# Effects of Leptin on Bovine Ovarian Granulosa and Theca Cell Steroidogenesis

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## Story in Brief

The effect of leptin on granulosa and theca cell steroidogenesis in cattle was evaluated using an in vitro system. Granulosa and theca cells were obtained from small (1-5 mm) and large (³ 8 mm) follicles of cattle, respectively, and cultured for 3 or 4 d. During the last 1 or 2 d of culture, cells were exposed to leptin and various doses of insulin in serum-free medium. Media were collected for quantification of steroid production and cell numbers were determined. Leptin had no effect on basal steroid production in cultures of granulosa or theca cells. However, at 10 ng/ml, leptin decreased insulin-stimulated steroid production by both granulosa and theca cells. These results indicate that leptin can directly inhibit steroidogenesis of bovine granulosa and theca cells. Additional studies are needed to determine if changes in systemic leptin concentrations could alter ovarian follicular growth.

Key Words: Leptin, Insulin, Steroidogenesis, 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). Insulin and gonadotropins are thought to play important roles in regulating the development of dominant follicles (Spicer and Echternkamp, 1995). Recently, leptin, a hormone secreted by adipocytes, has been suggested to be a possible metabolic mediator of reproduction (Spicer, 2001). Leptin binding sites exist in bovine granulosa and thecal cells (Spicer and Francisco, 1997; 1998). Leptin also inhibits steroid production by granulosa and theca cells (Spicer and Francisco, 1997, 1998). However, the effect of leptin on the dose-response to insulin on ovarian steroid production in cattle is unknown. Therefore, we set out to determine the effect of leptin on bovine granulosa and theca cell steroidogenesis induced by insulin.

## Materials and Methods

Ovaries were obtained at a local commercial slaughterhouse from beef and dairy cattle. Granulosa cells from small (1 to 5 mm) follicles and theca cells from large (³ 8 mm) follicles were collected and cultured as previously described (Spicer et al., 2000). 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 24 h (granulosa cells) or 48 h (thecal cells) in serum-free medium containing insulin (0, 10 or 100 ng/ml) with or without recombinant mouse leptin (10 ng/ml) and(or) purified bovine LH (0 or 100 ng/ml; theca cells) or ovine FSH (0 or 50 ng/ml; granulosa cells). Medium was changed every 24 h. For granulosa

cells, medium also contained 500 ng/ml of testosterone as an estradiol precursor. At the termination of each experiment, numbers of cells were determined using a Coulter counter. Medium was collected for quantification of estradiol or androstenedione as previously described (Spicer and Francisco, 1997; 1998).

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. Data were analyzed as a 2 x 3 factorial array using analysis of variance procedures. Steroid production data were expressed as pg of steroid produced per $10^5$ cells per 24 h.

## Results

In the absence of insulin, leptin had no effect (P>.10) on estradiol production by granulosa cells (Figure 1) or androstenedione production by theca cells (Figure 2). In contrast, 10 ng/ml of leptin reduced insulin (100 ng/ml)-induced granulosa estradiol production (P<.05) by 35% (Figure 1) and theca androstenedione production (P<.001) by 62.5% (Figure 2). Similarly, in the presence of 10 ng/ml of insulin, leptin (10 ng/ml) reduced granulosa estradiol production (P<.10) by 38% (Figure 1) and theca androstenedione production (P<.001) by 55.5% (Figure 2). In the absence of leptin, 100 ng/ml of insulin stimulated (P<.05) estradiol production by 6.4-fold and androstenedione production by 6.0-fold.

**Figure 1. Effect of 1-d treatment of 10 ng/ml of leptin on insulin-induced steroid production by granulosa cells from small bovine follicles. [a,b,c]Means without a common superscript differ (P<.05)**

