2014

Follicular Vascular Endothelial Growth Factor A Expression Before and After the LH Surge

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Summary

Granulosa cell expression of VEGFA isoforms in dominant bovine follicles was evaluated. Collection of granulosa cells via follicle aspiration revealed altered expression of the proangiogenic VEGFA_164 isoform but not the antiangiogenic VEGFA_164B isoform prior to and after the LH surge. Expression of VEGFA_164 declines as both the LH surge and ovulation approaches. In addition, VEGFA_164 and VEGFA_164B expression prior to the LH surge was positively correlated with FSHR and CYP19A1 expression, suggesting that VEGFA expression may be regulated by FSH. These data indicate differential expression of VEGFA isoforms may be an important feature of bovine dominant follicle development.

Introduction

Follicle stimulating hormone (FSH) promotes ovarian follicle growth including oocyte maturation and E_2 (estrogen) production by the granulosa cells in these follicles while a surge in the release of luteinizing hormone (LH) midway through the reproductive cycle stimulates ovulation of the dominant follicle and transformation of this follicle into a P_4 (progesterone)-secreting corpus luteum. Although selection of the dominant follicle is primarily regulated through these anterior pituitary hormones, growth factors are also important for dominant follicle development. For example, inhibition of vascular endothelial growth factor (VEGFA) has been shown to impair follicle development and block ovulation. However, both proangiogenic and antiangiogenic VEGFA isoforms exist and the majority of prior studies evaluating the role of VEGFA in follicle development have not differentiated between these different isoforms. The antiangiogenic "B" isoforms were named based upon their ability to inhibit the new blood vessel formation which is stimulated by the proangiogenic VEGFA. The current study evaluated the expression of proangiogenic and antiangiogenic *VEGFA* isoforms in granulosa cells of dominant follicles prior to and after the LH surge.

Procedure

All procedures were approved by the University of Nebraska–Lincoln Institutional Animal Care and Use Committee. Crossbred, non-lactating beef cows that were 75% MARC III (¼ Angus, ¼ Hereford, ¼ Pinzgauer, ¼ Red Poll) and 25% Red Angus/European composite background crossbreds were used in this study. Average age was 5.2 ± 2.4 years, and the weight range for breeding-age heifers and cows in this herd is approximately 850-1,400 lb.

Cows in the first experiment (n = 70) received 2 i.m. injections of $PGF_{2\alpha}$ (Lutalyse; prostaglandin F2 alpha; hormone that stimulates the regression of the corpus luteum and, thus, initiation of a new reproductive cycle) 14 days apart to synchronize estrus. Follicular fluid and granulosa cells were collected from dominant follicles with a minimum diameter of 10 mm via transvaginal, ultrasound-guided aspiration 6, 12, 18, 24, 30, 36, 48, 56, and 72 hours after the second injection of $PGF_{2\alpha}$. Blood samples were collected from a subset of 12 cows to determine the timing of the subsequent LH surge. In these cows, LH surges were detected between 56 and 72 hours following the second $PGF_{2\alpha}$ injection. To evaluate follicles prior to the LH surge, only follicles aspirated between 6 and 48 hours post-$PGF_{2\alpha}$ were analyzed.

Cows in the second experiment (n = 55) also received GnRH (Cystorelin; gonadotropin releasing hormone; hormone produced in the hypothalamus that stimulates release of FSH and LH from the anterior pituitary gland) 48 hours after the second $PGF_{2\alpha}$ injection to stimulate an LH surge. Dominant follicles were then aspirated 0, 3, 6, 12, 18, and 24 hours following GnRH. The peak of LH secretion has been shown to occur 2 hours after GnRH administration, and ovulation is induced between 22 and 32 hours following GnRH; therefore, aspiration of follicles 3 to 24 hours post-GnRH should occur after the stimulated surge of LH and just prior to ovulation.

