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The mRNA expression of the members of the IGF-system in bovine corpus luteum during induced luteolysis

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Abstract

The components of the IGF-system were shown to be differentially regulated in bovine antral follicles and corpora lutea (CL) during different stages of the estrous cycle, and to have important functions for specific stages. The aim of this study was to investigate the detailed pattern of mRNA expression of most constituents of the IGF-system and their possible involvement in prostaglandin (PG)F2α-induced luteolysis in the bovine CL. Therefore, cows in the mid-luteal phase (days 8–12) were injected with the PGF2α-analogue Cloprostenol, and CL were collected by transvaginal ovariectomy at 2, 4, 12, 48 and 64 h after PGF2α-injection. Real-time RT-PCR using SYBR Green I detection was employed to determine mRNA expressions of the following factors: ubiquitin (UBQ), insulin-like growth factor I (IGF I), IGF II, IGF-receptor type 1 (IGFR-1), growth hormone receptor (GH-R) and IGF-binding proteins-1–6 (IGFBP-1–6). Total extractable RNA decreased with ongoing luteolysis. IGFBP-1 mRNA was significantly up-regulated at 2 h after PGF2α and maximal at 4 h with a 34-fold increase. IGFBP-5 mRNA was significantly up-regulated after 12 h with a maximum of an 11-fold increase at 64 h. For GH-R, IGFR-1, IGF II, IGFBP-3 and -4 mRNA expression, we found a significant down-regulation in certain stages. There was a significant up-regulation for IGFBP-2 and -6 mRNA at 64 h after induced luteolysis. There were no significant changes in IGF I mRNA expression. In conclusion, the IGF-system with all its components seems to play an important role in the very complex process of PGF2α-induced luteolysis in bovine CL.
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Keywords: IGF; IGFBP; Bovine; Corpus luteum; Real-time RT-PCR

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1. Introduction

After ovulation, the corpus luteum (CL) develops within a few days to a highly secretory endocrine gland, producing the pregnancy sustaining hormone progesterone. The bovine CL consists of many different cell types: 52.3% endothelial cells and pericytes, 26.7% small luteal cells, 10% fibrocytes, 3.5% large luteal cells and 7.5% other cells (e.g. plasma cells, lymphocytes, leukocytes and other unidentified cells) [1]. Luteolysis is a very complex process of tissue regression. Functional luteolysis is characterized 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 and apoptosis. Weight loss, degeneration of luteal cells and pronounced oligonucleosome formation are seen at 24 and 48 h after PGF2α-induced luteolysis [2]. During ongoing luteolysis leucocytes and macrophages, which are responsible for the phagocytosis of cells and cell remnants [3], invade the corpus luteum. At late regression, proliferating leucocytes account for 70% of the total number of proliferating cells, mainly due to an increase in macrophages [4]. In the final regression stage, the CL becomes a non-vascularized scar out of connective tissue, which is deplete of cells. Thus, the CL undergoes drastic changes in tissue composition during regression.

Growth hormone (GH) acts in the body mainly via so called somatomedins, insulin-like growth factor I (IGF I) and insulin-like growth factor II (IGF II), but can also exert its effects by its own receptor GH-R. GH stimulates dose-dependently the progesterone release in bovine CL in vitro [5,6]. IGF I and IGF II are growth factors, that are involved in cell proliferation, mitogenesis and angiogenesis. IGFs protect different cell types against apoptosis including ovarian cells [7]. In luteal tissue IGF I and IGF II have stimulatory effects on progesterone secretion in rats [8], sheep [9], pigs [10] and cattle [11,12]. IGF I is nearly exclusively found in large luteal cells (LLC) and small luteal cells (SLC) and in a limited number of endothelial cells, whereas IGF II cannot be identified in LLC or SLC, but in perivascular fibroblasts of large blood vessels and pericytes of capillaries, as well as in fibroblasts in fine interlobular connective tissue [13]. GH-R, IGF I and IGF II mRNA expression was shown to be differentially regulated during estrous cycle and pregnancy in the bovine ovary [14,15]. Schams et al. [14,15] demonstrated a significant up-regulation of IGF I mRNA expression during early angiogenesis (day 1–4), whereas Woad et al. [16], beginning CL collection on day 5, did not find this up-regulation during early luteal phase.

