

INTERLEUKIN-2 AFFECTS STEROIDOGENESIS OF BOVINE OVARIAN GRANULOSA CELLS BUT NOT THECAL CELLS IN VITRO

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Story in Brief

The effects of recombinant bovine interleukin-2 (IL-2) on steroidogenesis of bovine ovarian granulosa and thecal cells were evaluated. Granulosa cells were examined from both small (surface diameter ≤ 5 mm) and large (≥ 8 mm) follicles, whereas thecal cells from only large follicles were utilized. IL-2 significantly attenuated FSH-induced estradiol production by cells from small but not large follicles. Moreover, IL-2 significantly attenuated FSH-induced progesterone production by granulosa cells from small and large follicles but had no effect on LH-induced progesterone or androstenedione production by thecal cells.

(Key Words: Interleukin-2, Ovary, Granulosa Cells, Thecal Cells, Steroidogenesis.)

Introduction

Interleukin-2 (IL-2) was one of the first hormones recognized in the immune system as having a role in T-cell growth and its secretion in the systemic circulation is increased during disease (for review see: Blecha, 1991; Minami et al., 1993). Recent studies suggest that IL-2 may be used as adjunct therapy with existing vaccines (Campos et al., 1993) and mastitis antibiotics (Daley et al., 1992) to improve therapeutic efficiency as well as a general immunostimulant when drying off dairy cows (Nickerson et al., 1992). Whether these treatments will alter reproductive functions is unknown. In spite of the fact that other cytokines such as tumor necrosis factor- α (TNF α) seem to affect ovarian cell function (Brannstrom and Norman, 1993), the role of IL-2 in ovarian steroidogenesis is practically unexplored. Hence, the objectives of our studies were to determine if IL-2 can directly affect ovarian function in cattle by evaluating the effects of IL-2 on steroidogenesis of granulosa and thecal cells in vitro. These studies may also provide useful insights as to the mechanism whereby disease alters reproductive efficiency.

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Materials and Methods

Ovaries of beef and dairy cattle obtained at slaughter from a nearby abattoir were brought to the laboratory on ice (within 120 min) and processed as previously described for obtaining granulosa cells (Alpizar and Spicer, 1994) and thecal cells (Roberts and Skinner, 1990). A cell suspension containing 1 to 3×10^5 viable cells, as ascertained by trypan blue exclusion method, was seeded in each well containing one ml of medium (Dulbecco's Modified Eagles Medium: Ham's F12 Medium, 1:1). Cultures of both granulosa and thecal cells were incubated at 38.5°C in a 5% CO₂ atmosphere, and the medium was changed every 22 to 26 h as described by Langhout et al. (1991). To obtain optimal attachment, cells were maintained in the presence of 10% FCS for the first 2 d of culture. After this time, cells were washed and incubations continued for 1 or 2 d in serum-free medium with or without added hormones, unless stated otherwise. For studies evaluating the effects of IL-2 on granulosa cells, cells were treated with 50 ng/ml of FSH, 1 µg/ml of insulin and 1 µg/ml of testosterone with or without .1, 1.0, 10 and 30 ng/ml of IL-2 for 1 d (i.e., from d 2 to 3 of culture). For studies evaluating the effects of IL-2 on thecal androstenedione and progesterone production, thecal cells were treated with 100 ng/ml of LH and 1 µg/ml of insulin with or without 0.1, 1.0, 10, or 30 ng/ml of IL-2 for 1 d (i.e., from d 2 to 3 of culture). At the termination of each experiment, the granulosa and thecal cells were counted with a Coulter counter as previously described (Langhout et al., 1991).

Functional aromatase activity was assessed during a 24-h exposure of granulosa cells to 1 µg/ml of testosterone as previously described (Spicer and Alpizar, 1994). The production of estradiol and(or) progesterone by the granulosa and thecal cells was assessed by radioimmunoassay. The androstenedione was quantified by solid-phase RIA kits.

Experimental data are presented as the least squares means \pm SE of measurements from replicated experiments. Each experiment was replicated three times with three replicates per treatment within an experiment.

Results

IL-2 inhibited FSH-induced estradiol production by granulosa cells from small follicles, whereas IL-2 had no significant effect on FSH-induced estradiol production by cells from large follicles (Figure 1). At 30 ng/ml of IL-2, FSH-stimulated estradiol production was 53% of control values in cultures of small-follicle granulosa cells.