Follicles with a follicular fluid E_2 to P_4 ratio less than 1 have been shown to be destined for degeneration rather than ovulation; thus, only follicles with an E_2 to P_4 ratio greater than 1 were utilized for data analysis. Total RNA was extracted from aspirated granulosa cells for quantitative RT-PCR to evaluate mRNA abundance for *VEGFA_164* and *VEGFA_164B*. Messenger RNA abundance was also evaluated for *CYP19A1* (aromatase; enzyme which converts androgens to E_2), *FSHR* (receptor which binds and mediates the actions of FSH), and *LHCGR* (receptor which binds and mediates the actions of LH). The constitutively expressed gene, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), was used as a control for RNA amplification. Data were analyzed by one-way ANOVA using JMP software and means for the different time points were compared using a Tukey-Kramer test. Differences in means were considered to be statistically significant at $P < 0.05$.

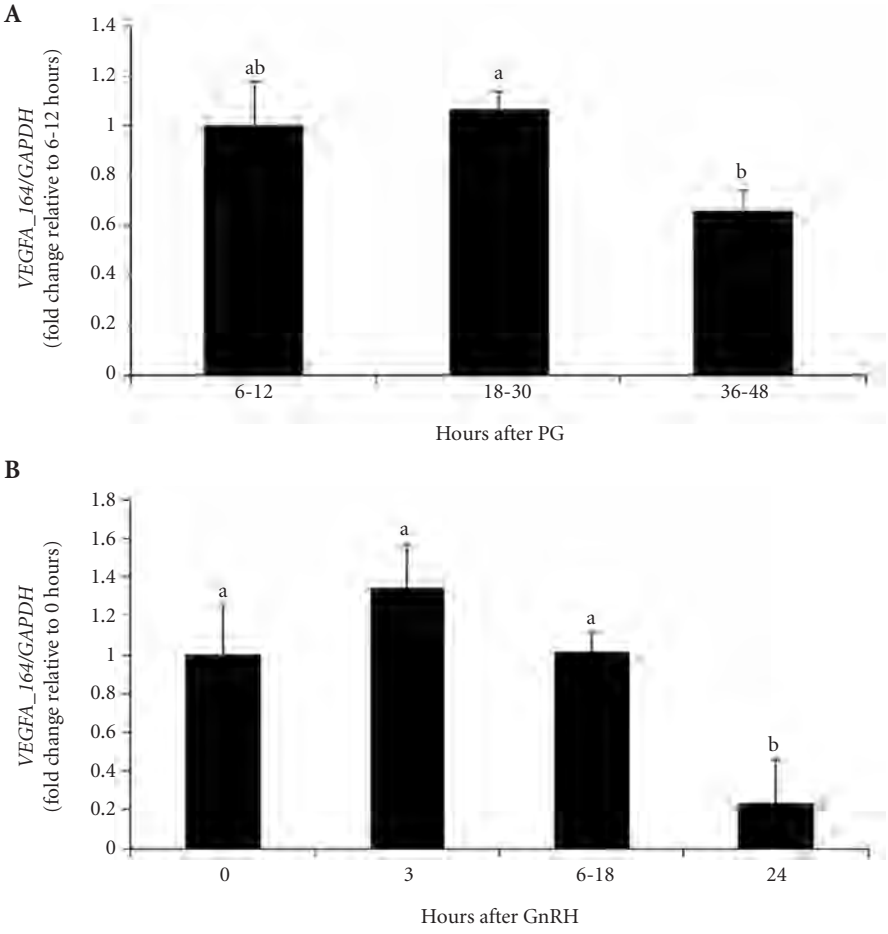

Figure 1. Quantitative RT-PCR was conducted to detect granulosa cell mRNA levels for *VEGFA_164* in dominant follicles 6 to 12 hours (n = 13), 18 to 30 hours (n = 40), 36 to 48 h ours (n = 11) after $PGF_{2\alpha}$ administration and prior to a LH surge (A) and 0 hours (n = 6), 3 hours (n = 9), 6 to 18 hours (n = 33), and 24 hours (n = 7) after GnRH administration (B). *GAPDH* was used as an endogenous control to account for differences in starting material. The mean normalized values obtained for granulosa cells aspirated 6 to 12 hours after $PGF_{2\alpha}$ (A) or 0 hours after GnRH (B) were set at 1 and the values for the other time points were calculated as a fold change. The subsequent means ± SEM are presented and different letters represent a statistically significant difference in LS Means ($P < 0.05$) between time points.