IGF-binding proteins (IGFBP) regulate the free IGF-concentration and influence the interaction between IGF and its receptors. They can stimulate and inhibit IGF-function and act as a storage pool for IGFs [17,18]. Some IGFBPs also seem to have IGF-independent properties, e.g. IGFBP-5 [19], or IGFBP-specific receptors, e.g. for IGFBP-3 [20,21]. The mRNA of all IGFBPs was detected in the bovine CL with differential regulation in IGFBP-3, -4 and -5 during estrous cycle and pregnancy [14,15]. In bovine luteal cells, IGFBP-2 and -3 inhibited IGF I-binding to its receptor and blocked the stimulatory effect of IGF I on progesterone secretion [22].

We suppose, that a subtle regulation of all components of the IGF-system plays an important role in luteolysis, which is characterized by arresting cell survival. The analysis of mRNA expressions during induced luteolysis allows us to draw conclusions concerning the possible function of the investigated members of the IGF-system.

2. Materials and methods

2.1. Collection of bovine corpora lutea (CL)

Cows at the mid-luteal phase (days 8–12) were injected i.m. with 500 μg of the PGF2α-analogue Cloprostenol (Estrumate®, Intervet, Germany). The CL were collected by transvaginal ovariectomy 2, 4, 12, 48 and 64 h ($n = 4$–5) after PGF2α-injection. Control CL were obtained from cows at the mid-luteal phase (days 8–12, $n = 5$) before PGF2α-injection. All CL were aliquoted, immediately frozen in liquid nitrogen and stored at −80 °C until RNA extraction.

2.2. Progesterone determination

Blood samples for progesterone (P) determination were taken from the jugular vein. The concentration of P in the blood plasma was measured after extraction with petrolether with an enzyme immunoassay using the second antibody technique [23]. Progesterone-6β-hydroxy-hemisuccinate-horseradish peroxidase was used as enzyme solution. The effective dose for 50% inhibition (ED_{50}) of the assay was 6 ng/ml. The intraassay coefficient of variation (CV) was 4–5% and the interassay CV 8–9%, respectively.

2.3. Total RNA extraction and reverse transcription

Small slices of deep frozen CL were cut and weighed. Total RNA from CL was extracted with peqGOLD TriFast (PeqLab, Erlangen, Germany) according to the manufacture's instructions. RNA was dissolved in water and spectroscopically quantified at 260 nm. The integrity of RNA was verified by optical density (OD) absorption ratio $OD_{260\,nm}/OD_{280\,nm}$ between 1.8 and 2.0, and by electrophoresis with ethidium bromide staining on a 1% denaturating agarose gel. Constant amounts of 1000 ng of total RNA were reverse transcribed to cDNA with 200 units of M-MLV Reverse Transcriptase (Promega corp., Madison, USA) according to the manufacturer's instructions.

2.4. Real-time PCR quantification

Primers were designed using the EMBL database (ubiquitin) or used according to literature [24,25]. Their sequences and expected PCR product length are shown in Table 1. Most of the primers [24] were multiple species primers, derived from bovine, ovine, human or mouse sequences, and produced an amplification product, which spanned at least two exons in a highly conserved coding region. These primers include all known alternatively spliced mRNA variants [24]. A master-mix of the following reaction components was prepared to the indicated end-concentrations: 6.4 μl water, 1.2 μl $MgCl_2$ (4 mM), 0.2 μl forward primer (4 μM), 0.2 μl reverse primer (4 μM) and 1.0 μl LightCycler Fast Start DNA Master SYBR Green I (Roche Diagnostics, Mannheim, Germany). Nine microlitres of the master-mix was put in glass capillaries and 1 μl PCR template containing 25 ng reverse transcribed total RNA was added. To ensure an accurate quantification of the desired product, a high temperature fluorescence measurement in a fourth segment of the PCR run was

Table 1
Forward (For) and reverse (Rev) primer sequences ($5' \rightarrow 3'$)

