January 2006

Vascular Endothelial Growth Factor mRNA Isoforms 120 and 164 are Differentially Regulated Prior to Ovulation

Robin A. Ten Broeck
University of Nebraska-Lincoln

Debra T. Clopton
University of Nebraska-Lincoln, dclopton1@unl.edu

Jeremy L. Martin
University of Nebraska-Lincoln

Rebecca C. Bott
University of Nebraska-Lincoln

Karl V. Moline
University of Nebraska-Lincoln, kmoline1@unl.edu

See next page for additional authors

Vascular Endothelial Growth Factor mRNA Isoforms 120 and 164 are Differentially Regulated Prior to Ovulation

Robin A. Ten Broeck
Debra T. Clopton
Jeremy L. Martin
Rebecca C. Bott
Karl V. Moline
Jeff W. Bergman
Andrea S. Cupp[1]

Summary

Vascular Endothelial Growth Factor (VEGF) is produced by cells surrounding the egg in the follicle prior to ovulation. If VEGF is inhibited, ovulation does not occur. The VEGF gene can be spliced to produce different protein isoforms which have specific functions. Our objective was to determine if VEGF 120 and 164 mRNA isoforms are differentially regulated in the preovulatory follicle. From our studies, VEGF isoforms are differentially regulated during both CL regression and after a simulated LH surge. Differences observed in VEGF isoform regulation may allow for manipulation of ovulation in the beef cow.

Introduction

Follicular development within the bovine ovary is a dynamic process. It begins prior to birth and continues throughout the cow's reproductive lifespan. Angiogenesis, the formation of new blood vessels, is crucial in the ovulatory follicle. The blood vessels supply the follicle with necessary nutrients for development and growth prior to ovulation. VEGF is expressed by granulosa cells (cells surrounding the egg) prior to ovulation in the bovine follicle and is an important factor in the regulation of normal angiogenesis in the developing follicle and corpus luteum. Several VEGF isoforms exist, the most common isoforms are VEGF 164 and VEGF 120. In other tissues such as the heart and lung VEGF isoforms have specific functions in vascular development. VEGF 120 is highly diffusible and recruits endothelial cells (precursor to blood vessel cells) in the establishment of blood vessels. VEGF 164 recruits endothelial cells that form the large blood vessels. However, it is not known how these isoforms function in the ovarian preovulatory follicle nor how inhibition of VEGF might alter ovulation. The objective of the current study is to determine if the two major VEGF mRNA isoforms, 120 and 164, are differentially regulated prior to ovulation in the granulosa cells of the dominant follicle.

Procedure

Trial 1

Two injections of $PGF_{2\alpha}$ (5mg/cow) were administered 14 days apart. $PGF_{2\alpha}$ induces regression of the corpus luteum initiating the LH surge and ovulation. A vaginal probe with needle attachment was used to collect follicular fluid and granulosa cells from the dominant follicle. Follicular aspirates were collected at 12, 18, 24, 30, 36, 48, 54, 60, 66, and 72 hours (n=average of 8) after the second injection of $PGF_{2\alpha}$. Messenger RNA was extracted from granulosa cells and samples were reversed transcribed to cDNA. Progesterone and estrogen concentrations were measured in follicular fluid. A ratio greater than 1 of estrogen to progesterone indicated that the follicle was still dominant. The mRNA expression of VEGF isoforms 120 and 164 were determined using real-time quantitative polymerase chain reaction (PCR) at each follicular aspirate time point. Data were analyzed using an ANOVA with SAS. Comparisons of means were analyzed using a Tukey-Kramer test. In 12 animals, blood samples were collected after the second injection of $PGF_{2\alpha}$ at two-hour intervals from 0 to 72 hours to determine when the LH surge occurred. The LH surge was detected in six of the 12 cows and occurred between 54 and 72 hours. Due to this variation, a second trial was conducted to obtain follicle aspirates after a simulated surge of LH by injecting GnRH (0.1mg/cow).

