Effects of Heparin and Insulin-Like Growth Factor (IGF) Binding Protein-3 on Bovine Ovarian Granulosa and Theca Cell IGF-I Receptors

L.J. Spicer

Story in Brief

The effects of heparin and insulin-like growth factor binding protein-3 (IGFBP-3) on granulosa and theca cell function in cattle were evaluated using a serum-free culture system. Granulosa and theca cells were obtained from large (≥ 8 mm) follicles collected from cattle and cultured for 4 d. During the last 2 d of culture, cells were exposed to heparin and(or) IGFBP-3 in serum-free medium and cells were collected for quantification of numbers of IGF-I receptors and cell numbers were determined. Heparin had no effect on numbers of specific ^{125}I-IGF-I binding sites in granulosa cells whereas heparin decreased numbers of ^{125}I-IGF-I binding sites in theca cells. At 50 ng/ml, IGFBP-3 increased numbers of specific ^{125}I-IGF-I binding sites of both granulosa and theca cells. These results indicate that IGFBP-3 can increase numbers of IGF-I receptors in cultured bovine granulosa and theca cells, whereas heparin decreases IGF-I receptor numbers in theca but not granulosa cells. Additional studies are needed to determine if these factors could alter ovarian follicular growth.

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

Introduction

The growth of dominant ovulatory follicles during the estrous cycle 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. Heparin binding sites exist in bovine granulosa cells and decrease with increasing follicle size (Ax et al., 1984; Bellin et al., 1987). Heparin also induces IGFBP-3 protease activity (Maile et al., 2000) and competes for IGFBP-3 binding to the cell surface (Devi et al., 2000). However, the role of heparin and IGFBPs in regulating IGF-I receptors in the dominant follicle in cattle is unknown. Therefore, we set out to determine the effect of heparin and IGFBP-3 on bovine granulosa and theca cell IGF-I receptor numbers.

Materials and Methods

Ovaries were obtained at a local commercial slaughterhouse from beef and dairy cattle. Granulosa and theca cells from large (≥ 8 mm) follicles were collected and cultured as previously described (Spicer et al., 1994). Briefly, isolated granulosa and theca 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 10 ng/ml of insulin with or without purified bovine heparin (0 or 10 μg/ml) and(or) recombinant human IGFBP-3 (0 or 50

ng/ml). At the termination of each experiment, numbers of cells were determined using a Coulter counter. In parallel cultures, cells were collected for determination of numbers of specific ^{125}I-IGF-I binding sites as previously described (Spicer et al., 1994).

Experimental data are presented as the least squares means ± SEM of measurements from three replicated experiments. In each repeated experiment treatments were replicated two times. Data were analyzed as a 2 x 2 factorial array using analysis of variance procedures. Numbers of IGF-I receptors were expressed as specific binding of ^{125}I-IGF-I in cpm per 10^5 cells.

Results

Because no significant interactions (P>.10) were observed between heparin and IGFBP-3 on numbers of IGF-I receptors in granulosa and theca cells, only main effects are summarized. Alone, 10 μg/ml of heparin had no effect (P>.10) on numbers of ^{125}I-IGF-I binding sites in granulosa cells (Figure 1). In contrast, 10 μg/ml of heparin decreased (P<.001) numbers of ^{125}I-IGF-I binding sites in theca cells (Figure 1). However, 50 ng/ml of IGFBP-3 increased (P<.05) numbers of ^{125}I-IGF-I binding sites in granulosa and theca cells by 26% and 20%, respectively (Figure 2). Granulosa or theca cell numbers were not affected (P>.10) by heparin or IGFBP-3 (data not shown).

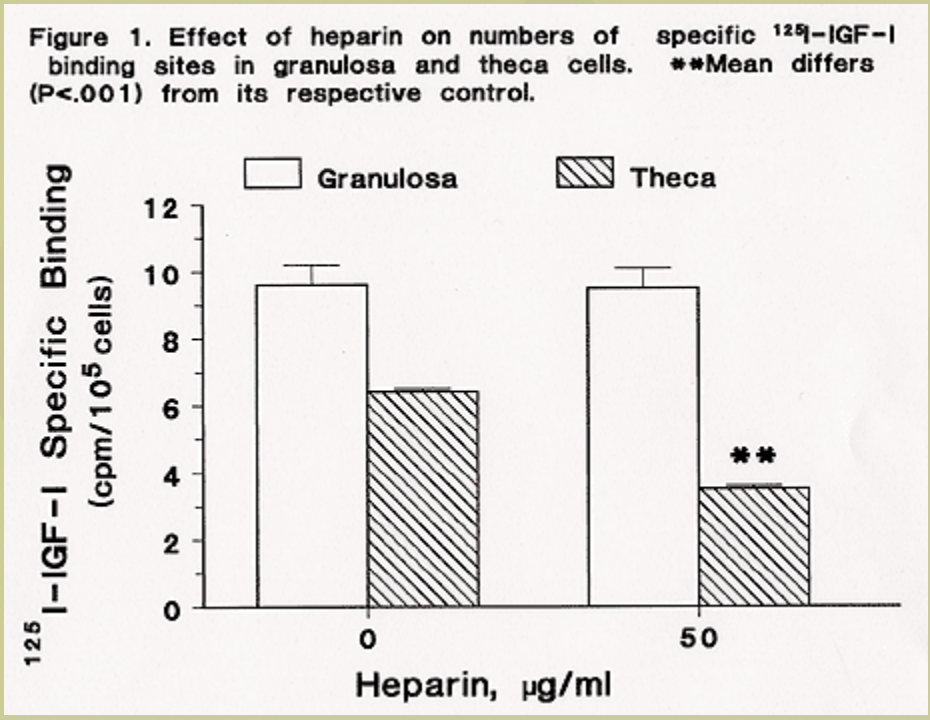

Figure 1. Effect of heparin on numbers of specific ^{125}I-IGF-I binding sites in granulosa and theca cells. **Mean differs (P<.001) from its respective control.

