Effect of Growth Hormone on Insulin-Like Growth Factor (IGF)-I and IGF Binding Protein Production by Bovine Ovarian Granulosa Cells

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Story in Brief

The effects of growth hormone (GH) on insulin-like growth factor-I (IGF-I) and IGF binding protein (IGFBP) production by granulosa cells of 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 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-I and IGFBP, and cell numbers were determined. In the presence or absence of 30 ng/ml of follicle-stimulating hormone (FSH), 300 ng/ml of GH had no effect on IGF-I and IGFBP production. However, FSH decreased IGF-I production, increased IGFBP-4 production, and had no effect on IGFBP-2 production. These results indicate that FSH has differential effects on IGF-I and IGFBP production by bovine granulosa cells, whereas GH has little or no effect on the ovarian IGF system in cattle. Locally produced IGF-I and IGFBP may play a role in regulating granulosa cell function during FSH-induced ovarian follicular growth in cattle. Understanding factors that regulate follicular growth will lead to the development of better synchronization systems for artificial insemination to maximize efficiency.

Key Words: Growth Hormone, 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, increased “free” insulin-like growth factor-I (IGF-I) concentrations, decreased IGFBP-2, -4 and –5, and increased numbers of granulosa cells (Spicer, 2004). 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; Mihm and Bleach, 2003). The IGF system, which includes IGF-I and –II and IGFBPs, is thought to play an important role in regulating the development of dominant follicles (Spicer and Echternkamp, 1995). In bovine granulosa cells, hormones regulate IGF-I and IGFBP production and its mRNA (Chamberlain and Spicer, 2001; Spicer and Chamberlain, 2002; Voge et al., 2004), but whether metabolic hormones like GH regulate the ovarian IGF system in cattle is unknown. By understanding the factors that regulate follicular growth, better synchronization systems for artificial insemination will be developed to help maximize reproductive efficiency. Therefore, we set out to determine if GH alters IGF-I or IGFBP production by granulosa cells of cattle.

Materials and Methods

Ovaries were obtained from beef and dairy cattle at a local commercial slaughterhouse. Granulosa cells from small (1 to 5 mm) follicles were collected and cultured as previously described (Langhout et al., 1991; Spicer and Chamberlain, 2000). 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 100 ng/ml of bovine insulin with or without bovine GH (0 or 300 ng/ml), ovine FSH (0 or 30 ng/ml), and GH plus FSH. 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-I by radioimmunoassay and IGFBP by ligand blotting.