IL-2 significantly reduced FSH-induced granulosa cell progesterone production by 24% and 43%, in cells from small and large follicles, respectively (Figure 2). In contrast, IL-2 had no effect on LH-induced

progesterone production by thecal cells which averaged 23 to 29 nanograms of progesterone per 10^6 cells (data not shown).

IL-2 had no significant effect on LH-induced androstenedione production by thecal cells, and averaged 131 to 147 picograms of androstenedione per 10^5 cells (data not shown).

Discussion

An inhibitory effect of recombinant bovine IL-2 (47% inhibition with 30 ng/ml of IL-2) on FSH-induced estradiol production by granulosa cells of small follicles in the bovine has been supported by studies of Spicer and Alpizar (1994), who found that addition of 100 ng/ml of recombinant human IL-2 brought about a 45% reduction in FSH-induced estradiol production by granulosa cells from small follicles; granulosa cells from large follicles in both reports showed no significant response to IL-2 on FSH-induced estradiol production. Thus, in spite of the fact that the amino acid sequence of bovine IL-2 shares only 65% homology with that of human IL-2 (Cerretti et al., 1986), similar effects were observed. The absence of an interaction between dosage and experiment indicates that the trend of inhibition was similar in all experiments conducted. In comparison, Adashi et al. (1989) and Kasson and Gorospe (1989) found that recombinant human IL-2 had no effect on FSH-induced estradiol production by rat granulosa cells. Similarly, Fukuoka et al. (1992) observed that human IL-2 had no significant effect on hCG-stimulated estradiol production by luteinized human granulosa cells. Collectively, these results indicate that IL-2 does not affect aromatase activity of highly differentiated granulosa cells from cattle and humans. Why IL-2 inhibits estradiol production by undifferentiated granulosa cells of cattle and not rats remains to be determined, but may be due in part to the fact that rat and human IL-2 share only a 50% amino acid homology (Cerretti et al., 1986). Similar to these differential effects of IL-2 on cells from small and large follicles, we have previously shown that granulosa cells from large bovine follicles are 40-times less sensitive to IL-6 than cells from small bovine follicles (Alpizar and Spicer, 1994).

An inhibitory effect of IL-2 on FSH-induced progesterone production by bovine granulosa cells in a dose-dependent manner is reported here for the first time. Similarly, a dose-dependent inhibitory effect of IL-2 on hCG-stimulated progesterone production by human granulosa cells has been reported by Wang et al. (1991), whereas in comparable studies, Fukuoka et al. (1992) found no effect of IL-2. In comparison, IL-2 has been shown to either stimulate (Kasson and Gorospe, 1989) or have no effect (Gottschall et al., 1988) on FSH-induced progesterone production by rat granulosa cells. Additional studies are required to clarify whether species differences in IL-2 structure account for these

variable effects of human IL-2 on rat granulosa cell progesterone production, since, as mentioned, rat and human IL-2 share only a 50% amino acid homology (Cerretti et al., 1986).

We observed that IL-2 had no significant effect on LH-induced androstenedione or progesterone production by thecal cells, and to our knowledge this is a new finding. Previous studies have not evaluated the effect of IL-2 on thecal androgen production, but IL-1 has been shown to inhibit hCG-induced androgen production by rat whole ovarian homogenates (Hurwitz et al., 1991). In cultured intact preovulatory rat follicles, recombinant human IL-2 had no effect on androstenedione accumulation (Brannstrom et al., 1993). Thus, it appears that the steroidogenic effect of IL-2 on bovine ovarian follicles is specific to the membrana granulosa. Whether IL-2 affects thecal cell steroidogenesis in other species remains to be determined.

In summary, the inhibitory effect of IL-2 on ovarian follicular steroidogenesis in cattle is confined to granulosa cells. The physiologic relevance of such a specific ovarian effect of IL-2 is unclear, since the levels of IL-2 in bovine follicular fluid and serum are unknown. Nonetheless, IL-2 may play a role in regulating follicular function during early stages of normal follicular growth as well as during disease states where systemic IL-2 may be elevated.