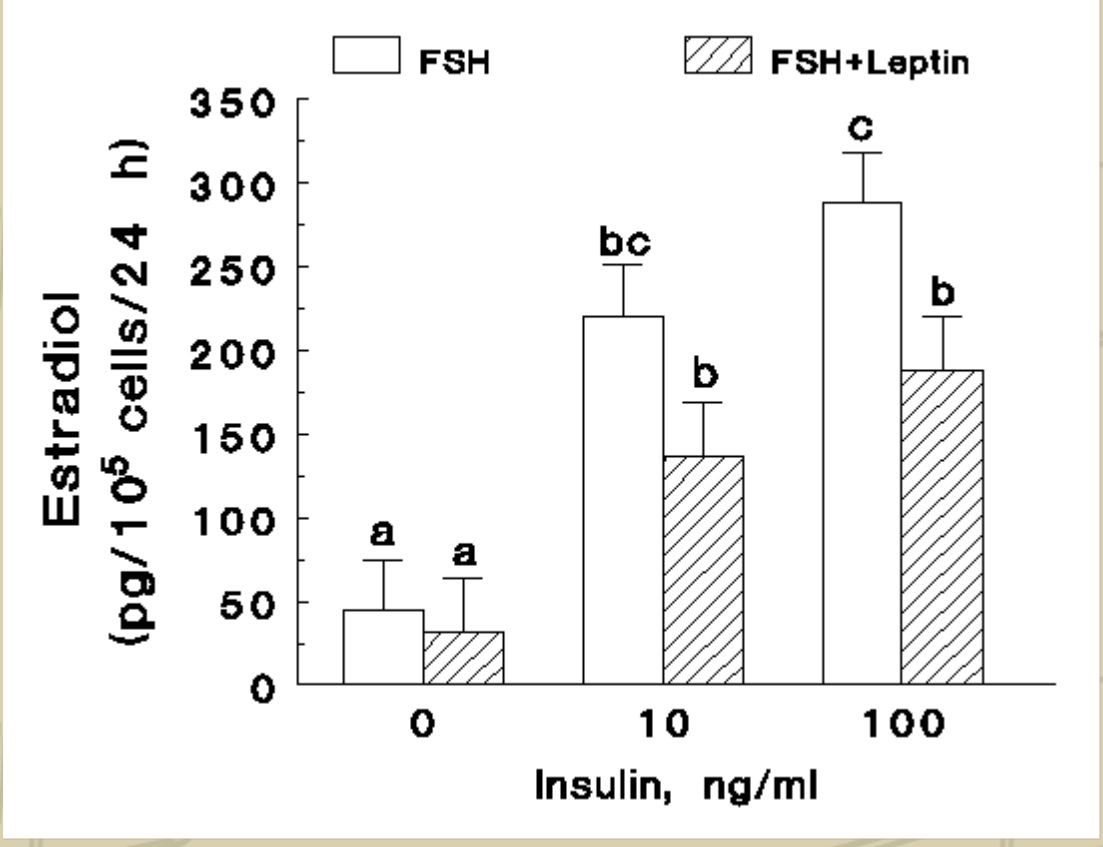
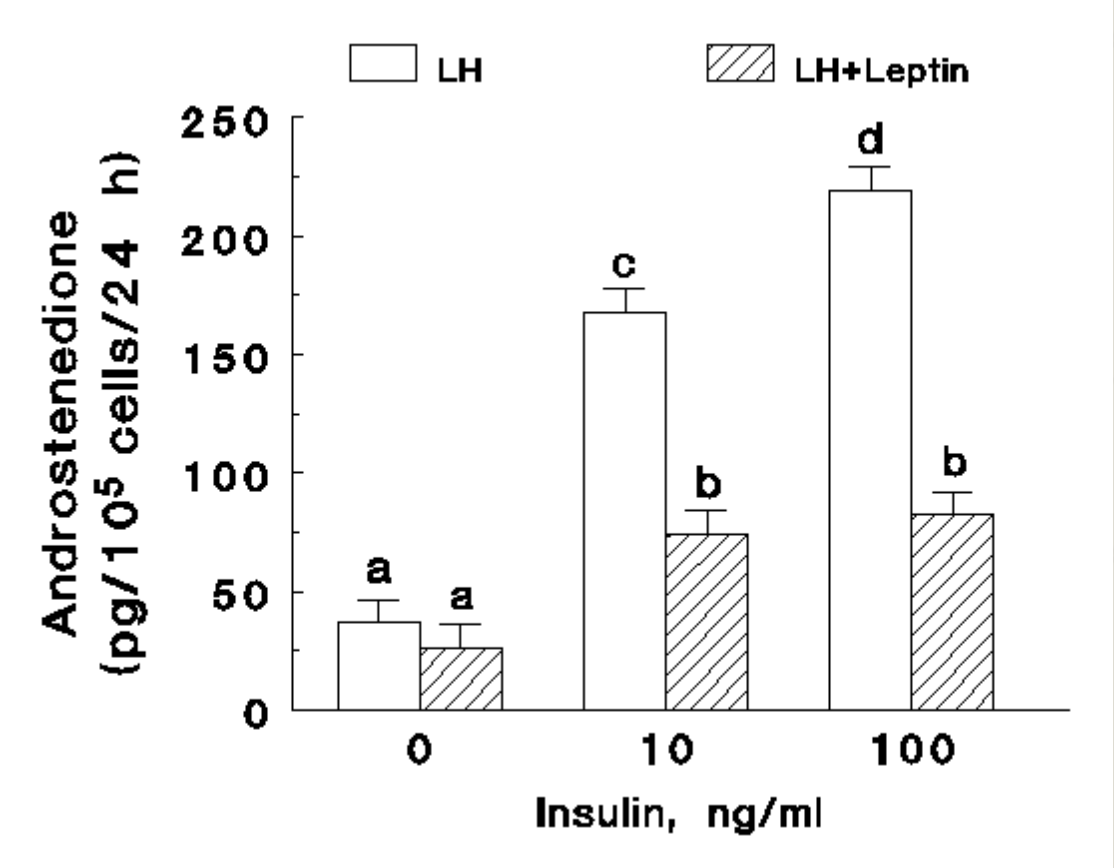


**Figure 2. Effect of 2-d treatment of 10 ng/ml leptin on androstenedione production by theca cells from large bovine follicles. [a,b,c,d]Means without a common superscript differ (P<.05)**




## Discussion

Results of the present study suggest that leptin, an adipocyte hormone found in plasma and follicular fluid, can inhibit granulosa and theca cell steroidogenesis induced by low (10 ng/ml) and high (100 ng/ml) concentrations of insulin. Previously, we have shown that leptin can inhibit steroidogenesis induced by a high dose of insulin in cultured bovine granulosa and theca cells (Spicer and Francisco, 1997; 1998). Thus, leptin may reduce estradiol production by ovarian follicles through a reduction in aromatizable androgen precursors from theca cells as well as through a reduction in aromatase activity in granulosa cells. Results of the present study also indicate that leptin does not alter gonadotropin-induced steroid production by theca and granulosa cells in the absence of insulin. Leptin inhibits IGF-I plus FSH-induced estradiol production by rat (Zachow and Magoffin, 1997) and human (Agarwal et al., 1999) granulosa cells, but not in bovine granulosa cells (Spicer et al., 2000). Further studies will be required to elucidate the hormonal specificity of leptin's inhibitory action within the bovine, human and rat ovary. In addition, further research will be required to determine if exogenous leptin or insulin could be used to alter reproductive efficiency in cattle.

## Literature Cited

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