Hypoxia-Related Regulation of Luteal Function: New Insights from Gene Expression and LPS Challenge Studies in Cattle

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Considering the role of the corpus luteum (CL) in the reproductive performance of cattle, comprehending its formation, maintenance and regression remains of utmost clinical importance. Reduced oxygen content has emerged as a significant factor influencing these processes. In this study, we explored the dynamics of CL functionality, investigating gene expression patterns of hypoxia-mediating and regulating factors during the luteal phase, and in response to *Escherichia coli* lipopolysaccharide (LPS) challenge, as well as inflammation related factors. LPS, a gram-negative bacterial endotoxin, frequently causes infertility and subfertility in livestock. When administered at 0.5µg/kg i.v., LPS affects CL function, decreases its steroidogenic capacities, and triggers systemic responses, without causing a life-threatening risk. Here: (1) CL samples were collected during different luteal stages (early, mid, and late); (2) CL biopsies from synchronized cows were obtained on day 10 of the first estrous cycle 12h after being treated i.v. with LPS or saline, and on day 10 of the subsequent cycle. Semi-quantitative real-time TaqMan PCR was performed to assess gene expression. Non-radioactive *in situ* hybridization (ISH) localized hypoxia inducible factor (HIF) 1α and its regulators. The transcript levels of *HIF1A* and its oxygen-sensitive regulators: PHD1 (*EGLN2*), PHD2 (EGLN1), PHD3 (*EGLN3*), FIH (HIF1AN), and *VHL* were assessed. The results demonstrated dynamic changes in gene expression patterns during the luteal lifespan, indicating active regulation of HIF1α transcriptional and functional availability. The luteal development was accompanied by increasing *HIF1A* transcription while it decreased in the late luteal phase. Its elevated levels at mid-luteal stage were accompanied by time-dependent expression of *EGLN2*, *HIF1AN* and *VHL*. In regressing CL, *VHL* and *EGLN3* were significantly downregulated. While the LPS challenge affected only the expression of *EGLN2* and *EGLN3* in opposite directions, with *EGLN2* being downregulated and *EGLN3* upregulated, we observed a significant increase in the response of endothelial cell-specific pro-inflammatory factors ICAM1 (*ICAM1*) and NFκB (*NFKB2*), suggesting that LPS-induced vascular inflammation occurs, with concomitant hypoxia-related effects, possibly modulated through *EGLN2* and *-3* expression. ISH revealed compartmentalization of signals regarding the distribution of HIF1α and its regulators within the bovine CL, emphasizing the cell-specific regulation of hypoxia-related factors. Large luteal cells presented predominant signals for *HIF1A*. These cells were shown to exhibit higher basal progesterone (P4) production, than small luteal cells and less responsiveness to LH stimulation, implying that the basal production of P4 in the CL and the constant provision of P4 remain under the control of HIF1α in large luteal cells. *HIF1A* also showed signals in capillary vessels, mostly in endothelial cells, as well as in the media of smaller vessels, where it was colocalized with the other regulatory factors. *EGLN2,* prevailed in vessels, whereas in luteal cells it was mostly targeted to large cells, thereby colocalized with *HIF1A*. *EGLN1* was abundantly present in larger vessels and ubiquitously distributed in small and large luteal cells, although it appeared weaker than in vessels. *EGLN3* and *VHL* followed the localization pattern of *EGLN1*, but appeared to prevail in the vessels. In conclusion, this study offers new insights into hypoxia-dependent regulation of luteal function, including the spatiotemporal distribution of signals encoding for HIF1α regulatory factors. It marks the first demonstration of HIF1α expression in the context of its regulators and reveals their dynamic cellular compartmentalization. Additionally, it broadens

our understanding of the LPS-mediated inflammatory responses in the CL, implicating possible hypoxia-mediated effects.