Effect of Resistin on Granulosa and Theca Cell Function in Cattle

D.V. Lagaly, P.Y. Aad, L.B. Hulsey, J.A. Grado-Ahuir and L.J. Spicer

Story in Brief

Resistin is an adipokine that has not been extensively studied in cattle, but has been implicated in insulin resistance, a condition associated with polycystic ovarian syndrome in humans. Determining the role of resistin in ovarian follicular function could help determine the reasons for cystic ovarian disease in cattle, a condition that causes large economic losses in the dairy industry. Experiments were conducted to determine the effect of resistin on steroidogenesis of theca and granulosa cells. Dose-response studies revealed that resistin had no effect on androstenedione production of large follicle theca cells, whereas resistin attenuated the stimulatory effect of IGF-I on progesterone and estradiol production of small follicle granulosa cells. These results indicate that resistin may preferentially inhibit steroidogenesis of granulosa cells.

Key words: Granulosa Cell, Resistin, Steroidogenesis, Theca Cell

Introduction

Reproductive efficiency in dairy cattle has been decreasing over the past few decades despite better management and feeding practices. Much of this reproductive failure is due to ovarian cystic disease. It is estimated that 5% to 20% of dairy cattle get ovarian follicular cysts (Gaverick, 1997; Peter, 2004). These cysts are defined as follicles which reach a size of at least 25 mm and fail to ovulate (Vanholder et al., 2006). The exact cause of this condition has not been identified; however, because the estrous cycle is controlled by hormones, it is likely that hormone imbalances are the cause of this condition (Gaverick, 1997; Peter, 2004; Vanholder et al., 2006).

Polycystic ovarian syndrome (PCOS) is a metabolic disorder in humans which is linked to insulin resistance and obesity, and is characterized by anovulation, hyperandrogenism and hyperinsulinaemia (Panidis et al., 2004; Yilmaz et al., 2005; Escobar-Morreale et al., 2006). Recent studies indicate that resistin, a newly discovered adipokine (Mitchell et al., 2005), may have a role in PCOS (Panidis et al., 2004; Munir et al., 2005). One study reported that levels of circulating serum resistin increased by 40% in women with PCOS and levels of serum resistin were positively correlated to serum testosterone levels (Munir et al., 2005). Another study reported greater resistin mRNA abundance in adipose tissue of PCOS vs. control women although serum resistin levels did not differ (Seow et al., 2007). When insulin response data were compared between dairy cows exhibiting cystic ovarian disease and cows with normal ovulatory cycles, it was observed that cows with ovarian cysts had a significantly lower insulin response to a glucose load (Opsomer et al., 1999). Insulin and insulin like growth factors (IGF) are known to play important roles in the follicular development (Spicer and Echternkamp, 1995). Therefore, the aims of this study were to determine the effects of resistin on steroid production in ovarian theca and granulosa cells.

Materials and Methods

Cell Culture. Ovaries from non-pregnant beef cows were collected from a local slaughterhouse, and based on surface diameter, theca and granulosa cells were collected from small (3 to 6 mm) and large (8 to 22 mm) follicles as previously described (Langhout et al., 1991; Stewart et al., 1995; Spicer and Chamberlain, 1998). Large follicles were bisected after aspiration of follicular fluid and granulosa cells separated from the theca interna via blunt dissection, and the theca interna was torn into small pieces, rinsed with basal serum-free medium (l:l Dulbecco's modified Eagle's medium and Ham's F12) and enzymatically digested for 1 h at 37°C as previously described (Stewart et al., 1995; Spicer and Chamberlain, 1998; Aad et al., 2006). Briefly, the non-digested tissue was filtered out through sterile syringe filter holders with metal screens of 149 μm mesh (Gelman, Ann Arbor, MI). Theca cells were then centrifuged at 50 x g for 5 min, supernatant discarded and the pellet washed with serum-free medium. The purity of theca cells prepared this way was >90% (Spicer and Stewart, 1996). Theca cells were re-suspended in serum-free medium containing collagenase and DNase I (Sigma Chemical Co.) at 1.25 mg/mL and 0.5 mg/mL, respectively, to prevent cell clumping prior to plating.

