mm), or large (>10 mm) follicles. However, studies from the same research group (13) have shown that a 1-d treatment with higher doses (≥50 ng/ml) of bST alone inhibited incorporation of [^{3}H]thymidine by bovine granulosa cells that were collected from large follicles but not those from small or medium follicles. Whether this decrease in [^{3}H]thymidine would have resulted in decreased cell numbers remains to be determined. In studies with rat granulosa cells (19, 21), ovine somatotropin had no effect on DNA content per well in the absence of insulin (19, 21). Thus, bST may only affect proliferation of granulosa cells when insulin is included in the medium.

Although the present and previous studies (13, 14, 24) establish the membrana granulosa and theca interna in cattle as sites of bST action, its physiologic relevance remains unclear. Mean concentrations of bST in the blood of dairy cattle are usually <20 ng/ml (6, 8, 15, 17, 26, 33) and thus fall below the effective doses of bST used on granulosa cells in the present and previous studies. Furthermore, bST concentrations rarely exceed 100 ng/ml in cattle treated with bST (6, 26, 33, 38) or treated with growth hormone-releasing factor (9, 25, 30). Thus, for untreated cattle

or cattle treated with bST or growth hormone-releasing factor, bST may not be a physiologically relevant promoter of granulosa cell function. However, physiologic concentrations of bST weakly influenced thecal cell functions, therefore implying that bST may be a physiologic effector of follicular function.

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REFERENCES

1 Ando, M., Y. Yoshimura, M. Iwashita, T. Oda, M. Karube, Y. Ubukata, M. Jinno, and Y. Nakamura. 1994. Direct ovarian effect of growth hormone in the rabbit. Am. J. Reprod. Immunol. 31:123.

2 Apa, R., A. Lanzone, F. Miceli, A. Caruso, S. Mancuso, and R. Canipari. 1994. Growth hormone induction of rat granulosa cell tissue-plasminogen activator expression and progesterone synthesis. Mol. Cell Endocrinol. 99:153.

3 Barreca, A., P. G. Artini, P. Del Monte, P. Ponzani, P. Pasquini, G. Cariola, A. Volpe, A. R. Genazzani, G. Giordano, and F. Minuto. 1993. In vivo and in vitro effect of growth hormone on estradiol secretion by human granulosa cells. J. Clin. Endocrinol. Metab. 77:61.

4 Carlsson, B., C. Bergh, J. Bentham, J.- H. Olsson, M. R. Norman, H. Billig, P. Roos, and T. Hillensjo. 1992. Expression of functional growth hormone receptors in human granulosa cells. Human Reprod. 7:1205.

5 Cole, W. J., K. S. Madsen, R. L. Hintz, and R. J. Collier. 1991. Effect of recombinantly-derived bovine somatotropin on reproductive performance of dairy cattle. Theriogenology 36: 573.

6 Crooker, B. A., M. A. McGuire, W. S. Cohick, M. Harkins, D. E. Bauman, and K. Sejrsen. 1990. Effect of dose of bovine somatotropin on nutrient utilization in growing dairy heifers. J. Nutr. 120:1256.

7 De La Sota, R. L., M. C. Lucy, C. R. Staples, and W. W. Thatcher. 1993. Effects of recombinant bovine somatotropin (Sometribove) on ovarian function in lactating and nonlactating dairy cows. J. Dairy Sci. 76:1002.

8 Enright, W. J., L. J. Spicer, D. J. Prendiville, M. G. Murphy, and R. M. Campbell. 1994. Interaction between dietary intake and ovariectomy on concentrations of insulin-like growth factor-I, GH and LH in plasma of heifers. Theriogenology 41:1231.

9 Enright, W. J., S. A. Zinn, L. T. Chapin, and H. A. Tucker. 1987. Growth hormone response of bull calves to growth hormone-releasing factor. Proc. Soc. Exp. Biol. Med. 194:483.

10 Esteban, E., P. H. Kass, L. D. Weaver, J. D. Rowe, C. A. Holmberg, C. E. Franti, and H. F. Troutt. 1994. Reproductive performance in high producing dairy cows treated with recombinant bovine somatotropin. J. Dairy Sci. 77:3371.

