Effect of Insulin-like Growth Factor (IGF)-I and Follicle-Stimulating Hormone (FSH) on Estradiol Production by Equine Ovarian Granulosa Cells

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Story in Brief

The objectives of this study were to determine if duration of culture and the hormones, insulin-like growth factor-I (IGF-I) and/or FSH, affect estradiol production by equine granulosa cells. Granulosa cells from small (6 to 15 mm) follicles were collected from cyclic mares and cultured 2 days in medium containing 10% fetal calf serum, washed, and then treated for an additional 1 or 2 days in serum-free medium with or without added hormones. IGF-I increased estradiol production by granulosa cells by about fivefold after 1 day of treatment and by 30-fold after 2 days of treatment, whereas FSH had little or no effect. These results indicate that IGF-I may be a more important regulator of aromatase activity in cultured equine granulosa cells than FSH.

Key Words: Granulosa Cells, Mare, Estradiol, Aromatase, Cell Culture

Introduction

The insulin-like growth factor (IGF) system, composed of IGF-I, IGF-II, IGF receptors and IGF-binding proteins (IGFBPs), plays an essential role in ovarian function (for reviews see Hammond et al., 1991; Giudice, 1992; Spicer and Echternkamp, 1995). In most species, IGF-I stimulates granulosa and thecal cell proliferation and mitogenesis, and synergizes with gonadotropins to stimulate granulosa and thecal cell steroidogenesis.

In the mare, one follicle is selected from a cohort of follicles to become dominant. After selection the dominant follicle continues to grow until ovulation, while the remaining cohort, or subordinate follicles become atretic and regress (for review see Ginther, 2000). Limited information is available regarding the physiological mechanism of follicle selection and maturation in the mare. During preovulatory development in the mare, follicular fluid IGF-I levels increase (Spicer et al., 1991; Davidson et al., 2002). In cattle, IGF-I and FSH synergizes to enhance estradiol production by granulosa cells during follicular development (Spicer et al., 2002). However, the role of IGF-I and FSH in regulating aromatase activity in granulosa cells of the mare is unclear. Therefore, the specific objective of this experiment was to determine whether IGF-I and/or FSH affect aromatase activity by equine granulosa cells.

Materials and Methods

The reagents used were as follows: Dulbecco's modified Eagle medium (DMEM), Ham's F-12, sodium bicarbonate, gentamicin, trypan blue, and fetal calf serum (FCS) all obtained from Sigma Chemical Company (St, Louis, MO.); ovine FSH (FSH activity 94 x NIH-oFSH-S1 U/mg) obtained from USDA Animal Hormone Program (Beltsville, MD); and recombinant human IGF-I obtained from R & D Systems (Minneapolis, MN).

In early July, 2002 ovaries were obtained at a commercial abattoir from six mares of various breeds and ages. The mares were classified as cyclic based on gross ovarian morphology and the ovaries were processed as previously described (Davidson et al., 2002). Granulosa cells from individual small follicles (6-15 mm) were collected and combined to form three pools of cells as previously described (Davidson et al., 2002). Granulosa cells were washed twice in serum-free medium by centrifugation at 200 X g (for 5 to 7 min) and resuspended in medium containing 1 mg/mL collagenase and 0.01 mg/mL DNase to disperse and prevent clumping of the cells. The number and viability of granulosa cells was determined using a hemocytometer and the trypan blue exclusion method, and averaged 71.5% of total granulosa cells.

Medium consisted of a 1:1 (vol/vol) mixture of DMEM and Ham's F-12 containing gentamicin, glutamine and sodium bicarbonate. Approximately 4 X 10^5 viable cells in 30 µL of medium were added to Falcon 24-well plates (No. 3047; Becton Dickinson and Co., Lincoln Park, NJ) containing 1 mL of medium with 10% FCS. Cultures were kept at 38.5°C in a 95% air-5% CO_2 atmosphere (Davidson et al., 2002). To obtain optimal attachment, cells were maintained in 10% FCS without added hormones for the first 2 d of culture. After 48 h, cells were washed twice with 0.5 mL of serum-free medium to remove FCS and non-adherent cells, and incubations continued in serum-free medium (0.5 mL) containing 500 ng/mL of testosterone (as an estradiol precursor) and 2.5 mg/mL of BSA with or without added hormones for an additional 48 h. Throughout the 4-d culture, medium was changed every 24 h. After the initial 48 h culture with 10% FCS, the medium was replaced with serum-free medium containing testosterone and either no hormone addition (control), IGF-I (0 or 100 ng/mL), FSH (0, 3 or 30 ng/mL), or IGF-I (100 ng/mL) plus FSH (0, 3, or 30 ng/mL) and incubated for an additional 48 h. Each treatment was applied to four replicate wells for each pool of cells. At the end of the first 24 h incubation with treatments (i.e., during d 2 to 3 of culture) medium was collected for estradiol measurement and fresh medium was added. Concentrations of estradiol in culture medium collected 24 h and 48 h after hormone treatments were determined with a double-antibody radioimmunoassay as previously described (Davidson et al., 2002). Numbers of granulosa cells were determined at the termination of experiments (i.e., d 4 of culture) using a Coulter counter (Model Zm; Coulter Electronics, Hialeah, FL) as previously described (Davidson et al., 2002). Data were analyzed using PROC MIXED of SAS with IGF-I and FSH and their interaction as main effects. Multiple mean comparisons (LSD) were performed only if a main effect was significant.

