Expression of pro-inflammatory cytokines, chemoattractant proteins and their rezeptors in bovine follicles before and after GnRH application and ovulation

H Kliem, D Schams, B Berisha

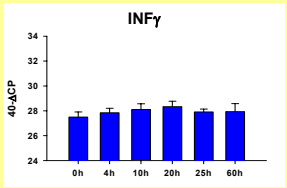
Physiology - Weihenstephan, Technical University Munich, Germany
(heike.kliem@wzw.tum.de)

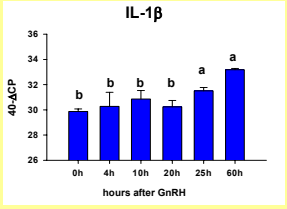
INTRODUCTION

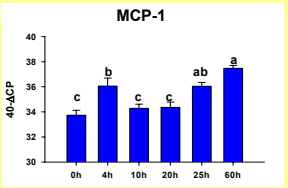
The rupture of a follicle during ovulation has been thought to be an inflammatory like process with the induction of pro-inflammatory cytokines and invasion of immune cells. The aim of this study was to evaluate the expression pattern of tumor necrosis factor α (TNFα), its two receptors (TNFα-R1, TNFα-R2), interferone γ (INFγ), interleukine-1β (IL-1β), monocyte chemoattractant protein-1 (MCP-1), its receptor CCR-2, eotaxin-3 and its receptor CCR-3 in time-defined follicle classes before and after GnRH application and after ovulation in the cow.

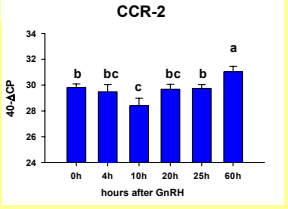
MATERIAL AND METHODS

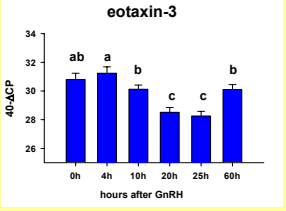
Ovaries containing preovulatory follicles or new corpora lutea (CL) were collected at approximately 0, 4, 10, 20 and 25h (follicles) and 60h (new CL) relative to injection of GnRH to induce an LH surge (n = 5 animals per group). The mRNA expression was evaluated by a two step real time PCR (Rotor-Gene 3000) and the data were normalised with the mean value of the three housekeeping genes ubiquitin, GAPDH and histone.











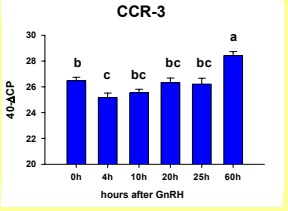
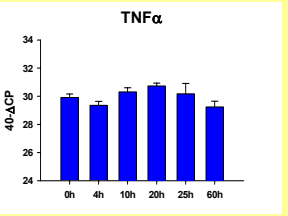
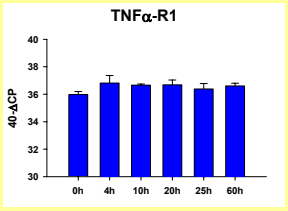


Fig. 1: mRNA expression data of INFγ, IL-1β, MCP-1, CCR-2, eotaxin-3 and CCR-3 in bovine follicles during different time points before (0h) and after GnRH application. 4h indicates the LH surge, 25h the ovulation and 60h the new CL (n=5/group); data are normalised to the mean value of ubiquitin, GAPDH and histone (ΔCP) and are expressed as 40 minus mean of normalised crossing point ± SEM. Different superscript letters indicate significant differences ($P < 0,05$).





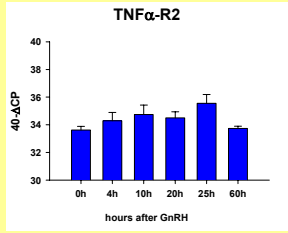


Fig. 2: mRNA expression data of TNFα and its two receptors in bovine follicles during different time points before (0h) and after GnRH application. 4h indicates the LH surge, 25h the ovulation and 60h the new CL (n=5/group); data are normalised to the mean value of ubiquitin, GAPDH and histone (ΔCP) and are expressed as 40 minus mean of normalised crossing point ± SEM. Different superscript letters indicate significant differences ($P < 0,05$).

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RESULTS

TNFα, its two receptors and INFγ showed no regulation, whereas IL-1β revealed an up-regulation at 25h (around ovulation) and 60h (new CL). The expression of MCP-1 was increased during the LH surge (4h) and further on around ovulation (25h) and in the new CL (60h). Its receptor CCR-2 was down-regulated 10h after GnRH application and increased again till its maximum expression at 60h. In contrast to MCP-1 showed eotaxin-3 its highest expression at 0h and 4h (LH surge) followed by a decrease at 20h and 25h (around ovulation) after GnRH injection. Its receptor CCR-3 was decreased around ovulation (25h) and increased in the new CL (60h).

DISCUSSION AND CONCLUSIONS

Of this data only IL-1β and MCP-1 seem to play an important role during ovulation, where macrophages seem to be actively involved in the tissue remodeling around the ovulation side. The increased expression of eotaxin-3 during the LH surge could trigger the invasion of eosinophils into the ovulating follicle, which might be necessary for an optimal angiogenesis in the developing CL.

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