Primer	Sequence ($5' \rightarrow 3'$)	Length (bp)	Reference
UBQ	For AGATCCAGGATA AGGAAGGCAT Rev GCTCCACCTCCAGGGTGAT	198	Accession number Z18245
IGF I	For TCG CAT CTC TTC TAT CTG GCC CTG T Rev GCA GTA CAT CTC CAG CCT CCT CAG A	240	Pfaffl et al. [24]
IGF II	For GAC CGC GGC TTC TAC TTC AG Rev AAG AAC TTG CCC ACG GGG TAT	205	Pfaffl et al. [24]
IGFR-1	For TTA AAA TGG CCA GAA CCT GAG Rev ATT ATA ACC AAG CCT CCC AC	314	Pfaffl et al. [24]
GH-R	For CCA GTT TCC ATG GTT CTT AAT TAT Rev TTC CTT TAA TCT TTG GAA CTG G	138	Pfaffl et al. [24]
IGFBP-1	For TCA AGA AGT GGA AGG AGC CCT Rev AAT CCA TTC TTG TTG CAG TTT	123	Pfaffl et al. [24]
IGFBP-2	For CAC CGG CAC ATG GGC AA Rev GAA GGC GCA TGG TGG AGA T	136	Pfaffl et al. [24]
IGFBP-3	For ACA GAC ACC CAG AAC TTC TCC TC Rev GCT TCC TGC CCT TGG A	194	Pfaffl et al. [24]
IGFBP-4	For GCC CTG TGG GGT GTA CAC Rev TGC AGC TCA CTC TGG CAG	342	Plath-Gabler et al. [25]
IGFBP-5	For TGC GAG CTG GTC AAG GAG Rev TCC TCT GCC ATC TCG GAG	257	Plath-Gabler et al. [25]
IGFBP-6	For AGA AAG AGG ATT TGC CTT Rev TCC GGT AGA AGC CCC TAT	324	Plath-Gabler et al. [25]

RT-PCR product length and reference of the investigated factors or of the according accession number in the EMBL-database.

performed [26]. The elevated temperature for fluorescence acquisition results in melting of unspecific products, e.g. primer dimers, and eliminating non-specific fluorescence signals. The following general real-time PCR protocol was employed: denaturation for 10 min at 95 °C, 40–45 cycles of a four segmented amplification and quantification program (factor-specific conditions are summarized in Table 2), a melting step by slow heating from 60 to 99 °C with a rate of 0.1 °C/s and continuous fluorescence measurement, and a final cooling down to 40 °C. Crossing Point (CP) values were acquired by using the "Second Derivative Maximum" method of the LightCycler software 3.3 (Roche Diagnostics). All CPs of the 32 samples ($n = 4$–5 per group) per investigated factor were detected in one run to eliminate interassay variance. Real-time PCR efficiencies were acquired by amplification of a standardized dilution series and the given slopes in the LightCycler Software 3.3 (Roche Diagnostics). The corresponding efficiencies (E) were then calculated according to the equation: $E = 10^{[-1/\text{slope}]}$ [27]. The specificity of the desired products in bovine CL was documented with a high resolution gel electrophoresis and analysis of the melting temperature, which accorded to previously published results [24,25]. For specific melting temperatures and PCR efficiencies see Table 3.

Table 2
Factor-specific conditions for LightCycler real-time PCR amplification and quantification of the investigated factors[a]

Factor	Denaturation, 15 s (°C)	Annealing, 10 s (°C)	Elongation, 20 s (°C)	Flourescence acquisition, 5 s (°C)	Cycle number
UBQ	95	60	72	86	40
IGF I	95	62	72	88	40
IGF II	95	62	72	88	40
IGFR-1	95	63	72	84	40
GH-R	95	58	72	76	45
IGFBP-1	95	58	72	82	45
IGFBP-2	95	58	72	88	45
IGFBP-3	95	58	72	86	45
IGFBP-4	95	66	72	89	45
IGFBP-5	95	64	72	92	40
IGFBP-6	95	64	72	88	45

[a] Ubiquitin (UBQ), insulin-like growth factor I (IGF I), IGF II, IGF-receptor 1 (IGFR-1), growth hormone receptor (GH-R) and IGF-binding proteins (IGFBP)-1–6.

2.5. Statistical analysis

The statistical significance of differences in mRNA expression of the examined factors was analyzed by the Relative Expression Software Tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR [28]. Therefore, significant differences were calculated by a pair-wise fixed reallocation test. The software computes an expression ratio in regard to the control group (here: days 8–12 of estrous cycle, before PGF2α-injection) and is normalized by a reference gene (ubiquitin). The mRNA expression data of ubiquitin showed no significant changes to the control group during any of the investigated stages of induced luteolysis. Thus, it was de-

Table 3
Product-specific melting temperature, real-time PCR efficiency in bovine CL, mean ($n = 31$) coefficient of variation in percentage (CV (%)) and range of crossing points (CP) of the investigated factors[a]