Approximately 2.0 x 10^5 viable cells were plated on 24-well Falcon multiwell plates (Becton Dickinson, Lincoln Park, NJ, USA) in a basal medium containing 10% FCS, 0.12 mM gentamycin, 2.0 mM glutamine, and 38.5 mM sodium bicarbonate (all obtained from Sigma Chemical Co.). Cells were cultured at 38.5°C in 10% FCS for the first 48 h with a medium change at 24 h. Cells were then washed twice with serum-free medium and various treatments (see below) applied in serum-free medium for 48 h with a medium change at 24 h. Depending on experiment, medium was collected for steroid radioimmunoassay (RIA) and cells were collected for cell enumeration (see below).

The hormones and reagents used in cell culture were: ovine LH (activity: 1.0 X NIH-LH-S1 U/mg) and ovine FSH (NIDDK-oFSH-20; activity: 175 X NIH-FSH-S1 U/mg) from the National Hormone and Pituitary Program (Torrance, CA), recombinant human IGF-I and recombinant mouse resistin from R&D Systems (Minneapolis, MN), testosterone from Steraloids (Wilton NH), and insulin (28 U/mg) and fetal calf serum (FCS) from Sigma Chemical Co. (St. Louis, MO).

Experimental Design. Experiment 1 was designed to determine the dose response of resistin on steroidogenesis of large-follicle theca cells. Theca cells were collected into separate pools, each containing cells from follicles of at least five individual cows. Cells were cultured for 48 h in 10% FCS, washed twice with serum-free medium as described earlier. Cells were treated with medium containing 0, 3, 10, or 100 ng/mL resistin in addition to IGF-I (30 ng/mL) and LH (30 ng/mL) for 48 h. Cells were counted and RIA was conducted on medium samples to determine treatment effects on progesterone and androstenedione production (see below).

Experiment 2 was designed to evaluate the dose response of resistin in the presence or absence of IGF-I on proliferation and steroidogenesis of small-follicle granulosa cells. Granulosa cells were collected into three pools, each containing cells from follicles of at least five individual cows. Cells were cultured for 48 h in 10% FCS, washed twice with serum-free medium as described earlier, and resistin (0, 10, 30 or 100 ng/mL) was applied for 48 h in the presence of testosterone (500 ng/mL; as an estrogen precursor) and FSH (30 ng/mL) with or without IGF-I (30 ng/mL).

Cells were counted and RIA conducted on medium samples to determine treatment effects on progesterone and estradiol production (see below). The type and concentrations of hormones used in these experiments were selected based on previous studies (Spicer and Chamberlain, 1998; Spicer and Aad, 2007).

Determination of Steroid Concentrations and Cell Numbers. Medium was collected from individual wells and frozen at –20°C for subsequent hormone analyses. Concentrations of progesterone, estradiol and (or) androstenedione in culture medium were determined by RIA as previously described (Langhout et al., 1991; Stewart et al., 1995; Spicer and Chamberlain, 1998).

Numbers of cells in the same wells that medium was collected were determined via Coulter counting (model Zm; Coulter Electronics, Hialeah, FL, USA) as previously described (Langhout et al., 1991; Stewart et al., 1995) and used to calculate steroid production on a ng or pg per 10^5 cell basis.

Statistical Analysis. Data are presented as the least squares means (± S.E.) of measurements from culture wells. Steroid production was expressed as ng or pg/10^5 cells per 24 h, and cell numbers at the termination of the experiment were used for this calculation. Specific differences in steroid production among treatments were determined using GLM procedure of SAS (Statistical Analysis Systems, Cary, NC) and Fisher's protected least significant difference procedure (Ott, 1977).

Results and Discussion

Experiment 1: Dose response of resistin on large-follicle theca cells. Resistin increased ($P<.05$) progesterone production at a dose of 10 ng/mL but 3 and 100 ng/mL were without effect ($P>.10$; Fig. 1A). None of the doses of resistin affected androstenedione production (Fig. 1B).

The up-regulation of progesterone production without a corresponding change in androstenedione production in Experiment 1 suggests that resistin may up-regulate cholesterol side-chain cleavage enzyme (CYP11A1) activity without affecting 17α-hydroxylase (CYP17A1) in theca cells. One previous study reported that resistin (at 10 ng/mL alone) did not affect basal CYP17A1 gene expression in cultured human theca cells, but did effect expression of CYP17A1 in combination with forskolin and forskolin plus insulin (Munir et al., 2005), suggesting that the ovarian response system to resistin may be induced by cAMP- dependent mechanisms. Because androstenedione production was unaltered by resistin in LH-treated bovine theca cells, perhaps species differences exist with regard to specific ovarian effects of resistin. Further studies will be required to verify these suggestions.

