Quantitative mRNA expression of the IGF-system members during induced luteolysis in the bovine.

TP Neuvians, MW Pfaffl, D Schams, B Berisha

Physiology Weihenstephan, Technical University Munich, Weihenstephaner Berg 3, D-85350 Freising-Weihenstephan, Germany

Background

Luteolysis is a very complex process of tissue regression. Functional luteolysis is characterised by a rapid decrease of serum progesterone levels within the first 8-12 h of prostaglandin (PG) F2α-induced luteolysis. Structural luteolysis is associated with weight loss, tissue remodelling, apoptosis and oligonucleosome formation, which is seen at 24 and 48 h after PGF2α-induced luteolysis.
Growth hormone (GH) acts in the body mainly via somatomedins, insulin-like growth factor I (IGF I) and IGF II, but also via its own receptor GH-R. IGFs are luteotropic and have anti-apoptotic effects on the corpus luteum (CL). Their actions are influenced by IGF-binding proteins (IGFBP), which can stimulate and inhibit IGFs and can also have IGF-independent, intrinsic properties.

Goal

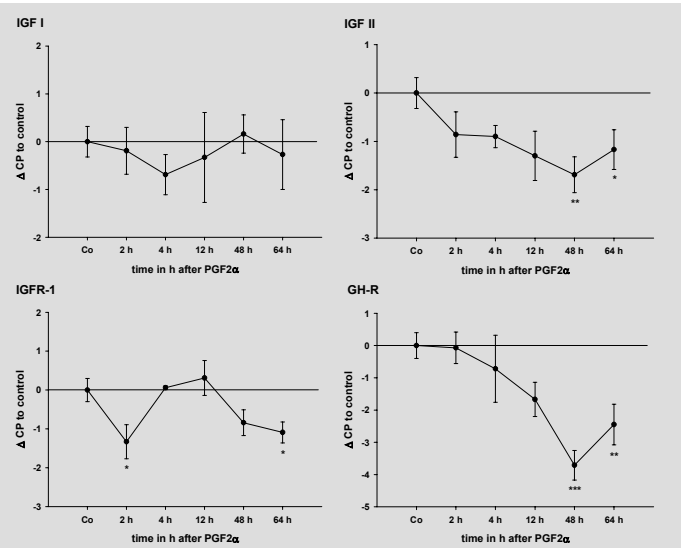
We studied the pattern of mRNA-Expression of the IGF-system members during induced luteolysis to further elucidate the role of local regulation factors, such as IGFs, in the bovine corpus luteum.

Material and Methods

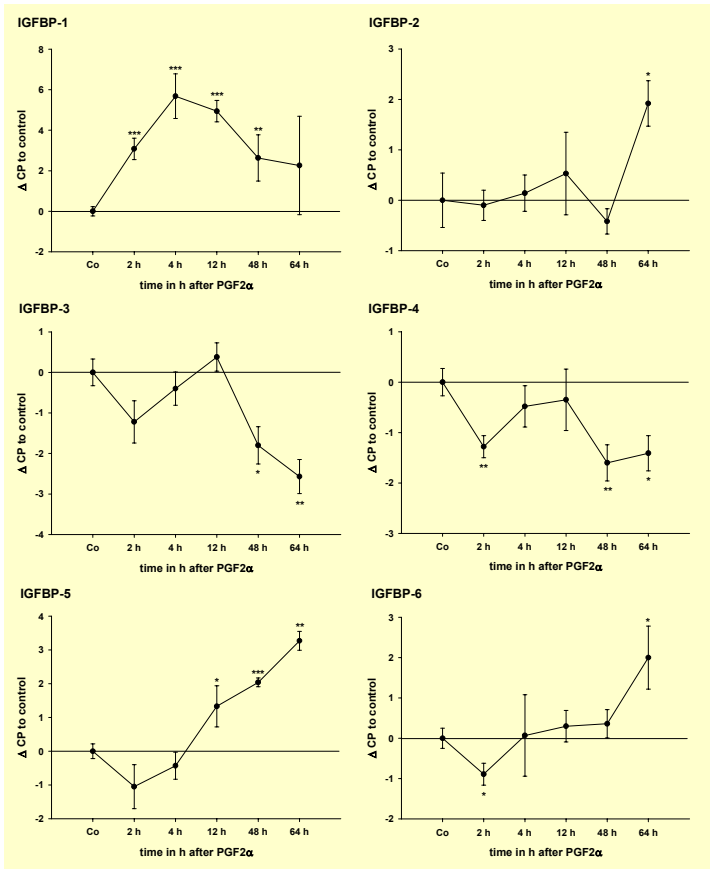
Collection and processing of bovine CL
Cows at the mid-luteal phase (days 8-12) were injected i.m. with 500µg of the PGF2α-analogue cloprostenol. CL were then collected by transvaginal ovariectomy 2, 4, 12, 48 and 64 h (n = 4-5/group) after PGF2α-injection. Control CL were obtained from cows at the mid-luteal phase (n = 5) before PGF2α-injection. All CL were immediately frozen in liquid nitrogen. Total RNA was extracted with peqGOLD TriFast.