Factor	Melting temperature (°C)	PCR efficiency	Mean CV (%)	CP range
UBQ	88.5	1.72	1.57	19.22–23.19
IGF I	90.4	1.69	3.30	27.59–31.42
IGF II	90.8	1.88	1.89	21.54–25.52
IGFR-1	87.4	1.87	1.47	23.17–27.68
GH-R	78.5	1.77	5.14	25.13–32.10
IGFBP-1	85.3	1.85	24.8	24.59–35.33
IGFBP-2	90.9	2.11	2.46	28.47–33.09
IGFBP-3	88.2	1.83	5.87	27.13–36.47
IGFBP-4	92.5	1.84	2.53	28.88–34.41
IGFBP-5	93.9	2.15	3.22	23.52–29.66
IGFBP-6	90.9	1.62	5.02	27.00–33.52

[a] Ubiquitin (UBQ), insulin-like growth factor I (IGF I), IGF II, IGF-receptor 1 (IGFR-1), growth hormone receptor (GH-R), IGF-binding proteins (IGFBP)-1–6.

termined to be suitable as a reference gene. REST also indicates coefficients of variation in percentage (mean values for CV (%) in Table 3) and standard deviations based on CPs of the target gene. The data are shown as the mean difference (Δ) $\pm$ S.E.M. of CPs between the control group and the following stages of PGF2α-induced luteolysis. A positive ΔCP means an earlier increase of fluorescence and therefore a higher concentration of the target gene. As the PCR amplification is a process with exponential character, a difference of 2 CPs signifies approximately a regulation by factor $E^{\Delta \mathrm{CP}}$ (with E: efficiency) and is indicated in the text according to the expression ratio calculated by REST.

3. Results

3.1. Progesterone blood levels during induced luteolysis

Peripheral blood levels of progesterone before PGF2α-injection averaged (mean$\pm$S.E.M.) 5.10 ± 1.38 ng/ml plasma and decreased 12 and 48 h after PGF2α application to 1.6 ± 0.65 ng/ml and 0.55 ± 0.43 ng/ml, respectively. Progesterone levels <1.0 ng/ml are basal levels and accord to the phase of the regressing CL, demonstrating the efficiency of induced luteolysis. The decrease of progesterone levels at 12 h after PGF2α application reflects the so called functional luteolysis.

3.2. Extractable RNA

The extractable RNA amount was dependent on the stage of luteolysis. With proceeding time, extractable RNA was reduced from a mean yield of 1.69 μg/mg wet weight before induced luteolysis to a mean yield of 1.06 μg/mg at 64 h after PGF2α. That signifies that there was a decline of 37% in total RNA yield in dependency of the stage of luteolysis, which was highly significant ($P < 0.01$) (Fig. 1).

3.3. Expression of IGF, IGF-receptor and GH-receptor during luteolysis

Expression data during induced luteolysis are shown as difference of CP in comparison to expression data of the control group (Co) before PGF2α-administration.

Expression ratios for IGF I were not significantly different to the control (Fig. 2). IGF II mRNA was continuously down-regulated during luteolysis, maximally and significantly three-fold at 48 h after PGF2α. At 64 h after PGF2α, IGF II mRNA was still significantly down-regulated, but to a lesser extent than at 48 h. The first detectable fluorescence increase for IGF II was six cycles earlier than that for IGF I, which means $\Delta \mathrm{CP} = 6$. Considering the efficiency of 1.88 for IGF II, this accords to a 44-fold ($E^{\Delta \mathrm{CP}}$) concentration of IGF II. The mRNA expression for IGFR-1 was significantly and maximally down-regulated (factor 2.2) at 2 h, increased slightly from 4 to 12 h and decreased significantly in the further progression of luteolysis (factor 2.1). The mRNA expression for GH-R decreased continuously during luteolysis, with significant changes to the control group at 48 and 64 h with a maximal down-regulation (nine-fold) at 48 h.