Experiment 2: Dose response of resistin on IGF-I-induced steroidogenesis of small follicle granulosa cells. IGF-I increased ($P<.05$) steroidogenesis (Fig. 2) of granulosa cells. Concomitant treatment with resistin at 10, 30 and 100 ng/mL decreased IGF-I- induced progesterone production by 29%, 28% and 41%, respectively (Fig. 2A). Resistin at 10, 30 and 100 ng/mL inhibited IGF-I-induced estradiol production by 34%, 29% and 55%, respectively (Fig. 2B). In the absence of IGF-I, resistin had no significant effect on progesterone production but weakly decreased estradiol production (Fig. 2).

Based on results of the present study, resistin most likely regulates the follicular function primarily through the granulosa cell and not the theca cell in cattle. Moreover, the present experiments showed that physiological concentrations of resistin (i.e., 10-30 ng/mL) were bioactive in granulosa cell cultures. Although not known for cattle, plasma levels of resistin in rats and women average 5-46 ng/mL (Caja et al., 2005; Papadopoulos et al., 2005; Majuri et al., 2007). At this concentration range, resistin had moderate inhibitory effects on granulosa cell steroidogenesis, suggesting a possible regulatory role in follicular development, especially estradiol production. These experiments indicate for the first time that resistin regulates steroidogenesis in granulosa cells to a greater extent than in theca cells. Since resistin effects on ovarian cells have not been extensively studied, these experiments provide additional support for a role of resistin in follicular development. Why resistin inhibits estradiol production from small follicles but stimulates progesterone production from large-follicle theca cells is unclear, but the results imply that the response system to resistin may shift from inhibitory to stimulatory as the follicle develops. Results of the present study are consistent with the hypothesis that increased resistin may contribute to development of cystic follicles via preferentially increasing progesterone production by theca cells and inhibiting estradiol production by granulosa cells. Further research will be required to determine the mechanism by which the response of resistin differs between theca and granulosa cells.

