

## INTRODUCTION

Locally produced growth factors may have important modulatory roles in final ovarian follicular growth and during corpus luteum (CL) formation and function. The aim of this study was to investigate the possible participation of fibroblast growth factor family members (FGF1, FGF2, FGF7) and their receptor variants (FGFR1IIIc, FGFR2IIIb, FGFR2IIIc) in porcine follicles during final follicular growth.

## MATERIAL AND METHODS

Classifications of follicles into four groups were created based on follicle diameter (2-3, 4-5, 6-7 and >8 mm) and according to the follicular fluid (FF) estradiol-17 $\beta$  (E) content. The mRNA expression was analysed by block reverse transcription polymerase chain reaction (RT-PCR) and quantitative real-time PCR (Rotor-Gene 3000). The hormone concentration was analysed by EIA, and protein localisation by immunohistochemistry. For immunohistochemistry of FGF2 ovaries were fixed in methanol-pure acetic and embedded in paraffin. As detection system the avidin – biotin complex labeled with horseradish peroxidase was used.



Fig. 2. Agarose gel electrophoresis. Representative sample of specific RT-PCR products: (1) FGF 1; 264 bp, (2) FGF 2; 161 bp, (3) FGF 7; 291 bp, (4) FGFR 1IIIc; 125 bp, (5) FGF 2IIIb; 298 bp; (6) FGFR 2IIIc; 113 bp, (M) 100bp Marker.

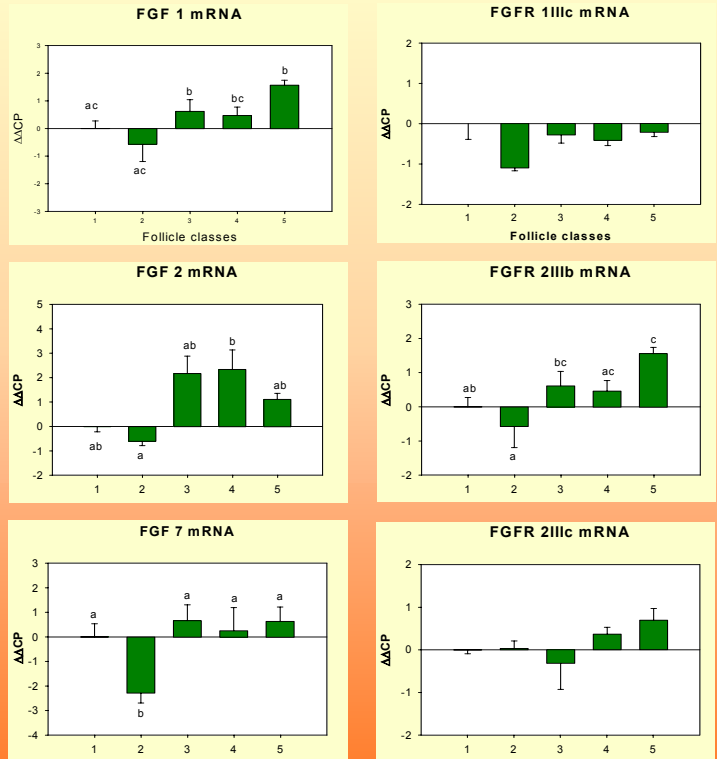
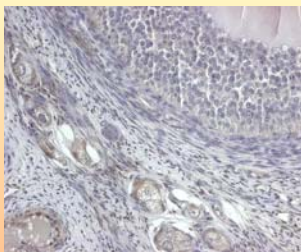
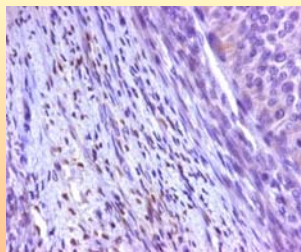


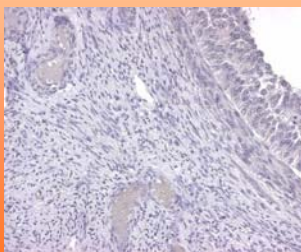
Fig. 1. The mRNA expression (RT-PCR; ΔΔCP, Housekeeper Ubiquitin) of fibroblast growth factor family members in porcine follicles of different development stage and ovary stroma tissue. (1) 2–3 mm; (2) 4–5 mm; (3) 6–7 mm; (4) 8–12 mm; (5) stroma. Data are expressed as a means ± SEM (n = 5 to 6 follicles / group; stroma: n = 5). Different superscripts denote statistical different values (p < 0.05).



FGF 2



FGF 2



Non Immune Serum

Fig. 3. Immunohistochemical localization of FGF 2 in the porcine ovary. Brown staining is visible in the nucleus and also weak in cytoplasm of stroma cells. There is also a good staining intensity in endothelial cells of blood vessels. Only weak staining in cytoplasm of basal granulosa cells.

## RESULTS AND DISCUSSION

Immunohistochemical staining for FGF2 occurred predominantly in stroma tissue, endothelial cells of blood vessel in theca interna and only weak staining in basal granulosa cells. The mRNA expression data obtained by block RT-PCR were confirmed by quantitative real-time PCR. The mRNA signals for FGF1 and FGF2 increased in large follicle groups with a significant difference. The mRNA expression of FGF7 was high already in small follicle group (2-3 mm), decreased significantly in follicle group 4-5 mm followed by a further significant increase in large follicles (>8 mm). The mRNA signal for the FGFR1IIIc and FGFR2IIIc during final follicle growth was without any regulatory change. There was a down-regulation of FGFR2IIIb mRNA expression in follicle group 4-5 mm, with further up-regulation in large follicle groups. mRNA expression in stroma tissue was mostly on a high level in comparison to mRNA expression in follicles. The different expression and localisation of FGF growth factor family members suggest that these local produced factors are involved in process of final growth of the preovulatory follicles.

## CONCLUSIONS

Fibroblast growth factor family members may be involved in the proliferation of capillaries (angiogenesis) that accompanies the selection and development of the preovulatory follicles, resulting in an increased supply of nutrients and precursors, and therefore supporting growth of the ovulatory follicles in pig ovary.