

Expression of some angiogenic factors in bovine follicles before and after LH surge



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INTRODUCTION

Angiogenesis is defined as the generation of new blood vessels through sprouting from already existing blood vessels in a process involving different angiogenic factor families. The aim of this study was to evaluate the expression patterns of hypoxia-inducible factor-1 alpha (HIF), vascular endothelial growth factor (VEGF) and angiopoietin (ANPT) system members (VEGF isoform 120, 164, 188AS, VEGFR-1, VEGFR-2 and ANPT-1, ANPT-2, Tie-1, Tie-2 receptors) in time defined follicle classes before and after GnRH application and after ovulation in cow.

MATERIAL AND METHODS

Ovaries containing preovulatory follicles or new corpora lutea (CL) were collected at 0h, 4h, 10h, 20h, 25h (follicles) and 60h (CL day 1-2) relative to injection of GnRH. Follicle samples were aliquoted, quickly frozen in liquid nitrogen and total RNA was isolated. Transcripts were amplified by RT-PCR and quantified relatively using real time PCR (Rotor-Gene 3000®). For better characterization of follicle classes estradiol, progesterone, prostaglandins (PGF2 α and PGE2) and VEGF concentrations were determined by EIA in follicular fluid (FF).

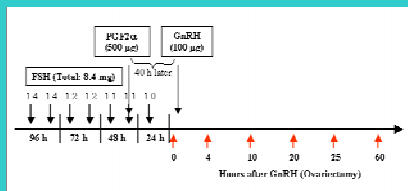


Fig 1. Time schedule of the treatment for multiple ovulation and ovary collection in cows. Ovaries containing preovulatory follicles or new corpora lutea (CL) were collected at approximately 0h, 4h, 10h, 20h, 25h (follicles) and 60h (new CL) relative to injection of GnRH to induce an LH surge (n=5 animals per group).

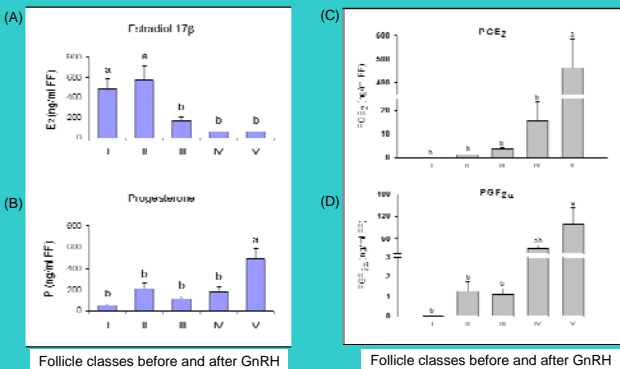


Fig 2. Concentration of (A) estradiol, (B) progesterone, (C) PGE2 and (D) PGF2 α in the FF of preovulatory follicles collected at (I) 0h, (II) 4h, (III) 10h, (IV) 20h, (V) 25h and (VI) 60h relative to injection of GnRH to induce an LH surge (n=5 animals per group). Different superscripts denote statistically different values (P<0.05).

RESULTS AND DISCUSSION

For a better understanding and characterisation of follicle classes used, estradiol, progesterone, PGF2 α and PGE2 were determined in FF. The mRNA of HIF decreased in follicles only during the periovation phase, followed by a significant increase in early CL tissue (data not shown). Transcripts of all VEGF isoforms were upregulated 4h after GnRH (during LH surge) followed by lowest expression around ovulation. The VEGF peptide content in FF increased 25h after GnRH. All VEGF isoforms as well as their receptors were upregulated again after ovulation. ANPT-1 mRNA decreased significantly in follicles during LH surge (4h after GnRH). ANPT-2 decreased 10h after GnRH and in the follicle group during ovulation. Tie-1 and Tie-2 mRNA expression decreased in follicle group during ovulation, with a further increase in early CL tissue. It is likely that the decrease of ANPT-1 and therefore the increase of the ANPT-2/ANPT-1 ratio during the LH surge is a basic mechanism of vascular remodelling in follicles during periovation. In conclusion, the temporal expression pattern of angiogenic factors ANPT and VEGF during periovation suggests them to be important mediators of the ovulatory process and the early CL formation (angiogenesis) in the cow.