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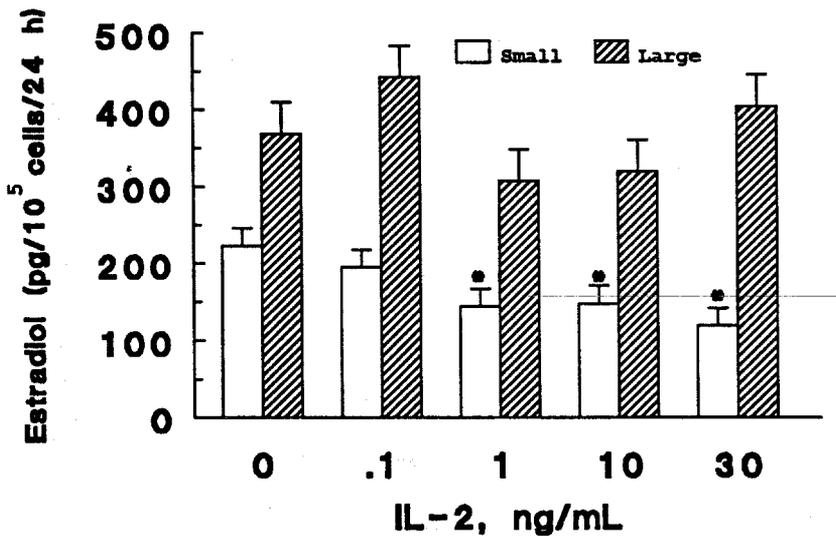


Figure 1. Effect of IL-2 on FSH-induced estradiol production (pg/10⁵ cells/24 h) by granulosa cells from small and large follicles. Granulosa cells were cultured for 2 d in the presence of 10% FCS as described in Materials and Methods and then treated with 50 ng/ml of FSH, 1 μ g/ml of insulin, and 1 μ g/ml of testosterone with or without the various doses of IL-2 for an additional 24 h. Values are means from four separate experiments for small follicles and three separate experiments for large follicles. Estradiol production in the absence of FSH but in the presence of insulin averaged 76 ± 23 and 299 ± 53 pg/10⁵ cells/24 h for cells from small and large follicles, respectively. *Within follicle size, mean differs ($P < .05$) from controls without IL-2.

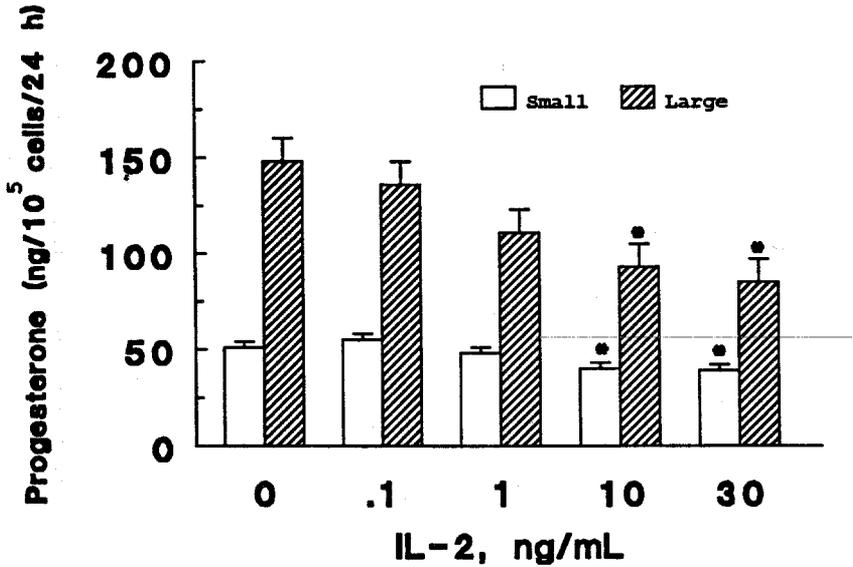


Figure 2. Effect of IL-2 on FSH-induced progesterone production (ng/10⁵ cells/24 h) by granulosa cells from small and large follicles. Cells were cultured as described for Figure 1. Progesterone production in the absence of FSH but in the presence of insulin averaged 40 ± 3 and 106 ± 12 ng/10⁵ cells/24 h for cells from small and large follicles, respectively. *Within follicle size, mean differs (P < .05) from controls without IL-2.