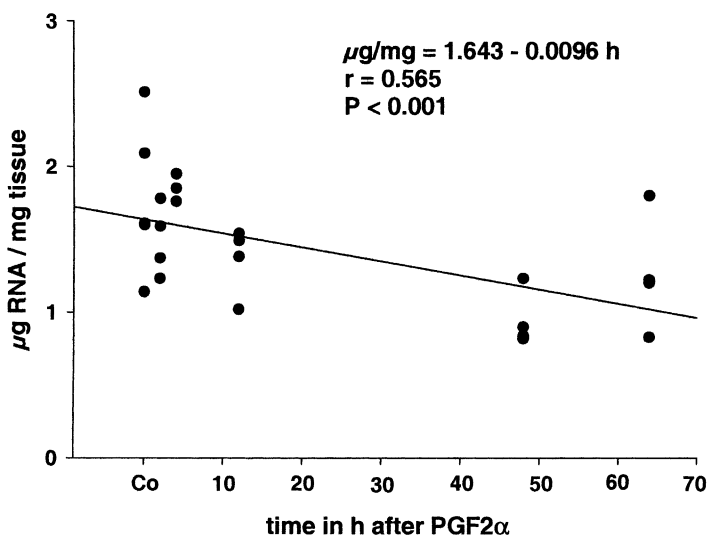

Fig. 1. RNA yield in microgram per milligram wet weight of corpus luteum tissue; data are shown as single data points with a regression over all data ($r = 0.583$, $P < 0.01$) during the progression of luteolysis.

3.4. Expression of IGFBPs during luteolysis

There was a massive up-regulation for IGFBP-1 mRNA expression, which became significant at 2 h and maximal at 4 h after induced luteolysis. The maximal up-regulation was 34-fold. The up-regulation was highly significant during the whole process of luteolysis except at 64 h (Fig. 3). The mRNA expression for IGFBP-2 showed an increasing tendency during luteolysis with a maximal and significant up-regulation by a factor of 3.8 at 64 h. At 48 and 64 h after PGF2α, IGFBP-3 mRNA was significantly down-regulated, maximally 4.8-fold at 64 h. The mRNA expression for IGFBP-4 decreased during luteolysis, significantly at 2, 48 and 64 h. The maximal down-regulation was 2.6-fold. At 4 and 12 h after induced luteolysis IGFBP-4 transcripts tended to be less down-regulated than during the other luteolytic stages. Expression data for IGFBP-5 were up-regulated during luteolysis. The up-regulation was significant at 12, 48 and 64 h and maximal 11-fold at 64 h. IGFBP-6 mRNA was variable during luteolysis. Foremost, it was significantly down-regulated at 2 h after PGF2α by a factor of 1.6. Maximal and significant up-regulation by a factor of 2.7 was found at 64 h. IGFBP-5 and -1 were more strongly expressed than IGFBP-6 and -3. IGFBP-2 and -4 were least expressed in bovine CL during luteolysis.

4. Discussion

The significant decrease of extractable total RNA during luteolysis, that accounts for 37%, seems to be physiological and reasonable, since the CL is converted from a highly active endocrine gland to a scar consisting of connective tissue, which is poor in cells and