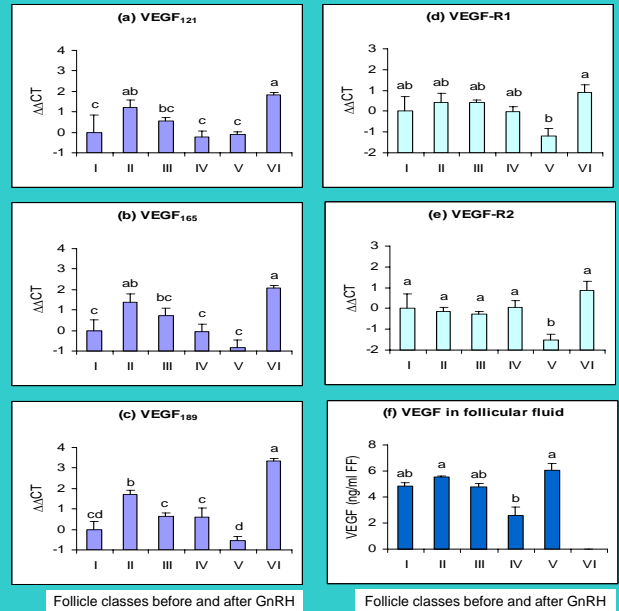


Fig 3. Expression of mRNA for VEGF system members (a,b,c,d,e) in preovulatory follicles collected at (I) 0h, (II) 4h, (III) 10h, (IV) 20h, (V) 25h (follicles) and (VI) 60h (new CL) relative to injection of GnRH to induce an LH surge (n=5 animals per group). The changes in mRNA expression for the different groups were calculated by normalization to UBQ and relative to the follicle group 0h. (f) VEGF protein concentrations in bovine follicular fluid (ng/ml FF) in preovulatory follicles. Results are presented as means+S.E.M (n=8-20 follicles/class). Different superscripts denote statistically different values (P<0.05).

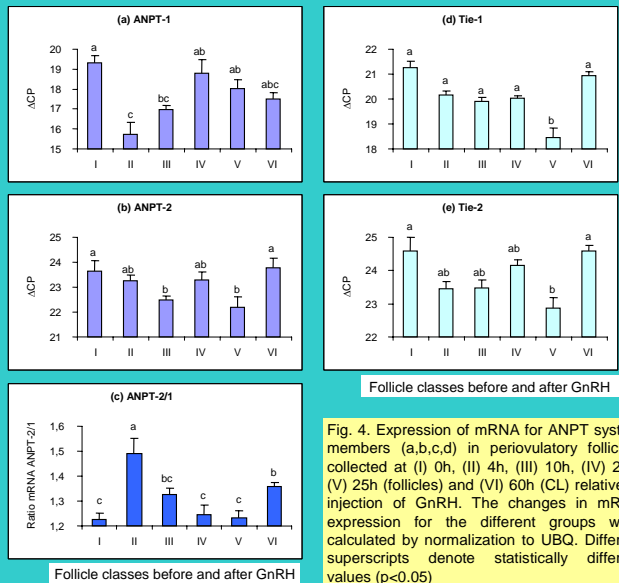


Fig 4. Expression of mRNA for ANPT system members (a,b,c,d) in preovulatory follicles, collected at (I) 0h, (II) 4h, (III) 10h, (IV) 20h, (V) 25h (follicles) and (VI) 60h (CL) relative to injection of GnRH. The changes in mRNA expression for the different groups were calculated by normalization to UBQ. Different superscripts denote statistically different values (p<0.05).