EFFECTS OF BOVINE SOMATOTROPIN ON OVARIAN FUNCTION IN CATTLE

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Story in Brief

The direct effect of bovine somatotropin (BST) on ovarian function in cattle was evaluated by using a serum-free culture system of bovine granulosa cells. Granulosa cells were obtained from small (1 to 5 mm) follicles collected from cattle and cultured for 4 days. Culture medium was collected for quantification of progesterone, and cell numbers were determined. In the presence of insulin, BST significantly increased granulosa cell numbers and progesterone production after 2 days of treatment. We conclude that BST may have direct effects on ovarian function in cattle.

(Key Words: Bovine Somatotropin, Granulosa Cells, Progesterone, Cattle.)

Introduction

Recent attempts to improve production efficiency in domestic animals has concentrated on the use of bovine somatotropin (BST) (Enright, 1989). However, long-term BST treatment has been reported to affect reproductive performance in cattle. Therefore, since direct effects of BST on bovine ovarian cells have not been reported previously, we set out to determine the effects of BST on cell growth and progesterone production of bovine granulosa cells.

Materials and Methods

Ovaries were obtained at a nearby commercial slaughterhouse from non-pregnant beef and dairy cattle. Granulosa cells from 1 to 5 mm follicles were collected by aspiration using a needle and syringe and washed three times in serum-free medium. At each wash, cells were separated from medium via centrifugation (200 x g for 10 min). Medium was a 1:1 mixture of Dulbecco's

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Modified Eagles Medium and Ham's F10 containing 0.12 mM gentamicin and 38.5 mM sodium bicarbonate. Between 2 and 4 x 10^5 viable cells in 20 to 50 μl of medium were added to plastic multiwell plates containing 1 ml medium. Cultures were kept at 37°C in a 95% air- 5% CO_2 atmosphere, and medium was changed every 24 hours. To obtain optimal attachment, cells were maintained in the presence of 10% fetal calf serum for the first 48 hours of culture. After this time, granulosa cells were washed twice with 0.5 ml serum-free medium and incubations continued in serum-free medium with or without added hormones. Hormonal treatments were maintained for 2 days.

At the termination of each experiment, numbers of cells were determined using a Coulter counter. Culture medium was also collected for determination of concentrations of progesterone (as a measure of steroidogenic capacity of cells) by radioimmunoassay.

Experimental data are presented as the least squares means ± SE of measurements from replicated experiments. Each culture well was a replicate and each experiment contained four replicates per treatment. Progesterone production was expressed as [ng.$(10^5$ cells$)^{-1}$.(24 h$)^{-1}$] using cell numbers at the termination of the experiment for the calculation.

Results

In serum-free medium with no additional hormones, BST (0, 10, 30, 100, 300 and 1000 ng/ml) had no significant effect on cell numbers (Figure 1A) or progesterone production (Figure 1B). However, when insulin was added, BST had a dose-dependent effect on cell numbers and progesterone production, compared with insulin treatment alone (Figure 1). The doses of 30, 100, 300 and 1000 ng/ml BST caused significant increases in cell numbers (Figure 1A). When 100, 300 or 1000 ng/ml BST was added to insulin-containing medium, a significant increase in progesterone production was observed (Figure 1B). When granulosa cells were cultured with 10% FCS during the 2-d BST treatment, no significant effect of BST was seen on cell proliferation or steroidogenesis (data not shown).

