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Effect of Insulin-Like Growth Factor-1 (IGF-1) on the expression of NFκB and cFLIP in Bovine Granulosa Cells

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Abstract

Infertility, often attributed to follicular atresia, is a growing problem in the agricultural industry. Programmed cell death, also known as apoptosis, is a contributing factor of follicular atresia. It occurs in both the granulosa cells and the oocyte that comprise ovarian follicles. Here, mechanisms influencing the process of apoptosis, via the death receptor Fas, were explored using bovine granulosa cells (bGCs) because Fas-induced apoptosis is a plausible mechanism of follicular atresia. Cell culture techniques, optimized for bGCs, were developed and used throughout the current study. In brief, cultures of bGCs were exposed to Fas ligand (100ng/mL) for 24 hrs. This induced cell death, as measured by MTS assay ($p=0.024$, $n=3$ experiments). Subsequent experiments in which doses of insulin-like growth factor-1 (IGF-1) were co-administered indicated that 100ng/ml IGF-1 provides the greatest protection against Fas-induced apoptosis ($p= 0.001$, $n=3$ experiments). Currently, we are testing the hypothesis that IGF-1 protects bGCs from Fas-induced apoptosis by stimulating the expression of cellular FLICE-like inhibitory protein (cFLIP) and the activation of nuclear factor-κB (NFκB), two molecules thought to protect granulosa cells from apoptosis. IGF-1-stimulated expression of cFLIP and NFκB will be assessed by immunoblots and in-cell western assays. This project was supported by the Hamel Center for Undergraduate Research (SD) and USDA grant no. 2013-67016-21071 (DHT).

Introduction

According to the Centers for Disease Control and Prevention, approximately 6.1 million women in the United States between the ages of 15 and 44 are infertile.[1] Most cases of infertility are attributed to problems with ovarian function and ovulation. The ovary nurtures follicles, which contain eggs surrounded by follicular cells called granulosa cells (Figure 1).[2]

Figure 1

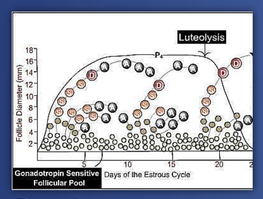

Figure 2

Ovarian function in women and cows is similar in that it is characterized by waves of follicular growth that occur prior to ovulation (Figure 2). During the last wave of follicular growth, one follicle is selected to become dominant, while the remaining follicles of the wave regress by a process known as follicular atresia.[3]

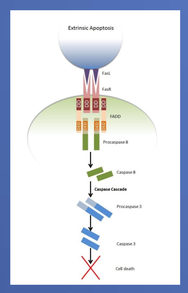

Figure 3

Within granulosa cells of the atretic follicles, Fas-Associated Death Domain (FADD) protein can be activated by Fas ligand binding (Figure 3). This activity converts pro-caspase 8 to cleaved caspase 8, which then initiates death signaling leading to apoptosis.[4] The molecule cFLIP can counteract the effects of pro-caspase 8 by competing for binding on FADD.[5]

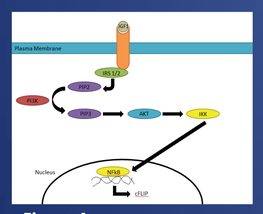

Figure 4

Insulin-like growth factor-1 (IGF-1) protects granulosa cells from apoptosis through the PI3K pathway (Figure 4). This pathway phosphorylates Akt, which is thought to prevent apoptosis by activating nuclear factor-κB (NFκB), and inducing the expression of anti-apoptotic proteins.[6] Here we speculate that one of the anti-apoptotic proteins regulated by NFκB is cFLIP.

Objectives

1. Validate that Fas ligand induces apoptosis of bovine granulosa cells
2. Determine an optimal dose of IGF-1 to prevent Fas-induced apoptosis of bovine granulosa cells
3. Determine whether IGF-1 stimulates the expression of cFLIP and NFκB as a mechanism to prevent Fas-induced apoptosis of bovine granulosa cells

Materials and Methods

Isolation of Bovine Granulosa Cells: Ovaries obtained from a slaughter house were dissected to obtain follicles 2-5 mm in diameter. The follicles were then either bisected and scraped or aspirated with an 18-gauge needle without dissection to obtain bGCs.

Cell Culture: bGCs were maintained in DMEM/F12 + 10% FBS at 37°C, 5% CO2, 95% air, 95% humidity in a T-25 until confluent, and then passaged into 96 well plates (cell death assays) or prepared as lysates for immunoblot experiments.

Cell Viability Assay: Culture medium was switched to serum-free containing ITS on day 1, pre-treated with IFN (50 ng/mL) and multiple doses of IGF-1 on day 2, then exposed to FasL (100 ng/ml) on day 3 for 24 hours. Cell viability was determined by measuring mitochondrial activity (MTS Assay, Promega; Madison, WI).

Immunoblots: Western blots of cell lysates were probed for cFLIP or NFkB normalized to β-actin using immunofluorescent antibodies. The proteins were detected via the LiCor system.

