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Renee M. McFee
University of Nebraska-Lincoln, rmcfee3@unl.edu

Robin A. Artac
University of Nebraska-Lincoln

William E. Pohlmeier Pohlmeier
University of Nebraska-Lincoln, wpohlmeier2@unl.edu

Jill G. Kerl
University of Nebraska-Lincoln

Vanessa M. Brauer
University of Nebraska-Lincoln

See next page for additional authors

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Authors

Renee M. McFee, Robin A. Artac, William E. Pohlmeier Pohlmeier, Jill G. Kerl, Vanessa M. Brauer, Robert A. Cushman, and Andrea S. Cupp

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Vascular Endothelial Growth Factor A (VEGFA) in Ovulatory Follicles

Renee M. McFee
Robin A. Artac
William E. Pohlmeier
Jill G. Kerl
Vanessa M. Brauer
Robert A. Cushman
Andrea S. Cupp[1]

Summary

Granulosa cells express vascular endothelial growth factor A (VEGFA), and VEGFA mRNA levels increase as bovine follicles reach preovulatory status. To further evaluate the role of VEGFA isoforms in follicular development, cows were either synchronized with a modified Co-Synch protocol (CIDR) or treated with melengestrol acetate (MGA) with subsequent aspiration of the dominant follicles. Higher mRNA levels for the antiangiogenic isoform, VEGFA_164B, along with AMH and CARTPT in E2-inactive follicles suggest that these factors are markers for unhealthy, atretic follicles. In contrast, higher mRNA levels for the proangiogenic isoform, VEGFA_164, in E2-active follicles indicate that this isoform may help predict healthy ovulatory follicles.

Introduction

Several factors are important for dominant follicle development. Granulosa cells express vascular endothelial growth factor A (VEGFA) and its receptors even though they are avascular follicular cells and *VEGFA* mRNA levels increase as bovine follicles reach preovulatory status. Follicular fluid anti-Müllerian hormone (AMH) levels decrease during antral follicle growth but then increase during early atresia of large follicles while granulosa cell expression of CART prepropeptide (CARTPT) is greater in estrogen (E2)-inactive follicles than E2-active follicles. The current study evaluated granulosa cell expression of *VEGFA_164* (proangiogenic) and *VEGFA_164B* (antiangiogenic) in dominant follicles in comparison with *AMH* and *CARTPT* expression.

Procedure

All procedures were approved by the University of Nebraska–Lincoln Institutional Animal Care and Use Committee. Nonlactating beef cows that were 75% MARC III (1/4 Angus, 1/4 Hereford, 1/4 Pinzgauer, 1/4 Red Poll) and 25% Red Angus/European Composite background crossbreds with an average age of 7.5 ± 2.7 years and weight of 1,200 lb were used in this study. Cows in the first treatment group (CIDR) were synchronized with the Co-Synch + CIDR timed artificial insemination (AI) protocol, except follicle aspiration was performed after synchronization rather than timed AI. The second group of cows was treated with melengestrol acetate (MGA) for 14 days and received three injections of ProstaMate (day 0, 7, and 14) to eliminate any luteal tissue prior to follicle aspiration. Aspiration of dominant follicles was performed transvaginally with the use of caudal epidural anesthesia and an endovaginal ultrasound transducer with an attached needle guide.

Follicular fluid E2 and progesterone (P4) levels were measured to determine E2-activity for each follicle. Total RNA was extracted from granulosa cells for quantitative RT-PCR to evaluate mRNA abundance for *VEGFA_164*, *VEGFA_164B*, *AMH*, and *CARTPT*. 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 differences were considered to be statistically significant at $P < 0.05$ unless otherwise stated.

Results

Using E2:P4 ratios, follicles were classified as E2-active (E2:P4 > 1; healthy) or E2-inactive (E2:P4 ≤ 1; atretic). In the CIDR group, mRNA levels for both *AMH* ($P = 0.0015$) and *CARTPT* ($P = 0.0004$) were greater in aspirated granulosa cells from E2-inactive follicles versus E2-active follicles (Figure 1C-D). Although *VEGFA_164B* mRNA levels were higher in E2-inactive follicles, and *VEGFA_164* was more abundant in E2-active follicles, these differences were not significant (Figure 1A-B). For the MGA-treated cows, mRNA levels for *VEGFA_164B* ($P < 0.0001$), *AMH* ($P = 0.007$), and *CARTPT* ($P = 0.0009$) were more abundant in E2-inactive follicles compared to E2-active follicles (Figure 1B-D). In addition, mRNA levels for *VEGFA_164* ($P = 0.02$) were greater in E2-active follicles than E2-inactive follicles (Figure 1A).

Evaluation of E2-active follicles between CIDR and MGA-treated cows did not reveal differences in mRNA levels for *VEGFA_164*, *VEGFA_164B*, or *AMH* and although *CARTPT* ($P = 0.11$) levels were higher in follicles from MGA cows; this difference was not significant (Figure 1A-D). For E2-inactive follicles, mRNA levels for *VEGFA_164* ($P = 0.04$) were more abundant in follicles from CIDR cows than MGA-treated cows (Figure 1A). Although not significant, mRNA levels for *VEGFA_164B* were higher in follicles from MGA-treated cows than CIDR cows (Figure 1B). No differences were seen in mRNA levels

