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Effects of Hormones on Insulin-Like Growth Factor-II Production by Bovine Ovarian Granulosa Cells

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Story in Brief

The effects of hormones on insulin-like growth factor-II (IGF-II) production by granulosa cells of cattle were evaluated using a serum-free culture system. Granulosa cells were obtained from small (1 to 5 mm) and large (³ 8 mm) follicles collected from cattle and cultured for 3 d. During the last 1 d of culture, cells were exposed to hormones in serum-free medium. Culture medium was collected and concentrated for quantification of IGF-II and cell numbers were determined. Granulosa cells from large follicles produced more IGF-II than those from small follicles. Alone, 100 ng/ml of either insulin or luteinizing hormone (LH) decreased IGF-II production, whereas 300 ng/ml of estradiol alone had no effect. Both LH and estradiol amplified insulin-induced decreases in IGF-II production. These results indicate that hormones can inhibit IGF-II production by cultured bovine granulosa cells. Thus, locally produced IGF-II may play a role in regulating granulosa cell function during ovarian follicular growth in cattle.

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

Introduction

The growth of dominant ovulatory and non-ovulatory follicles during the estrous cycle of cattle is characterized by increased estradiol secretion, decreased insulin-like growth factor-II (IGF-II) concentrations, 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 IGF-II, is thought to play an important role in regulating the development of dominant follicles (Spicer and Echternkamp, 1995). In human granulosa cells, hormones regulate IGF-II production and its mRNA (Voutilainen and Miller, 1987; Barreca et al., 1993; 1996). Whether IGF-II is acting as an autocrine or paracrine regulator of follicular function in cattle is unknown. Therefore, we set out to determine if IGF-II is produced by granulosa cells of cattle and if so, what hormones regulate its production.

Materials and Methods

Ovaries were obtained from beef and dairy cattle at a local commercial slaughterhouse. Granulosa cells from small (1 to 5 mm) and large (³ 8 mm) follicles were collected and cultured as previously described (Langhout et al., 1991; Spicer and Chamberlain, 1999). 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 24 h in serum-free medium containing 0.25% bovine serum albumin with or without bovine insulin (0 or 100 ng/ml), estradiol (0 or 500 ng/ml), ovine LH (0 or 100 ng/ml), insulin plus estradiol, and insulin plus LH. 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 and concentrated 10-fold for determination of concentrations of IGF-II 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. Production of IGF-II was expressed as ng per 10^5 cells per 24 h, respectively.

Results

Granulosa cells from large follicles produced 62% more IGF-II than did cells from small follicles ($P<.05$; Figure 1). Alone, 100 ng/ml of insulin decreased ($P<.05$) IGF-II production by 38% and 24% in cultures of small and large follicle granulosa cells, respectively (Figure 1). Alone, 100 ng/ml of LH decreased ($P<.05$) IGF-II production by 26% and 38% in cultures of small and large follicle granulosa cells, respectively (Figure 1). Also, in the presence of insulin, LH decreased ($P<.05$) IGF-II production by 35% and 32% below that of insulin alone in cultures of small and large follicle granulosa cells, respectively. In contrast, 300 ng/ml of estradiol alone did not affect IGF-II production (Figure 1). However, estradiol further inhibited ($P<.05$) insulin-induced decreases in IGF-II production by 35% and 41%, in small and large follicles, respectively.

Discussion

Granulosa cells of large follicles produced significantly more IGF-II than those of small follicles. In contrast, previous studies indicate that concentrations of IGF-II in follicular fluid do not differ between dominant and large subordinant follicles (Stewart et al., 1996) but are lower in large versus small follicles (Spicer and Echternkamp, 1995; Stewart et al., 1996). Reasons for these discrepancies between in vivo and in vitro studies are unclear but may be due to the removal of the inhibitory inputs on granulosa cells when isolated for in vitro culture. Nonetheless, the present studies indicate that the hormonal milieu of a particular follicle may influence the amount of IGF-II it produces.

