Hormonal Regulation of Pregnancy-Associated Plasma Protein-A Gene Expression in Ovarian Granulosa and Theca Cells of Cattle

P.Y. Aad, C.A. Santiago, J.L. Voge, and L.J. Spicer

Story in brief

Proteolytic enzymes such as pregnancy-associated plasma protein-A (PAPP-A) degrade insulin-like growth factor (IGF) binding proteins and increase bioavailability of IGF-I and -II during ovarian follicular development. The effects of luteinizing hormone (LH), estradiol and insulin on ovarian granulosa and thecal PAPP-A mRNA abundance were evaluated using an in vitro culture. Bovine granulosa and theca cells were collected from ovarian follicles and cultured for 3 d. During the last 1 d of culture, cells were exposed to hormones in serum-free medium. Abundance of PAPP-A mRNA was quantified using real-time RT-PCR. In theca cells, LH did not affect PAPP-A mRNA levels, whereas insulin decreased PAPP-A mRNA by 2-fold; in the presence of insulin, estradiol decreased thecal PAPP-A mRNA levels and amplified the insulin-induced inhibition of PAPP-A mRNA expression. Granulosa PAPP-A mRNA abundance was not affected by any of the hormones tested. Therefore, estradiol may act as a negative feedback regulator via thecal PAPP-A production to prevent excessive IGF-I – induced androgen production which in turn may prevent excessive estradiol production by granulosa cells within the follicle. Understanding the regulation of ovarian factors may eventually lead to development of new commercial products that can be used to increase reproductive efficiency in dairy cattle.

Key Words: Cattle, Ovary, mRNA, Estradiol, Insulin, Gene Expression.

Introduction

Insulin-like growth factor (IGF)-I and -II are potent inducers of ovarian follicular steroidogenesis and mitogenesis (Spicer and Echternkamp, 1995), and IGF binding proteins (IGFBPs) are inhibitors of IGF action (Spicer and Chamberlain, 1999). Therefore, changes in IGFBP within the follicle control the bioavailability of IGF-I or -II and control follicular development (Spicer, 2004). During follicular selection, pregnancy-associated plasma protein-A (PAPP-A) degrades IGFBPs in healthy estrogen-dominant follicles, and thus PAPP-A may be an important regulator of follicular growth and selection (Spicer, 2004). Investigation of the hormonal control of PAPP-A mRNA in granulosa and theca cells may reveal possible regulatory mechanisms involved in the control of follicular growth in cattle. Our objective was to determine the effect of gonadotropins, insulin and estradiol on PAPP-A gene expression in cultured bovine granulosa and theca cells.

Materials and Methods

Ovaries from pregnant and non-pregnant dairy and beef cows were collected from a local slaughterhouse. Granulosa and theca cells were collected from large (>7.9 mm) or small (1-5 mm) follicles and cultured as previously described (Spicer and Chamberlain, 1998).

Cells were cultured in 10 % fetal calf serum (FCS) for the first 48 h with a medium change at 24 h. Cells were then washed twice with serum-free media and then cultured for an additional 24 h in serum-free media containing various hormonal treatments in four experiments. Small- follicle granulosa cells were cultured with the following treatments: control (no additives), FSH (3 or 30 ng/mL), LH (30 ng/mL), estradiol (3 or 300 ng/mL), estradiol plus LH, or estradiol plus FSH in the presence or absence of insulin (Experiments 1 and 2). Large-follicle granulosa cells were cultured with the following treatments: control (no hormones), insulin (100 ng/mL), insulin plus LH (30 ng/mL), insulin plus FSH (30 ng/mL), insulin plus FSH plus low estradiol (3 ng/mL), or insulin plus FSH plus high estradiol (300 ng/mL) (Experiment 3). Large-follicle theca cells were cultured with the following treatments: control (no additives), insulin (100 ng/mL), LH (30 ng/mL), LH plus insulin, estradiol (300 ng/mL), or estradiol plus insulin (Experiment 4). At the end of the cell culture, cells were lysed in Trizol and RNA isolated. PAPP-A mRNA abundance was measured by real-time RT-PCR as described previously (Voge et al., 2004ab; Santiago et al., 2005).

Data were analyzed using the relative threshold method as described previously (Voge et al., 2004ab). Mean differences were determined by Fisher's protected least significant differences test, if significant treatment effects in ANOVA were detected. Data were presented as the least square means ± SEM.

Results

In the presence or absence of 100 ng/mL of insulin, FSH (3 or 30 ng/mL), LH (30 ng/mL), or estradiol (3 or 300 ng/mL) did not affect ($P>0.10$) PAPP-A gene expression in small-follicle granulosa cells (Figure 1 and 2). Also, LH plus estradiol or FSH plus estradiol in the presence or absence of insulin did not affect ($P>0.10$) PAPP-A mRNA levels in small-follicle granulosa cells. In addition, PAPP-A mRNA levels in large-follicle granulosa cells were not affected ($P>0.10$) by insulin alone or insulin plus FSH, LH or combinations of FSH with 3 or 300 ng/mL of estradiol in the presence of insulin (Figure 3). However, in large-follicle theca cells, treatments with 100 ng/mL of insulin decreased ($P<0.05$) PAPP-A mRNA levels, whereas 30 ng/mL of LH or 500 ng/mL of estradiol alone did not change ($P>0.10$) PAPP-A gene expression. In large-follicle theca cells, LH did not affect ($P>0.10$) the insulin-induced decrease in PAPP-A mRNA abundance, whereas estradiol induced a further decrease ($P<0.05$) in insulin-suppressed PAPP-A gene expression as compared to estradiol or insulin alone (Figure 4).