Progesterone Determination
The concentration of peripheral blood progesterone (P) levels was measured after extraction with petrolether using an enzyme immunoassay applying the second antibody technique.

RT-PCR
1 µg of total RNA was reverse-transcribed to cDNA with 200 units of M-MLV Reverse Transcriptase. Real-time PCR was performed using the LightCycler Fast Start DNA Master SYBR Green I Kit.
Efficiency corrected Crossing Points were acquired with the Second Derivative Maximum Method. The data were analysed by the Relative Expression Software Tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR.
Expression Data were normalised by Ubiquitin as a reference gene, which showed no significant changes in mRNA expression during induced luteolysis.



Expression data (mRNA) for IGF I, IGF II, IGF-R1 and GH-R during induced luteolysis
Data are shown as mean of crossing point difference (ΔCP) ± SEM between control group (Co = days 8-12, before PGF2α) and the following times after PGF2α (n = 4-5/stage); significances are indicated in relation to the control group; * = P < 0.05, ** = P < 0.01, *** = P < 0.001; the CP is the cycle number, where the acceleration of the fluorescence increase is maximal.



Expression data (mRNA) for IGFBP-1, -2, -3, -4, -5 and -6 during induced luteolysis
Data are shown as mean of crossing point difference (ΔCP) ± SEM between control group (Co = days 8-12, before PGF2α) and the following times after PGF2α (n = 4-5/stage); significances are indicated in relation to the control group; * = P < 0.05, ** = P < 0.01, *** = P < 0.001; the CP is the cycle number, where the acceleration of the fluorescence increase is maximal.

Results and Discussion

• Plasma progesterone levels decreased at 12 h after PGF2α and were below the basal level of 1.0 ng/ml at 48 h after PGF2α. This confirmed the efficiency of induced luteolysis.

• The 9 x down-regulation of GH-R during induced luteolysis could be a cause for the decline in progesterone plasma levels, but could also be a consequence of the degradation of the receptor expressing luteal and endothelial cells.

• The 3 x down-regulation of IGF II may contribute to a diminished cell survival of luteal cells and the nourishing vascular system. The slight up-regulation at 64 h may reflect its involvement in tissue remodelling of structural luteolysis.

• Down-regulation of IGF-R1 also represents the decreasing support of luteal survival factors. The slight increase at 4 and 12 h might be an attempt of counter-regulation to prevent luteolysis in the case of recovery or persistence of the CL.

• The 34 x increase of IGFBP-1 expression suggests a potentially inhibiting action on progesterone secretion during functional luteolysis by sequestering the stimulating IGFs.

• Both IGF-stimulating and –inhibiting, as well as intrinsic actions were described for IGFBP-5. The 11 x increase of IGFBP-5 mRNA during structural luteolysis seems to be associated with growth arrest and apoptosis.

• The role of IGFBP-3 and IGFBP-4 is discussed to be a kind of storage pool, which releases IGFs when necessary. Thus, a down-regulation (5 x and 3 x, respectively) would reduce the availability of IGFs. Whereas cell-associated IGFBP-3 stimulates IGF-effects, both soluble forms have inhibiting effects on IGF-action. Their role in bovine luteolysis is not clear and has to be further investigated.

• IGFBP-2 and IGFBP-6 both show an up-regulation (4 x and 3 x, respectively) during structural luteolysis. Both binding proteins inhibit mitogenic stimulation of IGF II on tumour growth and may have similar effects on the bovine corpus luteum.

In **Conclusion**, this study shows, that the fine tuning of IGFBPs is an important aspect in this complex physiological procedure of luteal regression. Although mRNA expression may not necessarily reflect protein concentrations, which are the actual biological effectors, the data can provide a useful basis for further study of luteal regression.

Supported by DFG (Scha257/14-2)