11 Gong, J. G., T. Bramley, and R. Webb. 1991. The effects of recombinant bovine somatotropin on ovarian function in heifers: follicular populations and peripheral hormones. Biol. Reprod. 45:941.

12 Gong, J. G., T. A. Bramley, I. Wilmut, and R. Webb. 1993. Effect of recombinant bovine somatotropin on the superovulatory response to pregnant mare serum gonadotropin in heifers. Biol. Reprod. 48:1141.

13 Gong, J. G., D. McBride, T. A. Bramley, and R. Webb. 1993. Effect of recombinant bovine somatotrophin, insulin-like growth factor-I and insulin on the proliferation of bovine granulosa cells in vitro. J. Endocrinol. 139:67.

14 Gong, J. G., D. McBride, T. A. Bramley, and R. Webb. 1994. Effects of recombinant bovine somatotrophin, insulin-like growth factor-I and insulin on bovine granulosa cell steroidogenesis in vitro. J. Endocrinol. 143:157.

15 Hall, J. B., K. K. Schillo, B. P. Fitzgerald, and N. W. Bradley. 1994. Effects of recombinant bovine somatotropin and dietary energy intake on growth, secretion of luteinizing hormone, follicular development, and onset of puberty in beef heifers. J. Anim. Sci. 72:709.

16 Hemken, R. W., R. J. Harmon, W. J. Silvia, W. B. Tucker, G. Heersche, and R. G. Eggert. 1991. Effect of dietary energy and previous bovine somatotropin on milk yield, mastitis, and reproduction in dairy cows. J. Dairy Sci. 74:4265.

17 Herbein, J. J., R. J. Aiello, L. I. Eckler, R. E. Pearson, and R. M. Akers. 1985. Glucagon, insulin, growth hormone, and glucose concentrations in blood plasma of lactating dairy cows. J. Dairy Sci. 68:320.

18 Hsu, C.- J., and J. M. Hammond. 1987. Concomitant effects of growth hormone on secretion of insulin-like growth factor-I and progesterone in cultured porcine granulosa cells. Endocrinology 121:1343.

19 Hutchinson, L. A., J. K. Findlay, and A. C. Herington. 1988. Growth hormone and insulin-like growth factor-I accelerate PMSG-induced differentiation of granulosa cells. Mol. Cell. Endocrinol. 55:61.

20 Ireland, J. J., and J. F. Roche. 1983. Development of nonovulatory antral follicles in heifers: changes in steroids in follicular fluid and receptors for gonadotropins. Endocrinology 112:150.

21 Jia, X.- C., J. Kalmijn, and A.J.W. Hsueh. 1986. Growth hormone enhances follicle-stimulating hormone-induced differentiation of cultured rat granulosa cells. Endocrinology 118:1401.

22 Kirkwood, R. N., P. A. Thacker, and K. Rajkumar. 1992. Effects of growth hormone and triiodothyronine on insulin-induced progesterone production by granulosa cells from prepubertal gilts. Can. J. Anim. Sci. 72:589.

23 Kuehner, L. F., D. Rieger, J. S. Walton, X. Zhao, and W. H. Johnson. 1993. The effect of depot injection of recombinant bovine somatotropin on follicular development and embryo yield in superovulated Holstein heifers. Theriogenology 40:1003.

24 Langhout, D. J., L. J. Spicer, and R. D. Geisert. 1991. Development of a culture system for bovine granulosa cells: effects of growth hormone, estradiol, and gonadotropins on cell proliferation, steroidogenesis, and protein synthesis. J. Anim. Sci. 69: 3321.

25 Lapierre, H., G. Pelletier, D. Petitclerc, P. Dubreuil, J. Morisset, P. Gaudreau, Y. Couture, and P. Brazeau. 1988. Effect of human growth hormone-releasing factor (1-29)NH_2 on growth hormone release and milk production in dairy cows. J. Dairy Sci. 71:92.

26 Lucy, M. C., J. C. Byatt, T. L. Curran, D. F. Curran, and R. J. Collier. 1994. Placental lactogen and somatotropin: hormone binding to the corpus luteum and effects on the growth and functions of the ovary in heifers. Biol. Reprod. 50:1136.