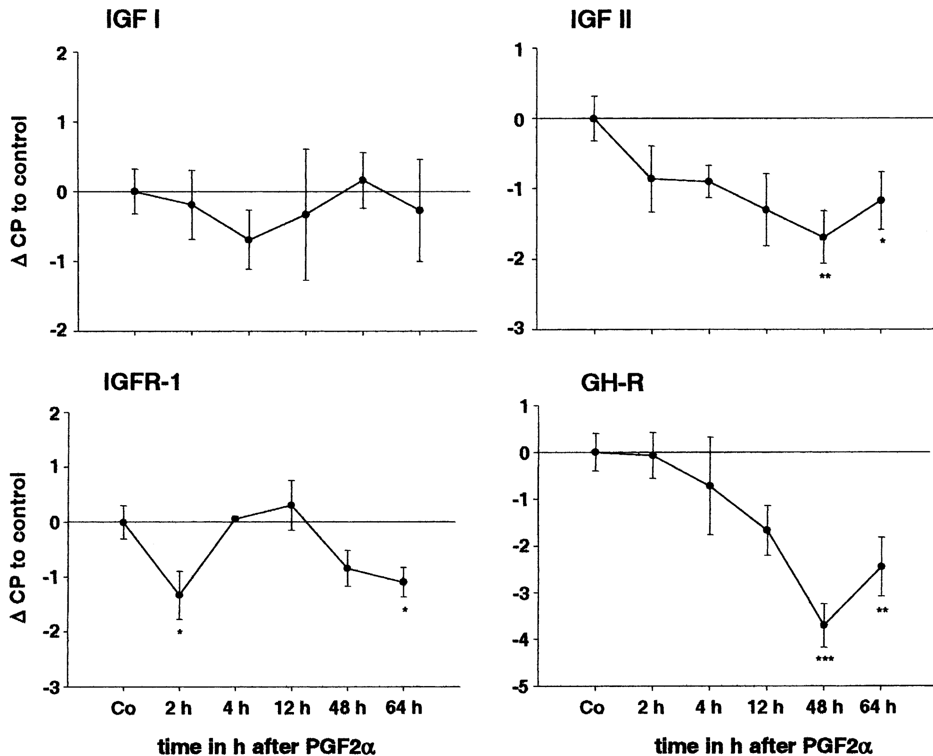

Fig. 2. Expression data (mRNA) for IGF I, IGF II, IGFR-1 and GH-R in bovine corpus luteum before (control group, Co) and after PGF2α-induced luteolysis on days 8–12; data are shown as mean of crossing point difference (ΔCP) ± S.E.M. between the control group and the following times in hours after PGF2α-administration ($n = 4$–5 per stage); significances are indicated in relation to the control group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

vessels. Thus, the fewer the number of active cells, the lower the amount of extractable RNA. We conclude, that the reorganization of the changing tissue composition during luteolysis is the reason for the decreasing amount of extractable RNA.

In the bovine CL in vitro, GH dose-dependently stimulates the progesterone release, mainly in the mid- and late-luteal phase [5,6]. During the estrus cycle GH-R expression is significantly up-regulated from days 5 to 18 when compared with days 1–2 and decreases during regression of the CL [14]. After induced luteolysis the decrease of GH-R mRNA becomes significant at 48 h after PGF2α and is notedly down-regulated during structural luteolysis. GH-R mRNA and protein are localized in large luteal cells and endothelial cells [29]. The down-regulation of GH-R during induced luteolysis could be a cause for the decline in progesterone plasma levels, since an important stimulant for progesterone release [5,6,30] cannot act anymore, but it could also be just the consequence of degradation of the cells, which are expressing this receptor.

Both IGF I and IGF II support the function of the bovine CL by anti-apoptotic effects and stimulation of progesterone secretion [11,12]. In our study IGF II is about 40-fold more