Discussion

This study revealed that insulin with or without LH decreased large-follicle theca PAPP-A mRNA abundance by 2-fold, and that estradiol amplified the insulin-induced inhibition of thecal PAPP-A mRNA expression. Previously, PAPP-A mRNA expression was detected in theca of human ovaries using in situ hybridization (Rhoton-Vlasak et al., 2003), but its hormonal regulation was not evaluated. Granulosa cell-derived estradiol may act as a paracrine negative feedback regulator to suppress PAPP-A mRNA production in theca cells which would cause less IGFBP-4 and/or -5 proteolysis and allow for less bioavailable IGFs for proliferation and steroid production by theca cells.

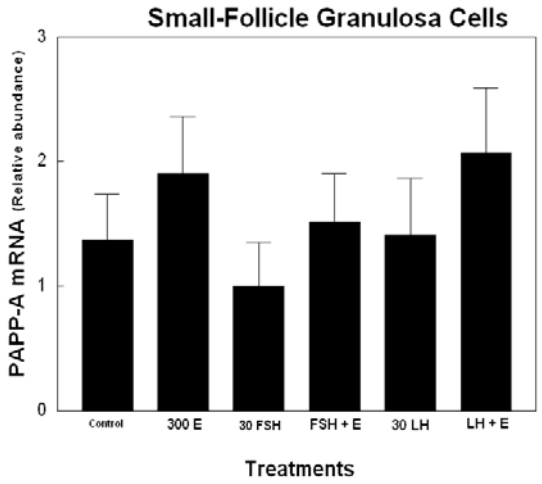

Figure 1: Lack of effect of LH, FSH and estradiol (E) on PAPP-A mRNA abundance in small-follicle (1-5 mm) granulosa cells. Means do not differ (P>0.10).

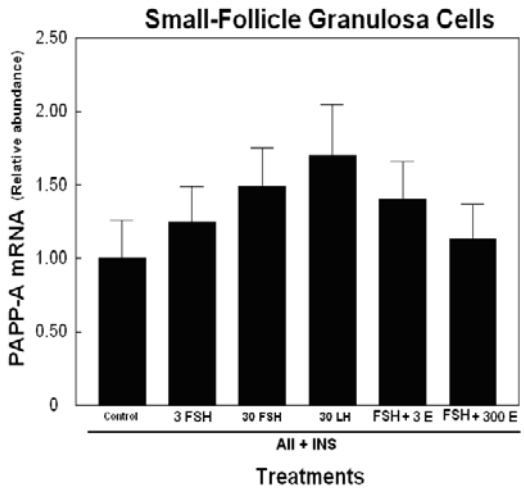

Figure 2: Lack of effect of LH, FSH and estradiol (E) in the presence of insulin (INS) on PAPP-A mRNA abundance in small-follicle (1-5 mm) granulosa cells. Means do not differ (P>0.10).

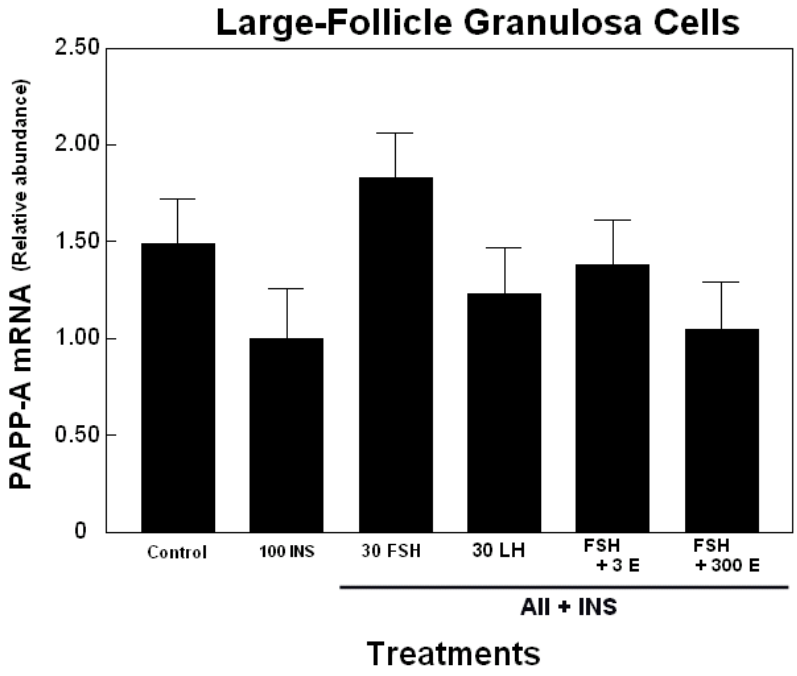

Figure 3: Lack of effect of insulin (INS), LH, FSH and estradiol (E) on PAPP-A mRNA in large-follicle (>7.9 mm) granulosa cells. Means do not differ (P>0.10).