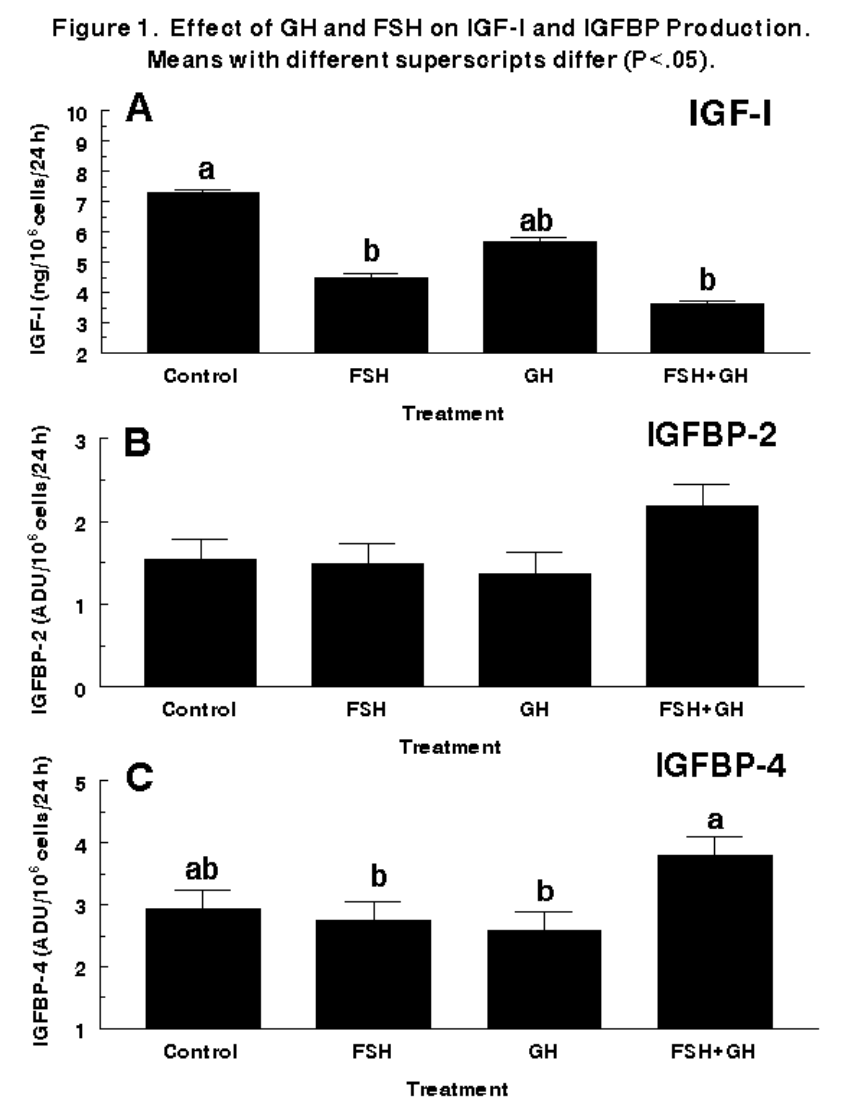
Experimental data are presented as the least squares means ± standard error of the measurements from three replicated experiments, and were analyzed as a 2 x 2 factorial ANOVA using general linear models procedure of SAS. In each repeated experiment, treatments were replicated three times. Production of IGF-I and IGFBP was expressed as ng and ADU per 10^6 cells per 24 h, respectively.

Results

In the presence or absence of 30 ng/ml of FSH, 300 ng/ml of GH had no effect ($P>.15$) on IGF-I and IGFBP production by cultures of small follicle granulosa cells (Figure 1). Alone, 30 ng/ml of FSH decreased ($P<.05$) IGF-I production by 38% (Figure 1A). Also, in the presence of GH, FSH increased ($P<.05$) IGFBP-4 production by 48% (Figure 1C). In contrast, FSH had no effect on IGFBP-2 production (Figure 1B).

Discussion

GH, an important metabolic hormone used to increase milk production, had no significant effect on production of IGF-I and IGFBP by granulosa cells of small follicles. Consistent with this finding, previous studies reported that GH has little or no effect on bovine granulosa cell proliferation and steroidogenesis (Langhout et al., 1991; Spicer and Stewart, 1996). In contrast, FSH decreased IGF-I production in the absence of GH and increased IGFBP-4 production in the presence of GH, and indicates that the hormonal milieu of a particular follicle may influence the amount of IGF-I and IGFBP it produces. Previous studies have shown inhibitory effects of FSH on IGF-I production by bovine granulosa cells (Spicer and Chamberlain, 2000). Perhaps the inhibitory effect of FSH is needed to prevent premature differentiation (e.g., estrogen production) of granulosa cells during growth of the cohort of follicles that follow the secondary surge of FSH in vivo. Also in agreement with the present study, FSH had no effect on IGFBP-2 or –4 production by bovine granulosa cells cultured in the presence or absence of insulin (Chamberlain and Spicer, 2001). Locally produced IGF-I and IGFBP may play a role in regulating granulosa cell function during FSH-induced ovarian follicular growth in cattle. Further research will be required to determine why the stimulatory effect of FSH on IGFBP-4 production is manifested only in the presence of GH. Understanding factors that regulate follicular growth will lead to the development of better synchronization systems for artificial insemination to maximize reproductive efficiency.



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