Table 1. Correlation coefficients for granulosa cell mRNA levels in dominant follicles following $PGF_{2\alpha}$ and GnRH administration.

Correlation Coefficients: Post-$PGF_{2\alpha}$

	FSHR	LHCGR	VEGFA_164	VEGFA_164B
CYP19A1	0.60[a]	0.47[c]	0.53[c]	0.35[d]
FSHR		0.77[a]	0.50[c]	0.46[d]
VEGFA_164				0.59[b]

n = 70

Correlation Coefficients: Post-GnRH

	VEGFA_164B
VEGFA_164	0.79[a]

n = 55

Letters represent correlation coefficients that are significant:
[a]$P < 0.0001$
[b]$P < 0.001$
[c]$P < 0.01$
[d]$P < 0.05$

Results

When granulosa cell gene expression was evaluated in dominant follicles not exposed to an LH surge (after PGF_2 administration), no differences were identified between early (6 and 12 hours), mid (18 to 30 hours) and late (36 and 48 hours) time points; therefore, data from these time points were combined for further analysis. This analysis revealed the relative abundance of *VEGFA_164* mRNA in granulosa cells was greater ($P = 0.0129$) in follicles aspirated 18 to 30 hours following PGF_2 administration compared to those aspirated 36 to 48 hours after PGF_2 (Figure 1). In addition, mRNA levels for *VEGFA_164* were strongly correlated ($P < 0.01$) with those for *VEGFA_164B* (0.59), *CYP19A1* (0.53), and *FSHR* (0.50). Positive correlations also were identified between the mRNA abundance of *VEGFA_164B* and mRNA levels for *CYP19A1* (0.35, $P = 0.0298$), and *FSHR* (0.46, $P = 0.0125$) (Table 1).

When gene expression was evaluated in dominant follicles following exposure to an LH surge (after GnRH administration), no differences were identified between the 6, 12, and 18 hour time points; therefore, data from these time points were combined for further analysis. This analysis determined the relative abundance of *VEGFA_164* mRNA in granulosa cells was lowest ($P = 0.0311$) 24 hours after GnRH (Figure 1). Likewise, the ratio of *VEGFA_164:164B* was lower in granulosa cells 24 hours post-GnRH compared to 3 ($P = 0.0155$) and 16 to 18 ($P = 0.0112$) hours post-GnRH (data not shown). Furthermore, a strong positive correlation (0.79, $P < 0.0001$) was identified between mRNA levels for *VEGFA_164* and *VEGFA_164B* (Table 1).

This study revealed altered expression of the proangiogenic *VEGFA_164* isoform but not the antiangiogenic *VEGFA_164B* isoform in bovine granulosa cells from dominant follicles prior to and after the LH surge. Before the LH surge, granulosa cell expression

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of *VEGFA_164* and *VEGFA_164B* mRNA was positively correlated with *FSHR* and *CYP19A1* expression in dominant follicles. Because FSH stimulates both *CYP19A1* expression and E_2 production in bovine granulosa cells, the correlation between *FSHR* and *CYP19A1* is not surprising. In addition, the reduction in *VEGFA_164* mRNA levels and the *VEGFA_164:164B* ratio 24 hours after administration of GnRH suggests the proangiogenic VEGFA isoforms may be initially important for the maintenance of preovulatory follicles but reduced proangiogenic *VEGFA* expression may be beneficial prior to ovulation. Increased vasculature will allow for the delivery of nutrients and hormones to developing follicles but blood vessel growth may need to be tempered to allow for rupture of the follicle wall and to limit ovulatory hemorrhage. Therefore, inappropriate VEGFA isoform expression may impair dominant follicle development and ovulation which would result in reduced reproductive efficiency.

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