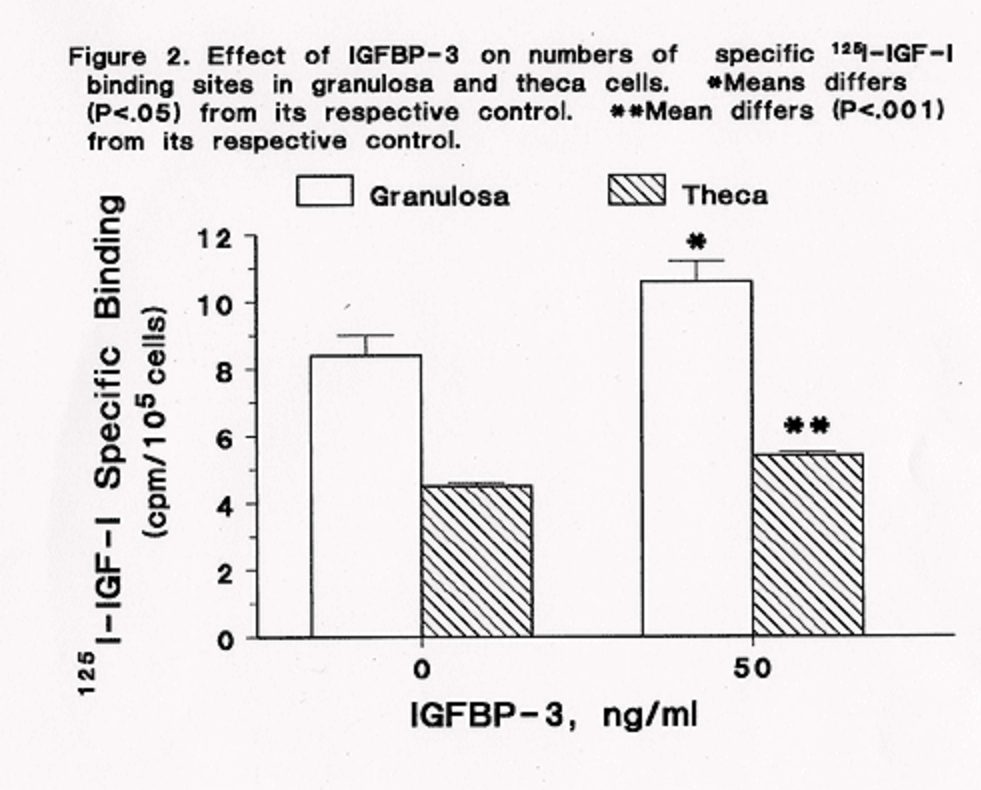

Figure 2. Effect of IGFBP-3 on numbers of specific ^{125}I-IGF-I binding sites in granulosa and theca cells. *Means differs (P<.05) from its respective control. **Mean differs (P<.001) from its respective control.

Discussion

Results of the present study suggest that IGFBP-3, the most abundant IGFBP found in plasma and follicular fluid, can alter numbers of IGF-I receptors in bovine granulosa and theca cells. Previously, we have shown that IGFBP-3 can inhibit IGF-I-induced steroidogenesis and cell numbers in cultured bovine granulosa and theca cells (Spicer et al., 1997; Spicer and Chamberlain, 1999). 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. Results of the present study indicate that heparin reduces the number of IGF-I receptors in theca but not granulosa cells. Because heparin competes for IGFBP-3 binding to the cell surface (Devi et al., 2000) and IGFBP-3 increased specific ^{125}I-IGF-I binding to both granulosa and thecal cells, perhaps the heparin-induced reduction in specific ^{125}I-IGF-I binding to thecal cells was due to direct competition of heparin for IGFBP-3 binding on thecal cells. Further studies will be required to verify this suggestion. Why heparin did not reduce specific ^{125}I-IGF-I binding in granulosa cells is unclear but may be due to lower heparin binding in granulosa cells (Ax et al., 1984). Heparin inhibits FSH-induced estradiol production by bovine granulosa cells (Vernon and Spicer, 1994) and heparin sulfate levels in bovine follicular fluid decrease significantly with increasing estradiol concentrations (Bushmeyer et al., 1985). Further research will be required to determine if exogenous heparin or IGFBP-3 could be used to enhance reproductive efficiency in cattle.

Literature Cited

Ax, R.L. et al. 1984. Endocr. Res. 10:63.

Bellin, M.E. et al. 1987. Biol. Reprod. 37:293.

Bushmeyer, S.M. et al. 1985. Endocrinology 117:879.

Devi, G.R. et al. 2000. Endocrinology 141:4171.

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

Maile, L.A. et al. 2000. Growth Horm. IGF Res. 10:71.

Spicer, L.J. et al. 1994. Mol. Cell. Endocrinol. 102:69.

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

Spicer, L.J. et al. 1997. Biol. Reprod. 56:1458.

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

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

Vernon, R.K. and L.J. Spicer. 1994. J. Anim. Sci. 72:2696.

Acknowledgements

The authors thank Wellington Quality Meats (Wellington, KS) for their generous donations of bovine ovaries, and B.E. Keefer for technical assistance.

[2001 Research Report | Animal Science Research Reports | Animal Science]