In vitro study of phoenixin-14 action on porcine luteal cells angiogenesis and apoptosis: role of GPR173 and kinases ERK1/2, AKT, AMPK pathways

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Phoenixin-14 (PNX-14) is a neuropeptide with a well-established function in reproductive processes at the hypothalamic-pituitary-gonadal axis. However, its role in the physiology of the ovary, and especially the corpus luteum (CL) function requires further research. Our previous study showed that PNX-14 and its putative receptor GPR173 are expressed in the porcine CL during the estrous cycle and PNX-14 has a luteotropic action by regulating the endocrine function of luteal cells. In the current study, we focused on understanding the role of PNX-14 in the process of angiogenesis and apoptosis occurring in the CL.

We performed *in vitro* culture of luteal cells isolated from the porcine CL from days 10-12 of the estrous cycle. Luteal cells were treated with PNX-14 at increasing doses 1-1000 nM and after 24 h of incubation, we checked the transcript level and secretion of angiogenic factors (VEGFA, bFGF2, ANG-1) as well as gene expression of apoptotic factors (caspase-3, -8, -9, Bax, Bcl-2), the activity of caspase 3/7 and level of DNA fragmentation. Additionally, using pharmacological inhibitors of extracellular signal-regulated kinases 1/2 (ERK1/2), protein kinase B (AKT), 5'AMP-activated protein kinase (AMPK) as well as silencing the GPR173 receptor by siRNA, we study the molecular mechanism of PNX-14 action on luteal cells angiogenesis and apoptosis. The results were analyzed by one-way ANOVA in GraphPad Prism 8.

We observed that PNX-14 increased the level of angiogenic factors in luteal cells: bFGF2 at all used doses and ANG-1 on gene level at 10 nM and its secretion at 1 nM. The effect of PNX-14 on bFGF secretion was mediated by GPR173 receptor and ERK1/2 and AKT. PNX-14 decreased gene expression of caspase-3, -8, and Bax, while had no effect on caspase-9 and Bcl-2 level. Additionally, caspase 3/7 activity was decreased after PNX-14 treatment, and its effect was mediated by GPR173, AKT, and AMPK. DNA fragmentation was downregulated only upon PNX-14 at 100 nM.

The current study confirmed that PNX-14 is a luteotropic factor in the CL of pigs by stimulation the process of angiogenesis and protects luteal cells against apoptosis.

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