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# EFFECTS OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-3 ON BOVINE OVARIAN GRANULOSA CELL PROLIFERATION AND STEROIDOGENESIS

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## Story in Brief

The effects of insulin-like growth factor binding protein-3 (IGFBP-3) and IGF-I on granulosa cell function in cattle were evaluated using a serum-free culture system. Granulosa cells were obtained from small (1 to 5 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 progesterone and cell numbers were determined. Alone, 10 ng/ml of IGF-I increased estradiol production by 7.5-fold, progesterone production by 1.7-fold and cell numbers by 2.2-fold, whereas 25 and 100 ng/ml of IGFBP-3 alone had no effect. However, 100 ng/ml of IGFBP-3 inhibited IGF-I-induced estradiol production by 45%, progesterone production by 14% and cell numbers by 11%. These results indicate that IGFBP-3 can inhibit IGF-I action in cultured bovine granulosa cells. Thus, IGFBP-3 may play a role in regulating granulosa cell steroidogenesis and proliferation during ovarian follicular growth in cattle.

Key Words: Insulin-like Growth Factor, Binding Proteins, Granulosa Cells, Estradiol, Cattle

## Introduction

The growth of dominant ovulatory and non-ovulatory follicles during the estrous cycle of cattle is characterized by increased estradiol secretion and increased numbers of granulosa cells. Increased estradiol secretion by the selected dominant ovulatory follicle causes estrus and induces an ovulatory surge of luteinizing hormone (LH), which subsequently induces ovulation and release of the oocyte (Ginther et al., 1997; Roche et al., 1998). The IGF system, which includes IGF-I and IGFBPs, is thought to play an important role in regulating the development of dominant follicles (Spicer and Echternkamp, 1995). At least four species of IGFBPs exist in follicular fluid with IGFBP-3 being the most abundant species. However, the role of IGF-I and IGFBPs in regulating production of estradiol by the dominant follicle in cattle is unknown. Therefore, we set out to determine the effect of IGF-I and IGFBP-3 on bovine granulosa cell proliferation and steroidogenesis.

## Materials and Methods

Ovaries were obtained at a local commercial slaughterhouse for beef and dairy cattle. Granulosa cells from small (1 to 5 mm) follicles were collected and cultured as previously described (Langhout et al., 1991). Briefly, isolated granulosa cells were cultured in medium containing 10% fetal calf serum for 48 h, washed with serum-free medium, and cultured for an additional 48 h in serum-free medium containing 500 ng/ml of testosterone (as an estrogen precursor) and .25 g/ml of bovine serum albumin with or without recombinant human IGF-I (0 or 10 ng/ml) and(or) IGFBP-3 (0, 25 or 100 ng/ml). At the termination of each experiment, numbers of cells were determined using a Coulter counter as previously described (Langhout et al., 1991). Culture medium was collected for determination of concentrations of estradiol and progesterone by radioimmunoassay.

Experimental data are presented as the least squares means ± SEM of measurements from

three replicated experiments. In each repeated experiment, treatments were replicated three times. Estradiol and progesterone production were expressed as pg and ng per $10^5$ cells per 48 h, respectively.

## Results

Alone, 10 ng/ml of IGF-I increased (P<.05) estradiol production by 7.5-fold, progesterone production by 1.7-fold, and cell numbers by 2.2-fold (Figure 1). In contrast, 25 and 100 ng/ml of IGFBP-3 had no effect on estradiol production, progesterone production, or cell numbers (Figure 1). However, 25 and 100 ng/ml of IGFB-3 inhibited (P<.05) IGF-I-induced estradiol production by 19% and 45%, respectively. IGF-I-induced progesterone production and cell numbers were not affected (P>.10) by 25 ng/ml of IGFBP-3, whereas 100 ng/ml of IGFBP-3 inhibited IGF-I-induced progesterone production by 14% (P<.05) and cell numbers by 11% (P<.10).

## Discussion

Similar to studies using granulosa cells of other species (reviewed in Spicer and Echternkamp, 1995), results of the present study suggest that IGFBP-3, the most abundant IGFBP found in plasma and follicular fluid, can inhibit IGF-I-induced increases in bovine granulosa cell estradiol and progesterone production and cell numbers without affecting basal cell function. Previously, we have shown that IGFBP-3 can inhibit IGF-I-induced steroidogenesis and cell numbers in cultured bovine thecal cells (Spicer et al., 1997). Thus, IGFBP-3 may reduce estradiol production by ovarian follicles through a reduction in aromatizable androgen precursors from thecal cells as well as through a reduction in aromatase activity in granulosa cells. Further research will be required to determine if exogenous IGF-I could be used to enhance reproductive efficiency in cattle.