Results

Fas Ligand Induces Apoptosis of bGCs

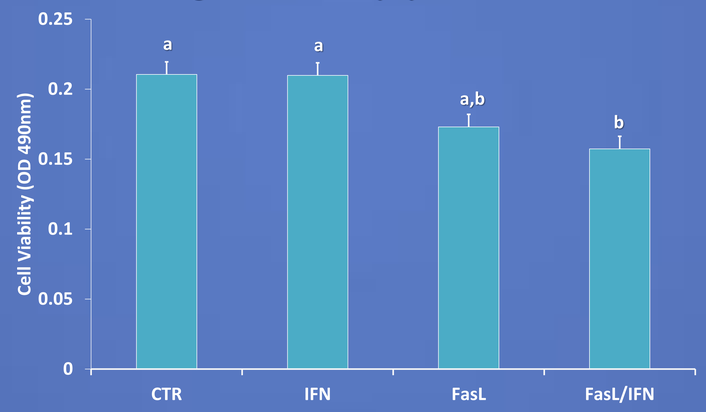

Figure 5. Exposure of bGCs to IFN (50 ng/mL) for 24 hrs followed by exposure to Fas ligand (100ng/mL) for 24 hrs. Different letters denote differences ($p=0.019$, $n=3$ experiments).

IGF-1 Dose-Dependently Inhibits Fas-Induced Apoptosis of bGCs

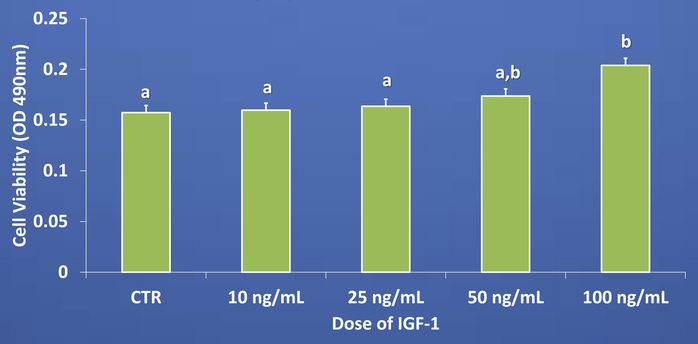

Figure 6. Effects of insulin-like growth factor-1 (IGF-1) on Fas-induced apoptosis. Cultures of bGCs were co-administered IFN (50 ng/ml) and FasL (100 ng/mL) following pretreatment with the indicated doses of IGF-1. Different letters denote differences ($p= 0.015$, $n=3$ experiments).

Results Continued

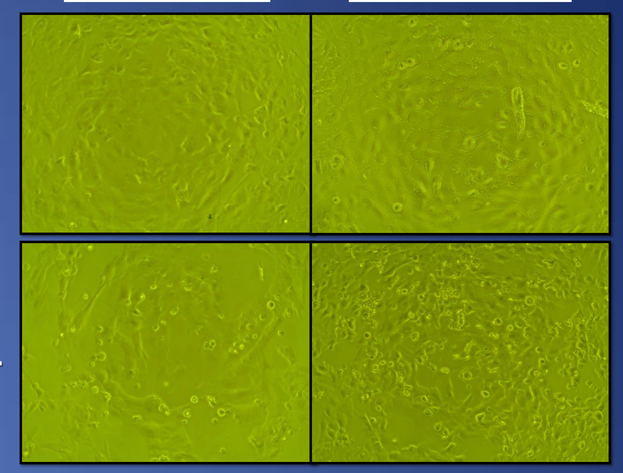

Figure 7. Effects of insulin-like growth factor-1 (IGF-1) on Fas-induced apoptosis. Cultures of bGCs were co-administered IFN (50 ng/ml) and FasL (100 ng/mL) following pretreatment with the indicated doses of IGF-1. Note the greater numbers of attached cells in the 100 ng/ml IGF-1 culture following IFN+FasL treatment.

Bovine Granulosa Cells Express cFLIP and NFkB

Figure 8. Immunodetection of cFLIP and NFkB in lysates of bGCs following SDS polyacrylamide gel electrophoresis (SDS-PAGE). Bands of appropriate molecular weight for cFLIP and NFkB (red bands, respectively) were detected relative to the housekeeping protein β-Actin (green). Quantitation of cFLIP and NFkB expression in bGCs following IGF-1 stimulation is ongoing.

Conclusion

The existence of anti-apoptotic/pro-survival molecules within granulosa cells of follicles has relevance to follicular development and atresia. In the current study, the observed protective effects of insulin-like growth factor-1 in bGCs, preventing Fas-induced apoptosis, are consistent with the up-regulated expression of such molecules, possibly cFLIP and/or NFkB. Further insight about these anti-apoptotic pathways has bearing on future therapeutic strategies to enhance follicular health within the ovary, and thus an overall improvement in female fertility.

Acknowledgements

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