Results of the present study suggest that estradiol, an important steroid found in follicular fluid, and LH can both amplify the insulin-induced decrease in bovine granulosa cell IGF-II production. Also, LH inhibited whereas estradiol did not affect basal IGF-II production. Because estradiol concentrations in follicular fluid are much greater in large versus small follicles (Spicer and Geisert, 1992; Stewart et al., 1996) and granulosa cells of large follicles contain greater levels of LH receptors than do granulosa cells of small follicles (Stewart et al., 1996), lower IGF-II levels found in large follicles may be a result of increased estradiol concentrations as well as increased responsiveness to LH. Previous studies have shown that LH stimulates whereas estradiol has no effect on IGF-II production by cultured human granulosa cells (Ramasharma and Li, 1987). Thus, species differences may exist with regard to hormonal control of granulosa cell IGF-II production, and indicate that locally produced IGF-II may play a role in regulating granulosa cell function during ovarian follicular growth. Further research will be required to determine if exogenous IGF-II could be used to enhance reproductive efficiency in cattle.

Literature Cited

Barreca, A. et al. 1993. J. Clin. Endocrinol. Metab. 77:61.

Barreca, A. et al. 1996. Fertil. Steril. 65:739.

Ginther, O.J. et al. 1997. Biol. Reprod. 55:1187.

Langhout, D.J. et al. 1991. J. Anim. Sci. 69:3321.

Ramasharma, K. and C.H. Li. 1987. Proc. Natl. Acad. Sci. 84:2643.

Spicer, L.J. and C.S. Chamberlain. 1999. Okla. Agr. Exp. Sta. Res. Rep. P-973:301.

Spicer, L.J. and S.E. Echternkamp. 1995. Dom. Anim. Endocrinol. 12:223.

Spicer, L.J. and R.D. Geisert. 1992. Theriogenology 37:749.

Stewart, R.E. et al. 1996. Endocrinology 137:2842.

Roche, J.F. et al. 1998. J. Anim. Sci. 76(Suppl. 3):16.

Voultilainen, R. and W.L. Miller. 1987. Proc. Natl. Acad. Sci. 84:1590.

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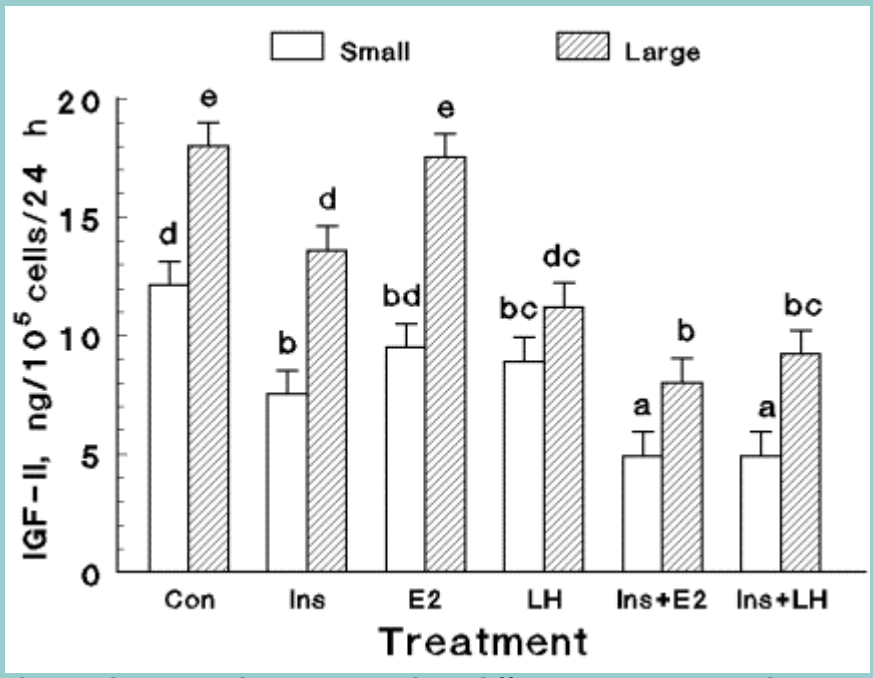

a,b,c Panel, means without a common letter differ (P<.05). Con=Control; IN=insulin; E2=estradiol; LH=luteinizing hormone.

Figure 1. Hormone effects on IGF-II production by granulosa cells of small (1 to 5 mm) and large (≥ 8 mm) follicles. Granulosa cells were obtained from bovine follicles, cultured for 2 d in the presence of 10% fetal calf serum, and then treated in serum-free media with 0.25 % of bovine serum albumin, and either, insulin (0 or 100 ng/ml), estradiol (0 or 300 ng/ml) and(or) LH (0 or 100 ng/ml) for an additional 1 d.

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