Trial 2

Two injections of $PGF_{2\alpha}$ (5mg/cow) were administered 14 days apart with an injection of GnRH (0.1mg/cow) administered 48 hours after the second injection of $PGF_{2\alpha}$. Follicular aspirates were collected via vaginal probe with needle attachment at 3, 6, 12, and 24 hours after GnRH (n=average of 3). Messenger RNA was extracted as described previously and expression of VEGF mRNA isoforms 120 and 164 were analyzed using quantitative real time PCR. Data were analyzed using an ANOVA with SAS. Comparisons of means were tested using a Tukey-Kramer test.

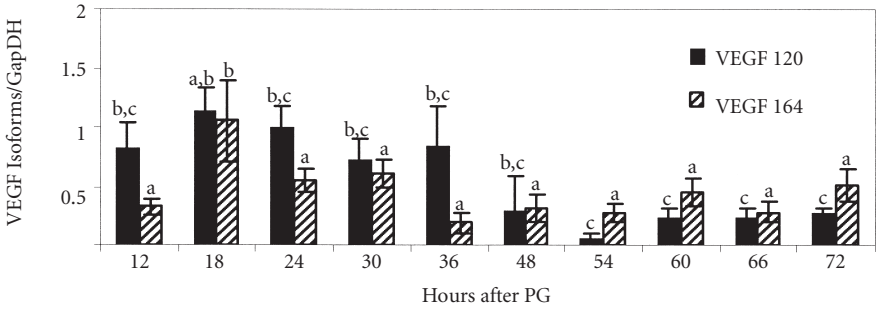

Figure 1. VEGF mRNA isoforms expression of 120 and 164 at different time points after second injection of PG. Different letters within each isoform at collection time points denote differences of $P < 0.01$.

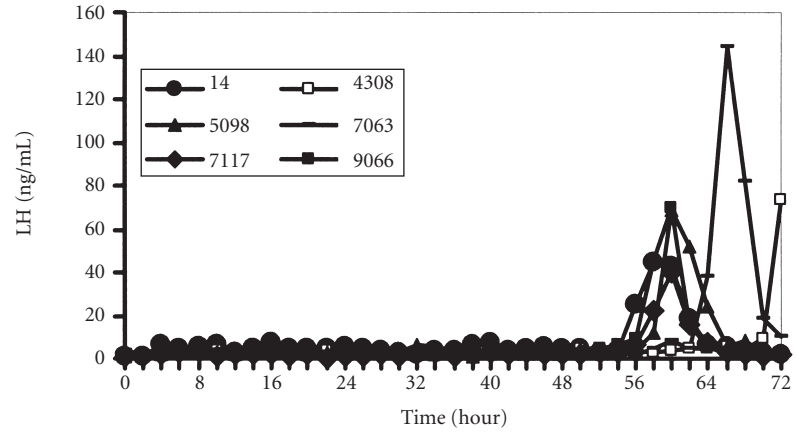

Figure 2. A subset of 12 cows were bled every two hours for 72 hours to determine timing of LH surge. This graph represents cows that displayed an LH surge within 72 hours.

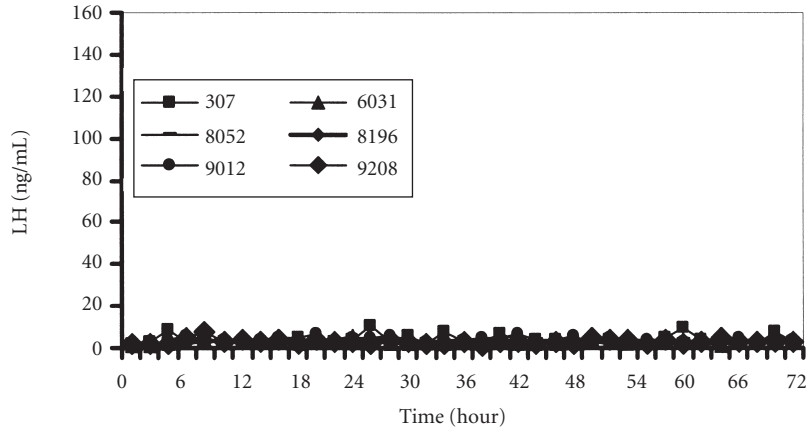

Figure 3. A subset of 12 cows were bled every two hours for 72 hours to determine timing of LH surge. This graph represents cows that did not display an LH surge within 72 hours.

