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Effects of Insulin and Luteinizing Hormone on Ovarian Granulosa Cell Aromatase Activity in Cattle

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Story in Brief

The direct effect of insulin and luteinizing hormone (LH) on ovarian granulosa cell function in cattle was evaluated by using a serum-free culture system. Granulosa cells were obtained from large (³ 8 mm) follicles collected from cattle and cultured for 4 d. During the last 2 d of culture, cells were exposed to testosterone in serum-free medium to assess aromatase activity. Culture medium was collected for quantification of estradiol, and cell numbers were determined. Insulin significantly increased estradiol production after 1 and 2 d of treatment. Alone, LH had no effect on estradiol production. However, 30 ng/mL of LH reduced the stimulatory effect of insulin on estradiol production after 1 d but not 2 d of treatment.

(Key Words: Insulin, Luteinizing Hormone, Granulosa Cells, Estradiol, Cattle.)

Introduction

Increased secretion of estradiol by growing dominant ovulatory and non-ovulatory follicles is a critical step during the estrous cycle that leads to the expression of estrus, release of an ovulatory surge of LH, ovulation of the follicle and release of the oocyte (Spicer and Echternkamp, 1986; Ginther et al., 1997). However, the hormonal factors that regulate production of estradiol by the dominant follicle in cattle are not completely understood. Estradiol is produced by bovine granulosa cells via the action of aromatase which is a granulosa-cell specific enzyme that converts androgens (e.g., testosterone) into estrogens (e.g., estradiol). Previous studies have shown that insulin is a potent stimulator of aromatase activity in cattle (Spicer et al., 1994) but direct effects of LH on aromatase activity have not been reported for cattle. Therefore, we set out to compare the effects of insulin and LH on aromatase activity of bovine granulosa cells.

Materials and Methods

Ovaries were obtained at a nearby commercial slaughterhouse from beef and dairy cattle. Granulosa cells from ³ 8 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). The medium was a 1:1 mixture of Dulbecco's modified Eagles medium and Ham's F12 containing gentamicin, glutamine and sodium bicarbonate. Approximately 2 x 10^5 viable cells were added to plastic multiwell plates containing 1 ml medium. Cultures were kept at 38.5° C in a 95% air- 5% CO_2 atmosphere, and medium was changed every 24 h. To obtain optimal attachment, cells were maintained in the presence of 10% fetal calf serum for the first 48 h of culture (Langhout et al., 1991). After this time, granulosa cells were washed twice with 0.5 ml of serum-free medium and incubations continued in serum-free medium containing 500 ng/ml of testosterone (as an estrogen precursor for aromatase) with or without added hormones. Hormonal treatments (i.e., insulin and LH) were maintained for an additional 1 or 2 d.

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 estradiol (as a measure of aromatase activity) by radioimmunoassay.

Experimental data are presented as the least squares means ± SE of measurements from replicated experiments. Each experiment was replicated three times with three replicates per treatment within an experiment. Estradiol production was expressed as (ng/10^5 cells/24 h) using cell numbers at the termination of the experiment for the calculation.

Results

In serum-free medium containing 500 ng/ml of testosterone and 50 ng/ml of ovine FSH, bovine insulin (100 ng/ml) increased cell numbers (data not shown) and FSH-induced estradiol production severalfold (Figure 1). This stimulatory effect of insulin was much more pronounced after 2 d (54-fold increase) than 1 d (6-fold increase) of treatment (Figure 1). In contrast, bovine LH had no effect on cell numbers (data not shown) or FSH-induced estradiol production, compared with control (Figure 1). Treatment with 3 and 30 ng/ml of LH caused significant reductions in the insulin-induced estradiol production after 1 d of treatment (Figure 1a). However, after 2 d of treatment, 3 and 30 ng/ml of LH had no significant effect on the insulin-induced increase in estradiol production.

Discussion

Results of the present study suggest that insulin and LH have direct effects on aromatase activity of bovine granulosa cells. Previous *in vitro* studies have shown that insulin can enhance (by 2- to 20-fold) estradiol production by rat (Davoren et al., 1985) and bovine (Spicer et al., 1994) but not porcine (Maruo et al., 1988) granulosa cells. This is the first report comparing the effects of concurrent treatment with insulin and LH on granulosa cell aromatase activity in cattle. Results of the present study also indicate that aromatase activity increases between d 1 and 2 of insulin treatment.