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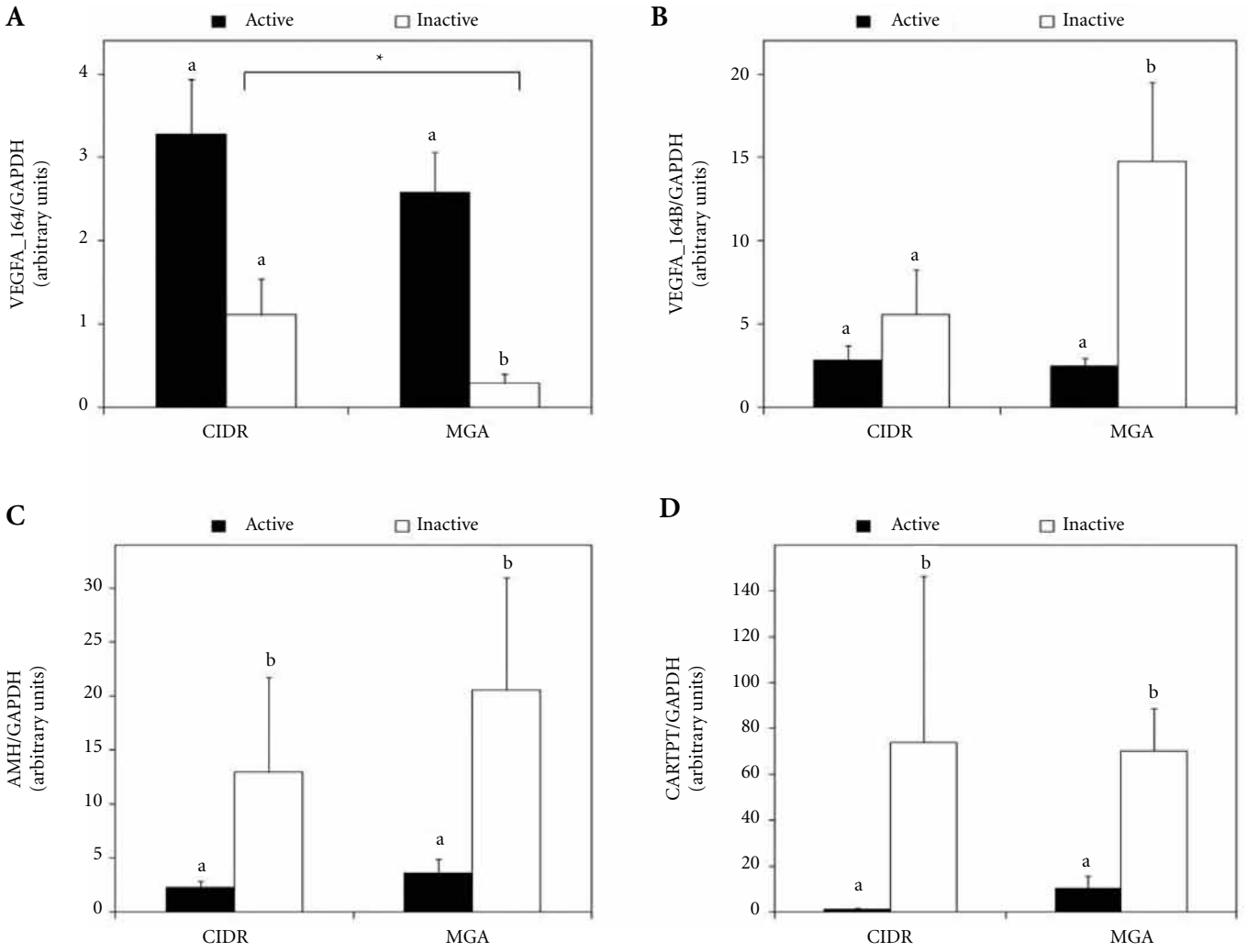

Figure 1. Granulosa cell quantitative RT-PCR results for *VEGFA_164* (A), *VEGFA_164B* (B), *AMH* (C), and *CARTPT* (D) for E2-active and E2-inactive dominant follicles from CIDR and MGA-treated cows. The mean ± SEM normalized values are presented. Different letters represent a statistically significant difference in means between E2-active and E2-inactive follicles for each treatment group ($P < 0.05$). Asterisks represent a statistically significant difference in means between CIDR and MGA-treated cows for each follicle type ($P < 0.05$).

between the two treatment groups for either *AMH* or *CARTPT* (Figure 1C-D).

Increased expression of *AMH* and *CARTPT* in E2-inactive follicles supports previous evidence that these factors are markers for unhealthy, atretic antral follicles. For *VEGFA*, higher levels of the antiangiogenic isoform (*VEGFA_164B*) were present in E2-inactive follicles and higher levels of the proangiogenic isoform (*VEGFA_164*) were present in E2-active follicles. Furthermore, *VEGFA_164* was more abundant in E2-inactive follicles from CIDR cows while *VEGFA_164B* was more abundant in E2-inactive follicles from MGA-treated cows. Treatment with MGA has been shown to promote the development of persistent dominant follicles and is associated with decreased ooctye viability. These data suggest that expression patterns for *VEGFA* isoforms may be used to predict the health status of dominant follicles.

[1]Renee M. McFee, research technician; Robin A. Artac, former graduate student; William E. Pohlmeier, research technician; Jill G. Kerl, research technician; Vanessa M. Brauer, research technician; Robert A. Cushman, physiologist, University of Nebraska–Lincoln Meat Animal Research Center, Clay Center, Neb.; Andrea S. Cupp, professor, UNL Department of Animal Science, Lincoln, Neb.

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