Discussion

Results of the present study suggest that BST has a direct effect on bovine granulosa cells. However, only in the presence of insulin did BST have an enhancing effect on both cell growth and progesterone production. Previous in vitro studies have shown that ST can enhance (by 1.5- to 15.0-fold) steroidogenesis by rat (Jia et al., 1986) and porcine (Hsu and Hammond,

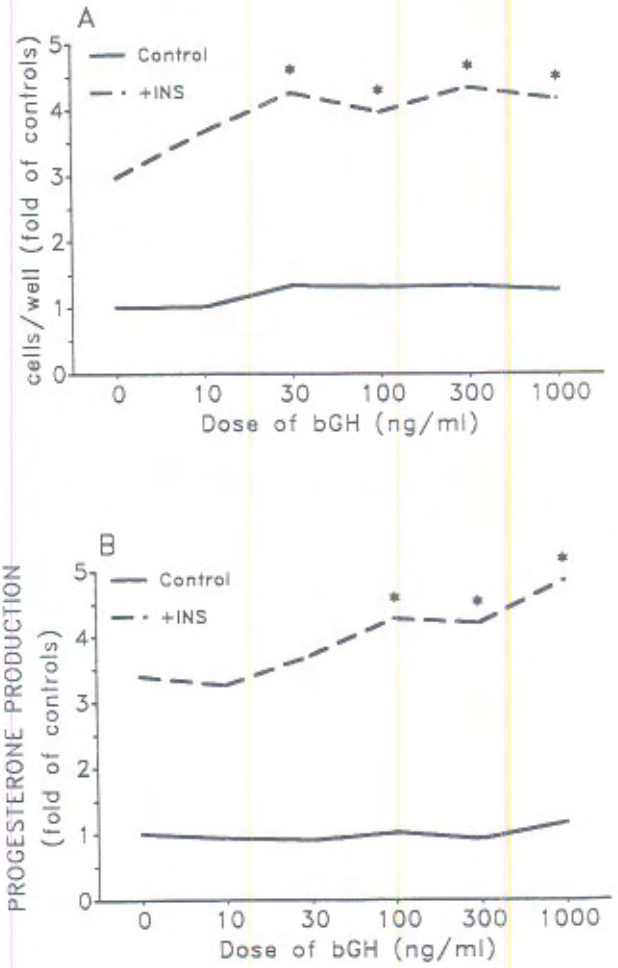

Figure 1. Effect of bovine somatotropin (BST; USDA-bGH-B1) on granulosa cell numbers (Panel A) and progesterone production (Panel B) in the absence or presence of insulin (INS) at d 4 of culture. Granulosa cells were obtained from small follicles and treated as described in Materials and Methods. Values are least squares means of quadruplicate culture wells from three separate experiments and expressed as fold of controls without insulin or BST. *, mean differs ($P < .05$) from treatment without addition of BST. Pooled SE = 0.18-fold of controls for Panel A and pooled SE = 0.17-fold of controls for Panel B. Cell numbers averaged 0.41 cells/well ($x10^5$) for controls without insulin or BST (Panel A). Progesterone production averaged 7 ng/10^5cells/24 h for controls without insulin or BST (Panel B).

1987) granulosa cells. This is the first report suggesting that ST affects granulosa cell proliferation in any species.

Although the present and previous studies establish granulosa cells as a site of ST action, its physiologic relevance remains unclear. Average concentrations of BST in beef and dairy cattle are usually less than 10 ng/ml, and thus fall below the effective doses of BST used in the present study. However in BST- and growth hormone-releasing hormone (GHRH)-treated cattle, BST levels usually exceed 100 ng/ml (Gluckman et al., 1987). Thus, in BST- and GHRH-treated cattle BST may be a physiologically relevant promoter of ovarian follicular function.

Literature Cited

Enright, W.J. et al. 1989. Effects of administration of somatotropin on growth, feed efficiency and carcass composition of ruminants: a review. In: K. Sejrsen, M. Vestergaard and A. Neimann-Sorensen (Ed.) Use of Somatotropin in Livestock Production. pp. 132-156. Elsevier Applied Science, London.

Gluckman, P.D. et al. 1987. Physiology of the somatotrophic axis with particular reference to the ruminant. J. Dairy Sci. 70:442.

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

Jia, X.C. et al. 1986. Growth hormone enhances follicle-stimulating hormone-induced differentiation of cultured rat granulosa cells. Endocrinology 118:1401.