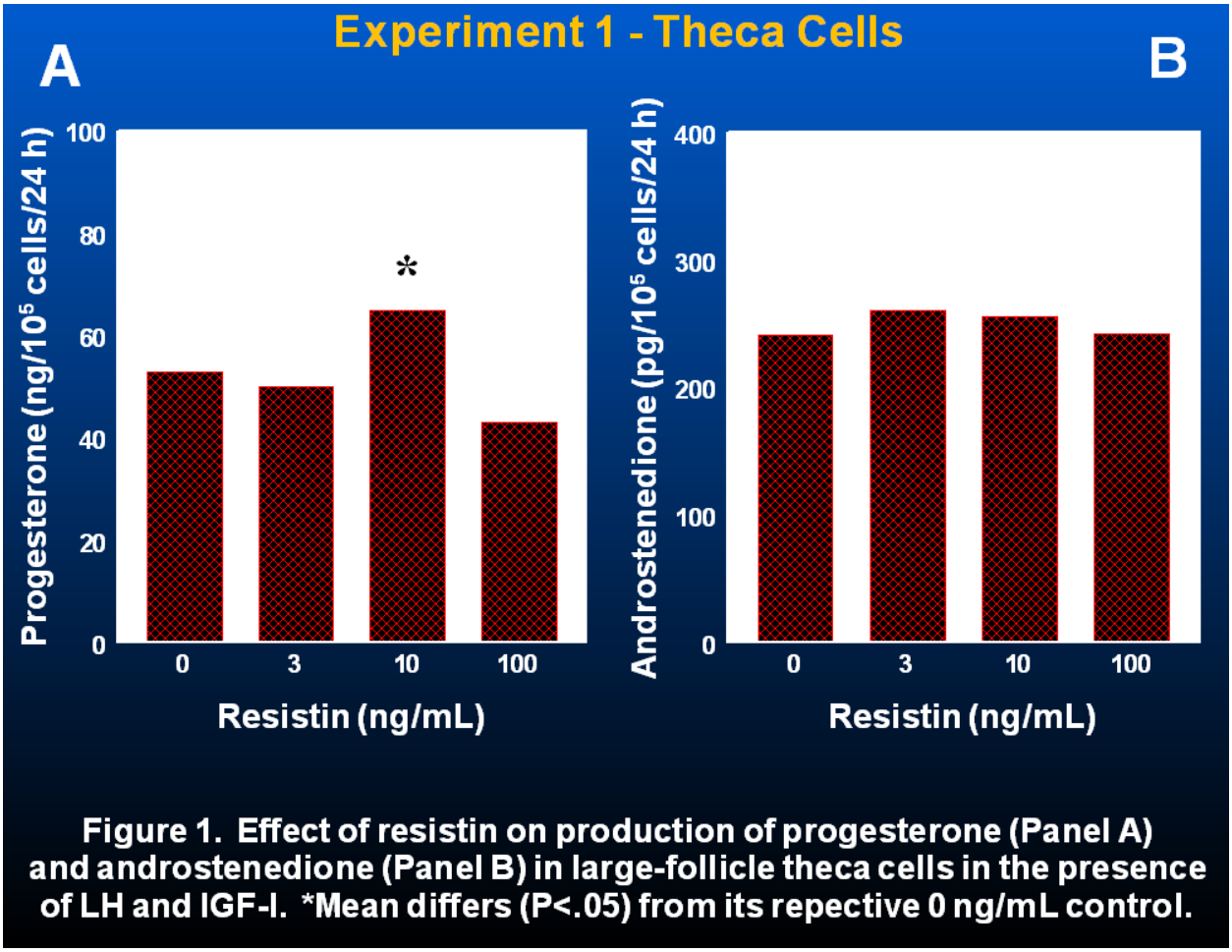

Figure 1. Effect of resistin on production of progesterone (Panel A) and androstenedione (Panel B) in large-follicle theca cells in the presence of LH and IGF-I. *Mean differs (P<.05) from its repective 0 ng/mL control.