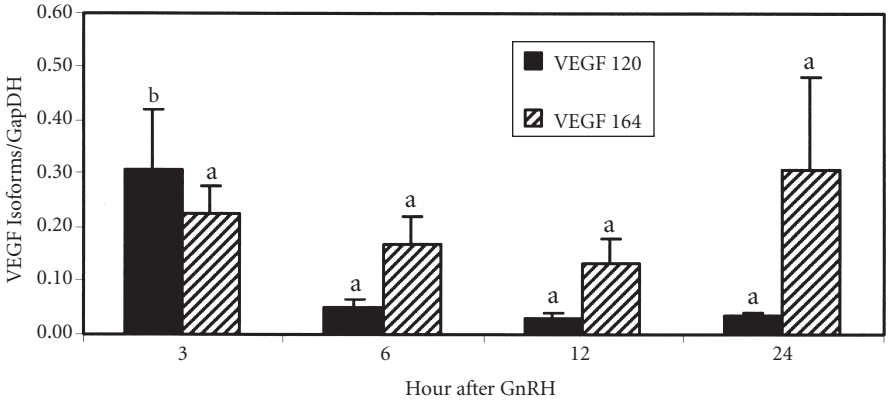

Figure 4. VEGF mRNA isoforms expression of 120 and 164 after injection of GnRH. Different letters within each isoform at collection time points denote differences of $P < 0.05$.

Results

In the first trial, the greatest concentration of VEGF 164 mRNA was observed at 18 hours post second injection of $PGF_{2\alpha}$ ($P < 0.01$; Figure 1). VEGF 164 is the predominant VEGF isoform responsible for the formation of large blood vessels. A peak in 164 isoform at 18 hour post-$PGF_{2\alpha}$ may contribute to the development of vasculature in the theca layer of the largest follicle on the ovary to establish its dominance. At 18 hours there also was an increase of VEGF 120 ($P < 0.01$) compared to time points 54, 60, 66, and 72 hour, but not compared to all other time points (Figure 1).

In the 12 cows bled to determine timing of the LH surge, 50% of the cows had an LH surge between 54 and 72 hours (Figure 2). In the other half of the cows, the LH surge did not occur within the 72-hour time period and presumably had occurred after 72 hours (Figure 3). Due to this variation in occurrence of the LH surge, data collected after the 54-hour time point was deemed not representative of all cows in the study. Luteinizing hormone has been shown to affect VEGF mRNA expression, therefore cows that had LH surges would have different VEGF mRNA expression profiles when compared to cows that did not have an LH surge at the 54 to 72 hour period. Thus a second trial was conducted to more accurately synchronize the LH surge by an injection with GnRH to determine how the LH surge would affect VEGF mRNA isoforms.

In trial 2, an increase in VEGF 120 mRNA expression ($P < 0.05$) was detected at 3 hours post GnRH (Figure 4). VEGF 120 is responsible for recruiting endothelial cells to develop initial blood vessels. The increase in VEGF 120 mRNA at 3 hours may represent recruitment of endothelial cells to develop blood vessels in the developing corpus luteum. There was no difference in VEGF 164 at any of the collections after GnRH. Thus, it appears that VEGF 164 and 120 isoforms maybe independently regulated at corpus luteum regression (trial #1) and after the LH surge (trial #2). Determining the role of VEGF isoforms during the preovulatory period may allow for better 1) synchronization of ovulation for timed-AI; and 2) corpus luteum formation which may reduce embryonic mortality in beef cattle.

[1]Robin A. Ten Broeck, graduate student; Debra T. Clopton, research technician; Jeremy L. Martin, graduate student; Rebecca C. Bott, graduate student; Karl V. Moline, cow/calf manager; Jeff W. Bergman, agriculture technician; Andrea S. Cupp, assistant professor, Animal Science, Lincoln.