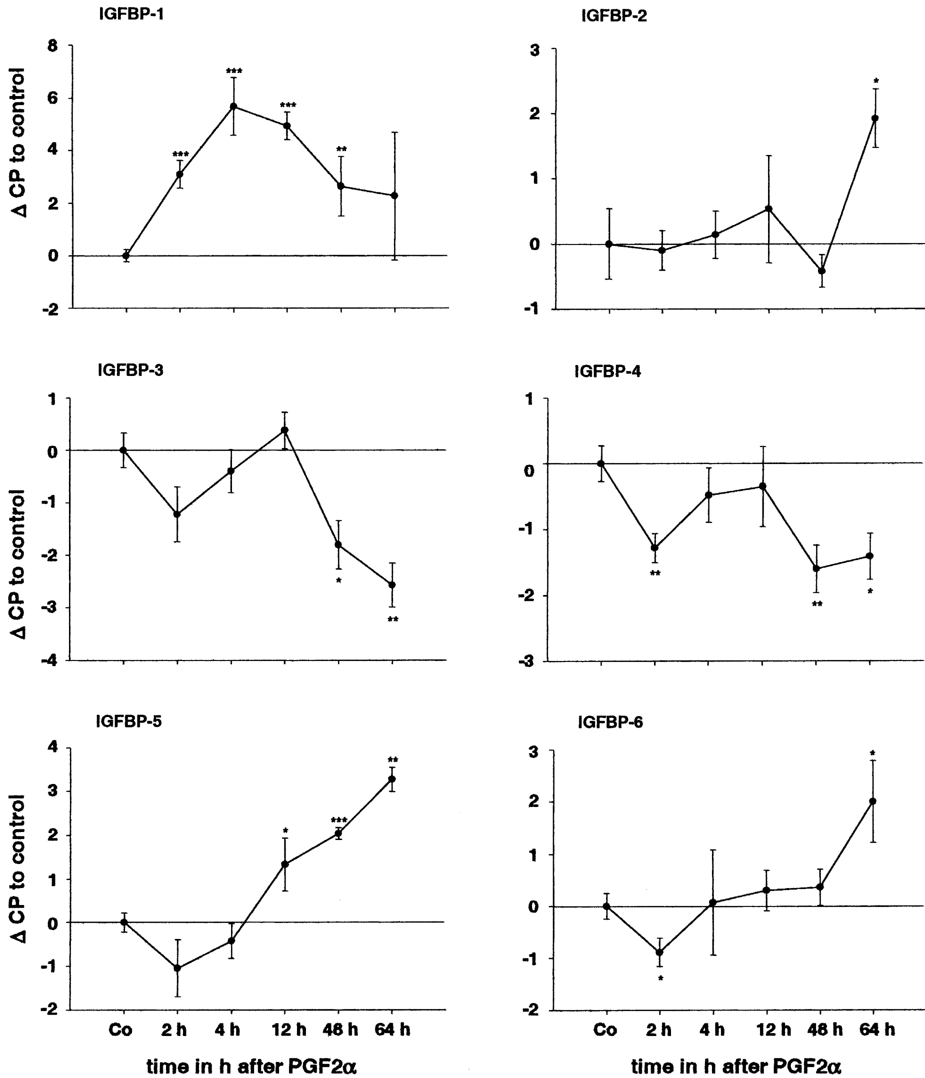

Fig. 3. Expression data (mRNA) for IGFBP-1–6 in bovine corpus luteum before (control group, Co) and after PGF2α-induced luteolysis on days 8–12; data are shown as mean of crossing point difference (ΔCP) ± S.E.M. between the control group and the following times in hours after PGF2α-administration ($n = 4$–5 per stage); significances are indicated in relation to the control group; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

strongly expressed in bovine luteal tissue than IGF I, and even slight regulation may have a relevant effect on luteal function. In contrast to Woad et al. [16], we found a significant three-fold down-regulation for IGF II mRNA especially during structural luteolysis. Probable reasons could be the applied methods, RT-PCR versus in situ hybridization, and the high sensitivity of the LightCycler-PCR. The down-regulation of IGF II can contribute to a

diminished cell survival of luteal cells. As Amselgruber et al. [13] assume for IGF II a major role in coordinating angiogenic processes and vessel maintenance, the loss of IGF II during luteolysis may affect primarily the nourishing vascular system. IGF II does not only seem to play an important role in embryonic growth and prenatal development [31], but also in other fast proliferating tissues like the CL. The reason for the unchanged expression level of IGF I may be its benefit for the reorganization of luteal tissue and the stimulation of immune cells. Einspanier et al. [32] detected the highest mRNA expression and protein concentration for IGF I on days 12–17 of the bovine estrous cycle and no significant changes between days 6–11 and 18–21, when spontaneous regression occurs. In our study, mRNA expression during induced luteolysis is compared to the expression on days 8–12. Thus, the observation of a significant decrease of IGF I during regression could be missed, because of the lack of the IGF I increase in the late-luteal phase (days 12–17). In contrast to our findings and those of Einspanier et al. [32], the mRNA expression for IGF I significantly increases 48 h after PGF2α-induced luteolysis in the study of Woad et al. [16]. In their study design, it is not mentioned, at which point of the estrus cycle the animals ($n = 3$) were treated with the prostaglandin analogue. Apart from different methods, different cycle stages at the time of induction of luteolysis may be responsible for different results.

IGFR-1 mediates most cellular effects of IGF I and II, whereas IGFR-2 serves for internalization and degradation of IGF II [33]. IGFR-1 shows partially significant down-regulations during luteolysis. This would confirm the assumption, that the support of the survival factors IGF I and IGF II is diminished. Interestingly there is, after a significant decrease at 2 h, a slight increase in IGFR-1 expression until 12 h after PGF2α. This might be an attempt of counter regulation to prevent luteolysis in the case of recovery or persistence of the CL. Generally, it is important to consider the rapidly changing tissue composition of the regressing CL. The same mRNA may be produced by different cells. For example, the increasing macrophages and leucocytes at late regression [4] could be the source of increasing mRNA expressions. The mRNA expression of IGFR-2 shows a slight down-regulation during functional luteolysis. During structural luteolysis this down-regulation is less distinct from control, and IGFR-2 mRNA increases again [34]. At the same time the down-regulation of IGF II mRNA becomes significant. Probably the decrease of IGF II during luteolysis is not only regulated by its mRNA transcription, but also by an increasing number of IGFR-2 receptors, which degrade IGF II protein.

There are primarily inhibiting actions on IGF function, which are accredited to IGFBP-1 [35]. Sayre et al. [36] determined a remarkable increase in IGFBP-1 mRNA and protein in bovine CL 24 and 48 h after induced luteolysis and proposed a potentially inhibiting action of IGFBP-1 on progesterone secretion. Regarding our results with a 34-fold increase of IGFBP-1 mRNA during luteolysis, we can confirm this assumption. Since the increase is significant at 2 h and maximal at 4 h after PGF2α, IGFBP-1 plays in all probability an important role yet in functional luteolysis.