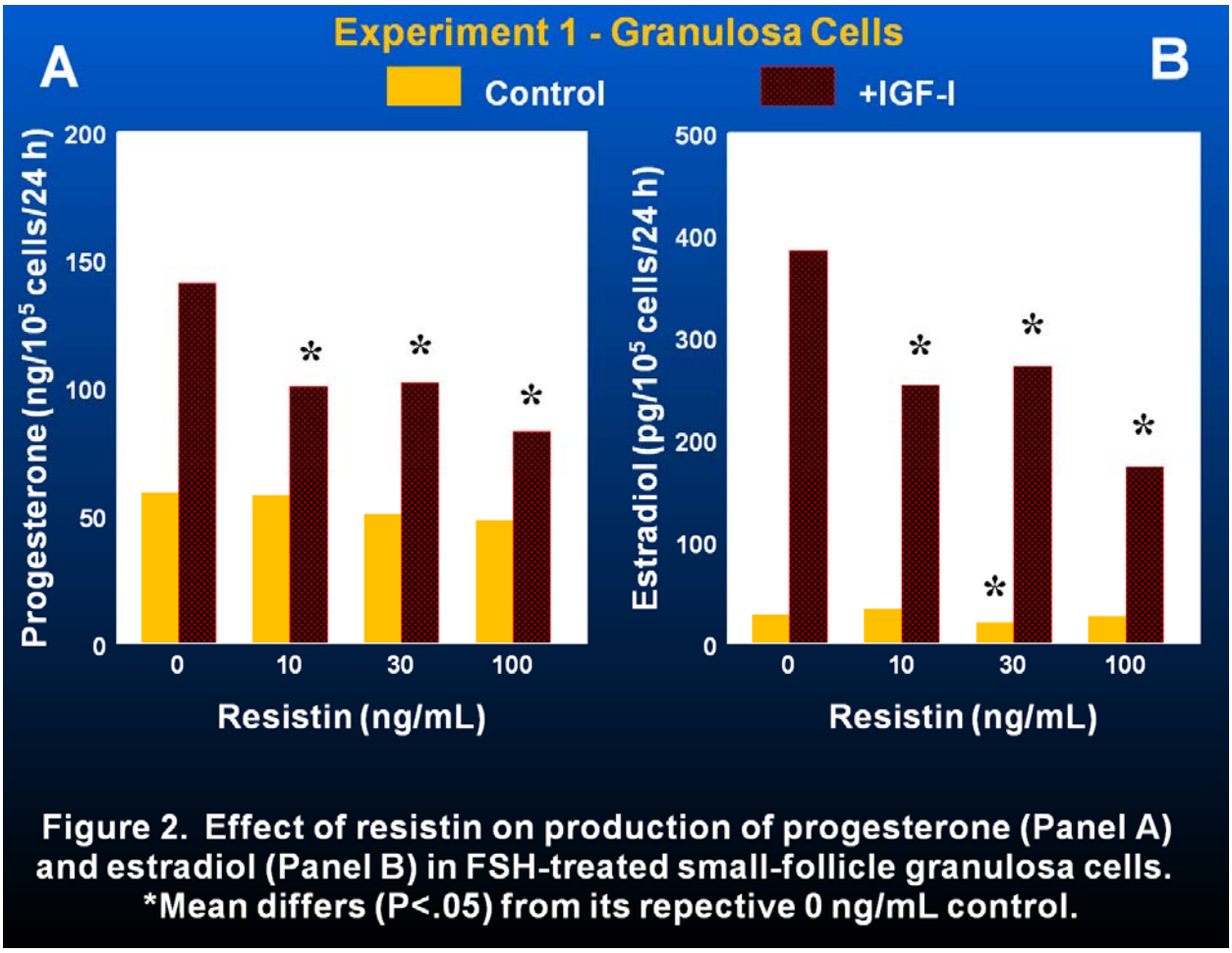

Figure 2. Effect of resistin on production of progesterone (Panel A) and estradiol (Panel B) in FSH-treated small-follicle granulosa cells. *Mean differs (P<.05) from its repective 0 ng/mL control.

Literature Cited

Aad P.Y. et al. 2006. Domest. Anim. Endocrinol. 31:357-372.

Caja S. et al. 2005. J. Endocrinol. 185:551-559.

Escobar-Morreale H.F. et al. 2006. Hum Reprod. 21:2257-65.

Garverick H.A. 1997. J. Dairy Sci. 80:995-1004.

Langhout D.J. et al. J. Anim. Sci. 1991. 69:3321-3334.

Majuri A. et al. 2007. Eur. J. Endocrinol. 156:263-9.

Mitchell M. et al. 2005. Repro. 130:583-597.

Munir I. et al. 2005. J. Clin. Endocrinol. Metab. 90:4852-4857.

Opsomer G. et al. 1999. Anim. Reprod. Sci. 56:211-222.

Ott, L. 1977. Pages 384-388 in An Introduction to Statistical Methods and Data Analysis. Duxbury Press, North Scituate, MA.

Panidis D. et al. 2004. Fertil. Steril. 81:361-366.

Papadopoulos D.P. et al. 2005. J. Clin. Hypertens. 7:729-733.

Peter A.T. 2004. Reprod. Domest. Anim. 39:1-7.

Seow K.M. et al. 2007. Reprod. 22:1100-6.

Spicer L.J. and Aad P. 2007. Biol. Reprod. 77:18-27.

Spicer L.J. and Chamberlain C.S. 1998. Endocrine. 9:153-161.

Spicer L.J. and Echternkamp S.E. 1995. Domest. Anim. Endocrinol. 12:223-245.

Spicer L.J. and Stewart R.E. 1996. J. Dairy Sci. 79:813-821.

Stewart R.E. et al. 1995. J. Anim. Sci. 73:3719-3731.

Vanholder T. et al. 2006. Reprod. Nutr. Dev. 46: 105-199.

Yilmaz M. et al. 2005. Hum. Reprod. 20:2414-2420.

Acknowledgements

The authors thank the Oklahoma State University Wentz Project scholarship program for financial support of D. Lagaly; Dustin Allen for laboratory assistance; and Creekstone Farms (Arkansas City, KS) for their generous donations of bovine ovaries.

Authors

D.V. Lagaly - Undergraduate student

P.Y. Aad - Graduate Student

L.B. Hulsey - Undergraduate student

J.A. Grado-Ahuir - Graduate Student

L. J. Spicer - Professor