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Summary

VEGF normally acts to stimulate vascular development (angiogenesis) and is necessary for ovulation of the dominant follicle. The inhibitory isoform blocks the actions of VEGF angiogenic isoforms; therefore, the objectives of the experiments were to identify the bovine inhibitory isoform, VEGF164b and to determine its expression prior to and after the LH surge. VEGF164b mRNA was upregulated prior to but did not change after the LH surge. Therefore, VEGF164b may be necessary for preparation of the dominant follicle prior to ovulation.

Introduction

During deviation of the dominant follicle from the subordinate follicles, the dominant follicle develops an extensive vasculature network in the theca layer that surrounds the basement membrane. The granulosa cell layer surrounding the oocyte within the basement membrane of the follicle is avascular at this time point. VEGF is expressed by granulosa cells prior to ovulation in the bovine follicle and working via the theca cells to increase vascularization, providing nutrients to the developing follicle. VEGF aids in cell migration, proliferation, and increased microvascular permeability through the stimulation of endothelial cells which are cells that form blood vessels.

Recent studies have identified an anti-angiogenic inhibitory VEGF splice variant, VEGF164b. Vascular Endothelial Growth Factor 164b is the inhibitory isoform of bovine VEGF164. The VEGF inhibitory isoform blocks actions of VEGF pro-angiogenic isoforms.

The objective of the current study was to identify VEGF164b and to determine the expression pattern of VEGF164b in granulosa cells of the bovine follicle prior to and after the LH surge. The hypothesis was that VEGF164b isoform would be differently expressed and may be used to regulate folliculogenesis during the bovine estrous cycle.

Procedure

In the first trial, cows were administered two injections of PGF_{2α} 14 days apart and follicular aspirates were collected at 12, 18, 24, 30, 36, 48, 54, 60, and 72 hours (n=average of 8) after the second injection of PGF_{2α} to obtain granulosa cells prior to the LH surge. Messenger RNA was extracted from granulosa cells and samples were reverse transcribed to cDNA. Progesterone and estrogen concentrations were measured in follicular fluid. A ratio greater than 1 of estrogen to progesterone indicated the follicle was still dominant. The mRNA expression of VEGF164b isoform was determined using real-time quantitative polymerase chain reaction (PCR) at each follicular aspirate time point.

Blood samples were collected every two hours from 12 cows to determine when the cows had an LH surge. Since all the cows had an LH surge from 56 hours to greater than 72 hours, the granulosa cells collected prior to 56 hours were used in the analysis.

A second trial was conducted to obtain granulosa cells after a GnRH induced LH surge. Cows were injected with two injections of PGF_{2α} (25 mg/cow) and 48 hours after the second injection of PGF_{2α} administered GnRH (100 µg/cow). Follicular aspirates were collected at 3, 6, 12, 18, and 24 hours after GnRH (n=average of 10). Messenger RNA was extracted as described previously and expression of VEGF mRNA isoform 164b was analyzed using quantitative real time PCR.

Quantitative RT-PCR data for both trials were analyzed using an ANOVA with SAS. Comparisons of means were tested using a Tukey-Kramer test.

Results

Analysis of newly identified bovine VEGF164b

The VEGF164b inhibitory isoform was subcloned and sequenced in our laboratory. Conventional RT-PCR confirmed the presence of VEGF164b in bovine granulosa cells from dominant follicles (Figure 1). We speculate that every angiogenic isoform of VEGF has an anti-angiogenic isoform that regulates their functions.

Quantitative Real-time PCR (QRT-PCR) for granulosa cells

In the first trial, mRNA expression was measured at 12, 18, 24, 30, 36, 48, and 54 hours post second injection of PGF_{2α} (PG). In Trial 1, the inhibitory isoform, VEGF164b, increased at 18 hours ($P<0.05$); Figure 2.