The effect of insulin and LH on bovine granulosa cells is likely physiologically relevant. Average concentrations of insulin and LH in beef and dairy cattle are usually less than 10 ng/ml (Richards et al., 1989; 1991) except at the time of the ovulatory surge of LH during which LH concentrations can achieve $\geq$ 30 ng/ml (Richards et al., 1991). Studies *in vivo* have shown that insulin injections increase estradiol concentrations in follicular fluid of cattle (Simpson et al., 1994), and that estradiol concentrations in follicular fluid decrease after the LH surge in cattle (Voss and Fortune, 1993). Thus, insulin and LH may be physiologically relevant regulators of ovarian follicular estradiol production in cattle. Further research will be required to determine if exogenous insulin could be used to enhance reproductive efficiency in cattle.

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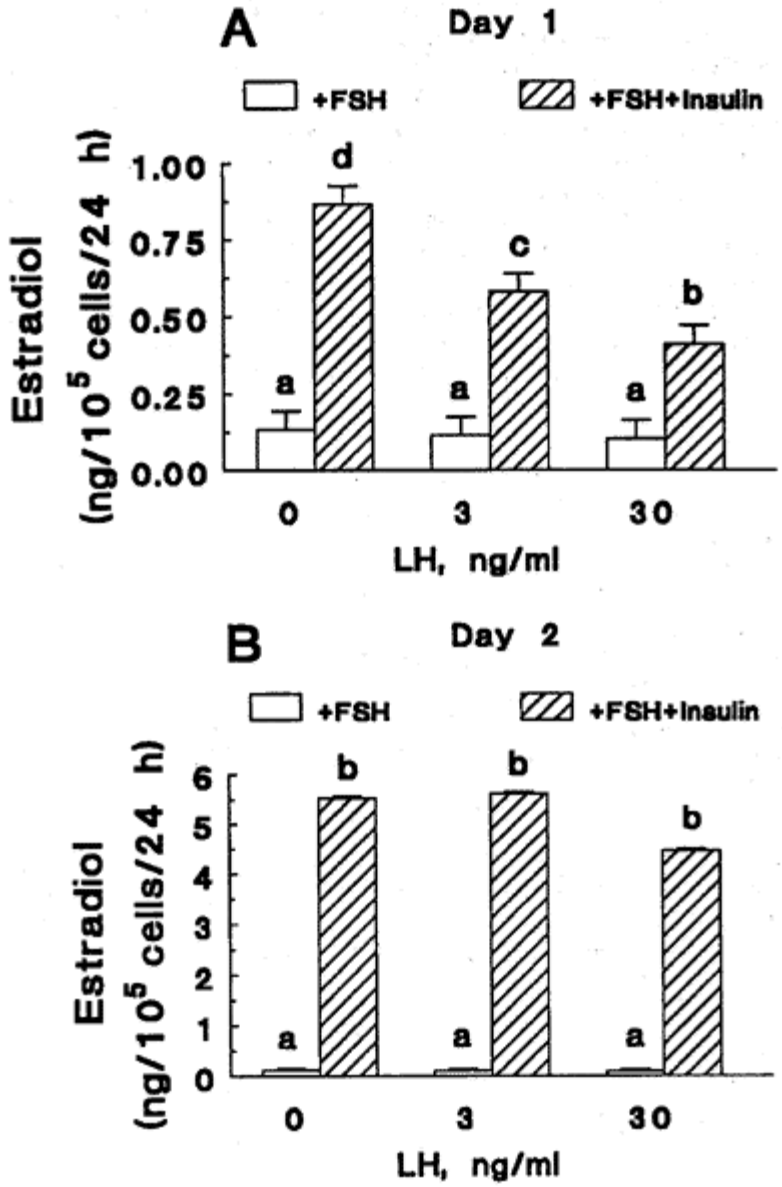

Figure 1. Effect of 1-d (Panel A) and 2-d (Panel B) treatment with bovine insulin and luteinizing hormone (LH) on granulosa cell estradiol production. Granulosa cells were obtained from large follicles. [a,b,c,d] Within a panel, means without a common letter differ (P<.05).

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