Both IGF-stimulating and -inhibiting actions are described for IGFBP-5. Moreover IGF-independent actions and a specific receptor which mediates intrinsic actions of IGFBP-5 are discussed [19,37,38]. In ovine granulosa cells and bovine mammary tissue IGFBP-5 is associated with growth arrest and apoptosis [38–40]. Our results show a significant increase (11-fold) of IGFBP-5 mRNA during structural luteolysis, when oligonucleosome formation

and cell degradation occur [2]. It suggests itself to presume, that IGFBP-5 is involved in apoptosis in bovine luteolysis.

These two IGFBPs, IGFBP-1 and -5, show only a weak expression (IGFBP-1), or a relative constant expression (IGFBP-5), respectively, during estrous cycle and pregnancy in bovine CL [14,15]. During induced luteolysis, they show the most strongly and the most varying expression when compared to the other IGFBPs, and seem to gain in importance when regression occurs.

IGFBP-3 has been shown to be both stimulatory and inhibitory on IGF effects, whereas for IGFBP-4 primarily inhibiting effects on IGF-action are known, presumably to protect the cell from over-stimulation [33,41]. Whereas IGFBP-3 can have intrinsic action on the cell by an own receptor, IGFBP-4 is a soluble, extra-cellular IGFBP, which inhibits the receptor interaction by sequestering IGF [18]. In the bovine ovary, IGFBP-3 seems to have rather inhibiting functions, as IGF I-stimulated progesterone secretion is blocked [22]. The down-regulation of inhibitory IGFBP-3 and -4 would mean a decreased inhibition of IGF-action. In this case, one would assume that an increased IGF-action may support leucocytes and macrophages in the remodeling of the regressing tissue. On the other hand, cell-associated IGFBP-3, in contrast to soluble IGFBP-3, raises IGF I action [41], and a down-regulation would reduce the survival effect of IGFs on the CL. The main intraovarian IGF (IGF II) is significantly down-regulated during structural luteolysis, and IGFBP-3 and -4 mRNA expressions seem to follow this course. Maybe IGFBP-3 and -4 generally serve as a kind of storage pool, which releases plasma IGFs by a fine tuned mechanism when necessary.

IGFBP-2 and -6 mRNA expressions show a slight, partially significant tendency for up-regulation during structural luteolysis. In bovine CL, IGFBP-2 mRNA is only weakly expressed [14,15]. Whereas IGFBP-6 is rather lowly expressed during estrus cycle, it is more strongly expressed than IGFBP-3 and -4 during induced luteolysis. IGFBP-6 binds IGF II with a 100-fold higher affinity than IGF I, and potentially represses tumor growth by inhibiting IGF II, which is supposed to be an autocrine tumor growth factor [41,42]. In non-small cell lung cancer, that is mainly stimulated by IGF II, IGFBP-2 inhibits the binding of IGFs to their receptor [43]. Possibly both IGFBPs are supposed to inhibit stimulating effects of IGF II on the regressing luteal tissue. Although there are important species specific differences in how IGF-system components change in response to PGF treatment, the inhibitory role of IGFBP-2 in PGF2α-induced luteolysis is supported by the findings of Nicholson et al. [44] in pig.

To summarize, IGFBP-1 seems to have an important task in preventing IGF from further support of the CL during functional luteolysis. When structural luteolysis begins, IGFBP-5 expression was activated, and the mRNA of IGFBP-2 and IGFBP-6, as potential inhibitors of IGF II action, was up-regulated. Further on, the expression of GH-R and IGF II was significantly reduced, which means that the support for CL function is directly stopped. In conclusion this study shows, that the fine tuning of IGFBPs is an important aspect in such a complex physiological procedure like luteal regression. Considering that IGF-action is influenced by differential expression of its factors IGF I and IGF II, their receptors, six different binding proteins, specific IGFBP-proteases, and that IGFBPs may have intrinsic actions by own receptors, there are a lot more components to be taken into account and to be investigated in further research. Of course we recognize that mRNA expression may not necessarily reflect protein concentrations, which are the actual biological effectors.

Nevertheless, expression data can provide a useful basis for further study of this complex system.

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