## Literature Cited

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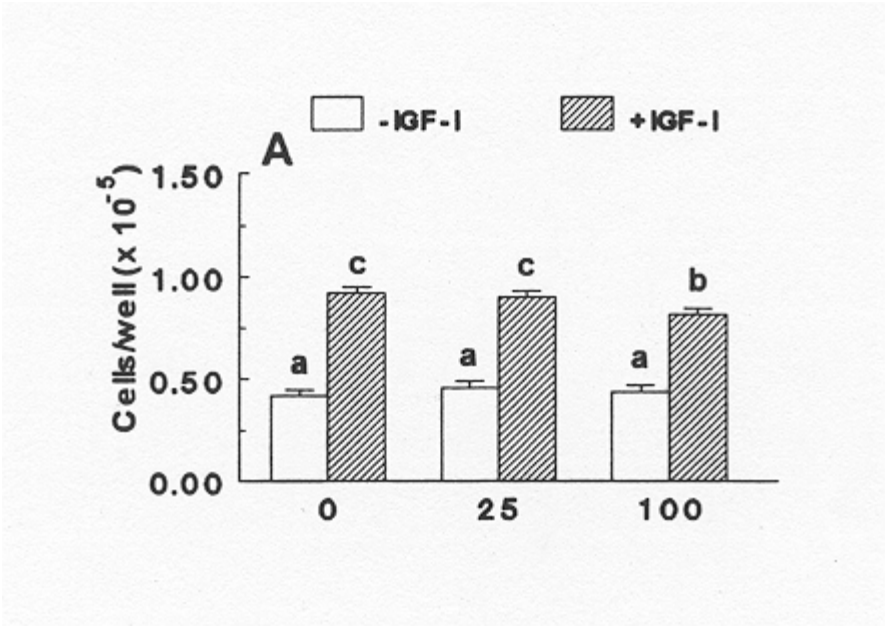
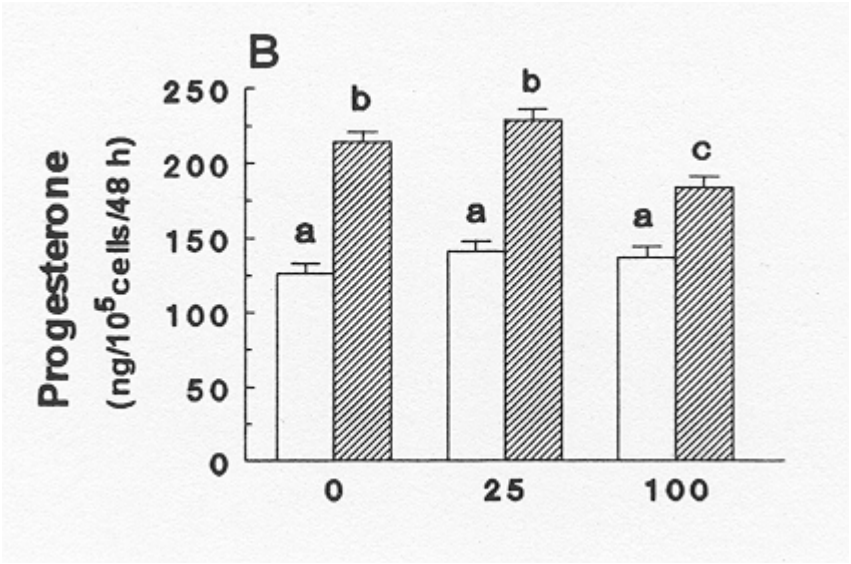

-IGF-I
+IGF-I
A
Cells/well (x 10$^{-5}$)
1.50
1.00
0.50
0.00
a
c
a
c
a
b
0
25
100


B
Progesterone
(ng/10$^5$cells/48 h)
250
200
150
100
50
0
a
b
a
b
a
c
0
25
100

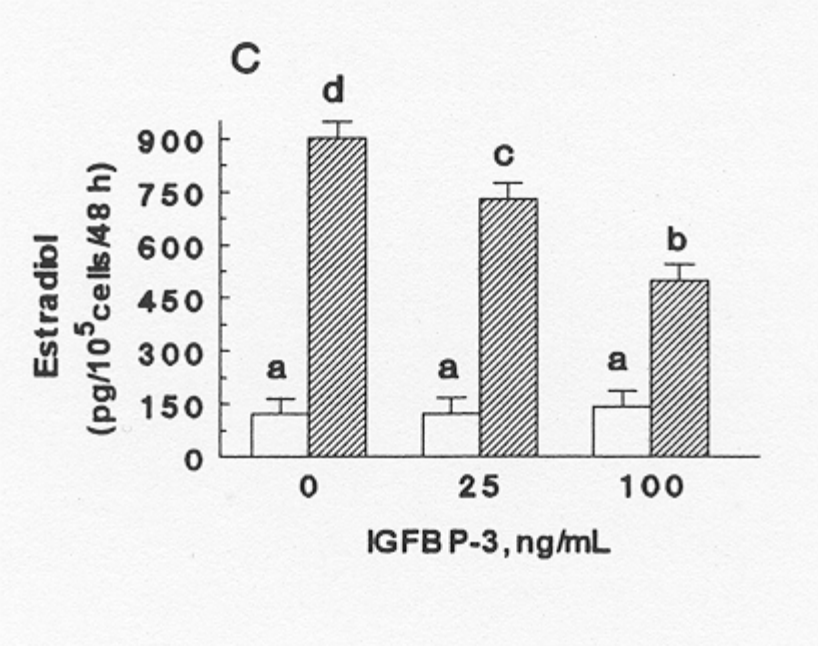



**Figure 1. Dose-response of IGFBP-3 on IGF-I-stimulated granulosa cell numbers (Panel A), progesterone production (Panel B), and estradiol production (Panel C). Granulosa cells were obtained from small follicles, cultured for 2 d in the presence of 10% fetal calf serum, and then treated in serum-free media with .25 g/ml of bovine serum albumin, 500 ng/ml of testosterone, IGF-I (0 or 10 ng/ml) and IGFBP-3 (0, 25 or 100 ng/ml) for an additional 2 d. [a,b,c]Within a panel, means without a common letter differ (P<.05).**

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