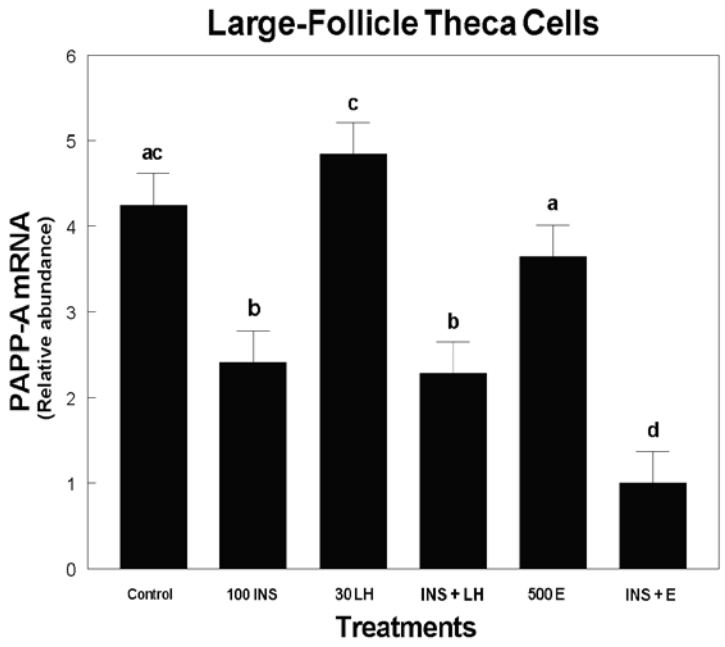

Figure 4: Effect of insulin (INS), LH, estradiol (E), or their combinations on PAPP-A mRNA in large-follicle (>7.9mm) theca cells. Means without a common letter differ (P<0.05).

In small- and large-follicle granulosa cells, PAPP-A mRNA levels were not significantly affected by any of the hormones tested including insulin, gonadotropins, or estradiol. In contrast, PAPP-A mRNA levels in cultured granulosa cells of late pre-antral rat follicles were increased by FSH within 12 to 24 h (Matsui et al., 2004), and PAPP-A mRNA levels in whole ovarian extracts increased between 4 and 36 h post-PMSG treatment in mice (Hourvitz et al., 2002). Previously, Conover et al. (2001) reported that hCG had no effect on PAPP-A protein levels secreted by human granulosa cells, but Hourvitz et al. (2002) showed that hCG treatment in vivo increased whole ovarian PAPP-A mRNA abundance in mice. These differences in the response of granulosa cell PAPP-A mRNA levels to FSH and LH/hCG may be species, dose or culture condition specific.

In conclusion, levels of PAPP-A mRNA in granulosa cells were not regulated by the main reproductive hormones, whereas theca cell production of PAPP-A mRNA was regulated negatively by insulin and estradiol. Insulin and estradiol work in concert to decrease thecal PAPP-A mRNA. The selective decrease in thecal PAPP-A gene expression induced by estradiol may lead to a decrease in proteolysis of IGFBP-4/-5 and thus less bioavailable IGFs within the theca layer. We hypothesize that estradiol acts as a negative paracrine feedback regulator to prevent excessive IGF-I – induced androgen production, and hence prevent excessive estradiol production by granulosa cells. This control of thecal PAPP-A production likely maintains desirable levels of IGFBP-4 and -5 and subsequently free IGF-I /-II within the follicle during follicular development and selection in cattle. Understanding the regulation of ovarian factors may eventually lead to development of new commercial products that can be used to increase reproductive efficiency in dairy cattle.

Literature Cited

Conover, C.A. et al. 2001. Endocrinology 142: 2155.

Hourvitz, A. et al., 2002. Endocrinology 143: 1833.

Matsui, M. et al., 2004. Endocrinology 145: 3686.

Rhoton-Vlasak, A. et al. 2003. Fertil. Steril. 79: 1149.

Santiago, C.A. et al. 2005. Domest. Anim. Endocrinol. 28:46-63.

Spicer, L.J. 2004. Biol. Reprod. 70:1223.

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

Spicer, L.J., and C.S. Chamberlain. 1999. Domest. Anim. Endocrinol. 16:19.

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

Voge, J.L. et al. 2004a. Domest. Anim. Endocrinol. 26:241.

Voge, J.L. et al. 2004b. Peptides 25:2195.

Acknowledgements

The authors thank Dustin Allen for technical assistance, Mikkelson Beef Inc. (Oklahoma City, OK) for their generous donations of bovine ovaries, and the Fulbright Foreign Student Program of the U.S. Department of State Bureau of Educational and Cultural Affairs for financial support (to Pauline Aad and Consuelo Santiago).

Authors

Aad, P.Y. – Graduate Student

Santiago, C.A. – Graduate Student

Voge, J.L. – Graduate Student

Spicer, L.J. – Professor