27 Lucy, M. C., R. L. De La Sota, C. R. Staples, and W. W. Thatcher. 1993. Ovarian follicular populations in lactating dairy cows treated with recombinant bovine somatotropin (Sometribove) or saline and fed diets differing in fat content and energy. J. Dairy Sci. 76:1014.

28 Nitray, J., A. V. Sirotkin, J. Poltarsky, and J. Bulla. 1993. The effect of recombinant somatotropic hormone on secretion of ovarian hormones of sexually mature gilts in vivo and in vitro. Vet. Med. (Prague) 38:53.

29 Ott, L. 1977. Multiple comparisons. Page 384 *in* An Introduction to Statistical Methods and Data Analysis. Duxbury Press, North Scituate, MA.
30 Plouzek, C. A., W. Vale, J. Rivier, L. L. Anderson, and A. Trenkle. 1988. Growth hormone-releasing factor on growth hormone secretion in prepubertal calves. Proc. Soc. Exp. Biol. Med. 188:198.
31 Roberts, A. J., and M. K. Skinner. 1990. Hormonal regulation of thecal cell function during antral follicle development in bovine ovaries. Endocrinology 127:2907.
32 SAS® User's Guide: Statistics. Version 6 Edition. 1988. SAS Inst., Inc., Cary, NC.
33 Schams, D., U. Winkler, M. Theyerl-Abele, and A. Prokopp. 1989. Variation of BST and IGF-I concentrations in blood plasma of cattle. Page 18 *in* Use of Somatotropin in Livestock Production. K. Sejrsen, M. Vestergaard, and A. Neimann-Sorensen, ed. Elsevier Appl. Sci., New York, NY.
34 Schemm, S. R., D. R. Deaver, L. C. Griel, Jr., and L. D. Muller. 1990. Effects of recombinant bovine somatotropin on luteinizing hormone and ovarian function in lactating dairy cows. Biol. Reprod. 42:815.
35 Spicer, L. J., E. Alpizar, and S. E. Echternkamp. 1993. Effects of insulin, insulin-like growth factor I, and gonadotropins on bovine granulosa cell proliferation, progesterone production, estradiol production, and(or) insulin-like growth factor I production in vitro. J. Anim. Sci. 71:1232.
36 Spicer, L. J., and W. J. Enright. 1991. Concentrations of insulin-like growth factor I and steroids in follicular fluid of preovulatory bovine ovarian follicles: effect of daily injections of a growth hormone-releasing factor analog and(or) thyrotropin-releasing hormone. J. Anim. Sci. 69:1133.
37 Spicer, L. J., W. B. Tucker, and G. D. Adams. 1990. Insulin-like growth factor-I in dairy cows: relationships among energy balance, body condition, ovarian activity, and estrous behavior. J. Dairy. Sci. 73:929.
38 Stelwagen, K., D. G. Grieve, B. W. McBride, and J. D. Rehman. 1992. Growth and subsequent lactation in primigravid Holstein heifers after prepartum bovine somatotropin treatment. J. Dairy Sci. 75:463.
39 Villa-Godoy, A., T. L. Hughes, R. S. Emery, W. J. Enright, A. D. Ealy, S. A. Zinn, and R. L. Fogwell. 1990. Energy balance and body condition influence luteal function in Holstein heifers. Domest. Anim. Endocrinol. 7:135.
40 Webb, R., J. G. Gong, A. S. Law, and S. M. Rusbridge. 1992. Control of ovarian function in cattle. J. Reprod. Fertil. 45(Suppl.):141.
41 Yoshimura, Y., Y. Nakamura, N. Koyama, M. Iwashita, T. Adachi, and Y. Takeda. 1993. Effects of growth hormone on follicle growth, oocyte maturation, and ovarian steroidogenesis. Fertil. Steril. 59:917.
42 Zhao, X., J. H. Burton, and B. W. McBride. 1992. Lactation, health, and reproduction of dairy cows receiving daily injectable or sustained-release somatotropin. J. Dairy Sci. 75:3122.