Results

After 24 h of treatment, IGF-I stimulated ($P<0.05$) granulosa cell estradiol production by 4.6- to 5.3-fold (Figure 1). After 48 h of treatment, IGF-I stimulated estradiol production by 27- to 39-fold (Figure 1). FSH treatment alone had no effect ($P>0.10$) on estradiol production by granulosa cells. The IGF-I-induced increase in estradiol production was significantly greater in the presence of 30 vs 3 ng/ml of FSH (Figure 1). Basal estradiol production decreased 52% between 24 h and 48 h of treatment, whereas IGF-I-stimulated estradiol production increased 2.6- to 4.5-fold between 24 h and 48 h of treatment (Figure 1).

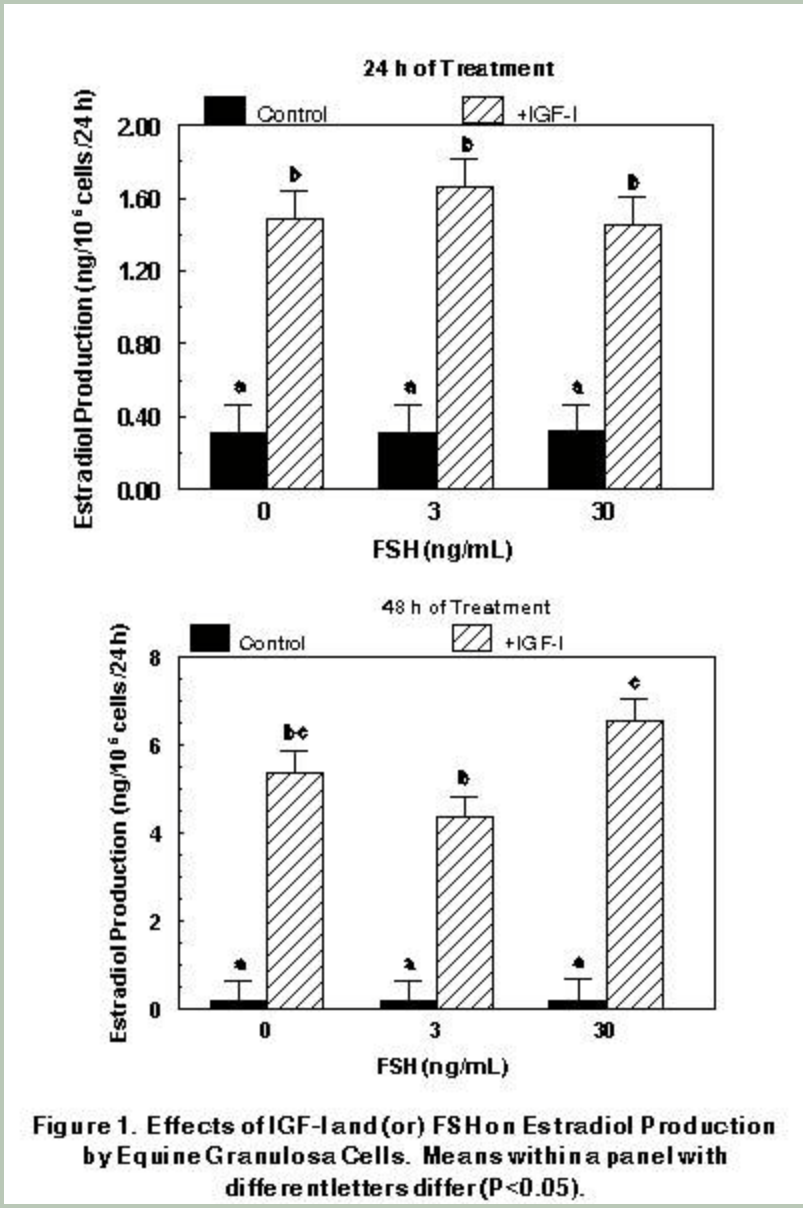

Figure 1. Effects of IGF-I and (or) FSH on Estradiol Production by Equine Granulosa Cells. Means within a panel with different letters differ (P<0.05).

Discussion

The present study revealed that estradiol production by equine granulosa cells was differentially regulated by IGF-I and FSH. We found that IGF-I dramatically increased estradiol production in small-follicle (6 to 15 mm) granulosa cells whereas FSH had little or no effect. Similarly, insulin stimulates estradiol production by small-follicle equine granulosa cells (Davidson et al., 2002). In cattle, IGF-I and FSH synergized to stimulate estradiol production by granulosa cells (Spicer and Chamberlain, 1998; Spicer et al., 2002). FSH alone did not affect estradiol production by small-follicle granulosa cells in the present and a previous study (Davidson et al., 2002). However, in the presence of IGF-I, 30 ng/ml of FSH enhanced the stimulatory effect of IGF-I on estradiol production by equine granulosa cells as compared to 3 ng/ml of FSH. Sirois et al. (1991) reported that in the presence of insulin, FSH was unable to stimulate estradiol production in large equine follicles during the early estrous phase but was able to significantly stimulate

progesterone production by granulosa cells from large (early and late estrus stage) follicles. Also, equine granulosa cell responsiveness to FSH in terms of estradiol and progesterone production decreased as follicle diameter increased in early to late estrus follicles (Davidson et al., 2002). The present study indicates that IGF-I plays a more important role than FSH in the regulation of aromatase activity in equine granulosa cells.

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Acknowledgments

The authors gratefully acknowledge Bel-Tex (Ft. Worth, TX) for their generous donation of equine ovaries; N. R. Mason (Lilly Research Laboratories, Indianapolis, IN) for the generous donation of estradiol antiserum.