A second trial was conducted to more accurately simulate the LH surge by an injection with GnRH and to

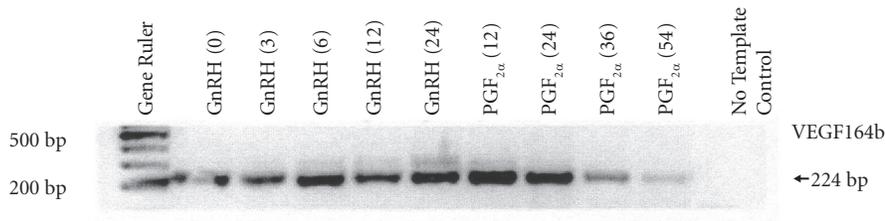


Figure 1. Conventional reverse transcription PCR to detect VEGF164b isoform in bovine granulosa cells collected at 0, 3, 6, 12 and 24 hours after GnRH injection (Trial 2) or 12, 24, 36 and 54 hours after a second injection of PGF₂ (Trial 1).

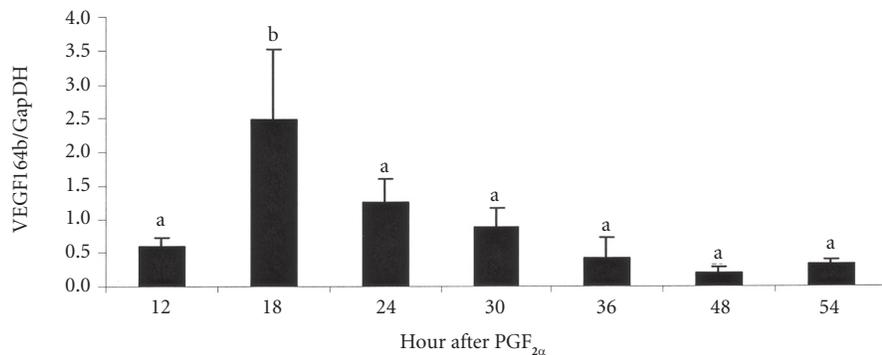


Figure 2. Expression of mRNA for anti-angiogenic VEGF164b isoform using QRT-PCR hours after second injection of PGF_{2α}. VEGF isoforms were normalized to GapDH. Messenger RNA expression at each time point is denoted by different letters.

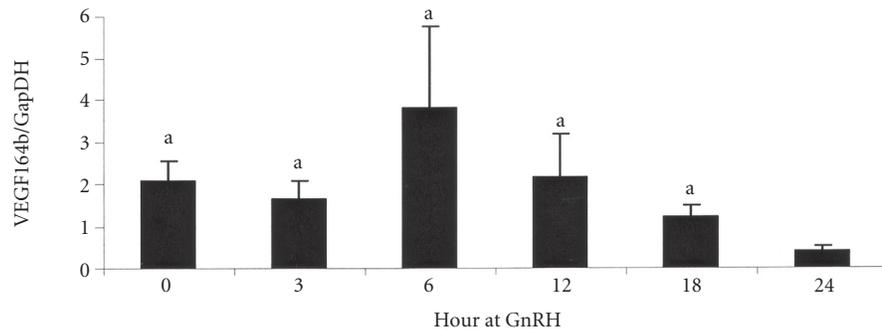


Figure 3. Expression of mRNA for anti-angiogenic VEGF164b isoform using QRT-PCR hours after second injection of GnRH (Trial 2). VEGF isoforms were normalized to GapDH. There were no differences in expression of VEGF164b mRNA as denoted by the same letter for each time point.

collect follicle aspirates after the LH surge. In the second trial, there was no difference in VEGF164b at any of the time points after GnRH (Figure 3). Thus, it appears that the inhibitory isoform may be regulated after corpus luteum regression (Trial 1) but not after the LH surge (Trial 2).

Conclusion

The current studies demonstrate VEGF mRNA isoform expression patterns prior to and after the LH surge. VEGF164b increases 18 hours after CL regression. From these results, we speculate that VEGF mRNA isoform expression is finely regulated by ovarian growth factors and steroid hormones to provide for necessary vascular development and follicle progression. These studies establish a role for VEGF anti-angiogenic isoforms prior to ovulation in the bovine follicle. It is possible that we may be able to use VEGF anti-angiogenic isoforms to manipulate follicle development to more accurately time ovulation in synchronization or superovulation protocols of beef females.

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