

INTRODUCTION

Apoptosis or programmed cell death is an important event in determining the lifespan and function of the Corpus luteum (CL) in several species. It is well established that apoptosis plays a critical role in structural regression of the CL. The aim of this study was to specify the expression of various apoptotic and anti-apoptotic factor family members in the bovine CL during PGF2 α induced luteolysis.

MATERIAL AND METHODS

Cows in the mid-luteal phase (days 8-12) were injected with the PGF2 α -analogue Cloprostenol, and CL were collected by transvaginal ovariectomy before and 2, 4, 12, 24, 48 and 64 h after PGF2 α -injection. Any factor showing a significant ($P < 0,05$) increase or decrease at 2h after PGF2 α application was also investigated at 0,5h after induced luteolysis. The mRNA expression was detected by quantitative real time PCR (Rotor Gene 3000). Investigated genes were FAS-Ligand (FAS-L) and tumor necrosis factor α (TNF α), which are the ligands of the extrinsic apoptotic pathway and their receptors FAS antigen and tumor necrosis factor α receptor 1 (TNFR1). As representatives of the mitochondrial pathway, BAX, BCL-X_L, p53, Smac/Diablo and Survivin and three central caspases (Caspase3, -6, -7) were

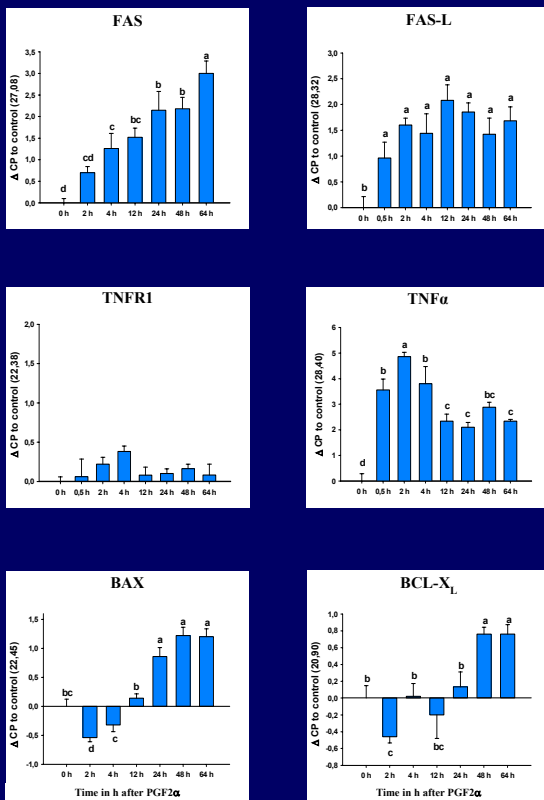


Fig. 1: mRNA expression data of FAS, FAS-L, TNFR1, TNF α , BAX, BCL-X_L during induced luteolysis; data are shown as mean of crossing point difference (Δ CP) \pm SEM between control group (0h) and the following times in hours after PGF2 α administration (n=3-5/stage). Different superscript letters indicate significant differences ($P < 0,05$).

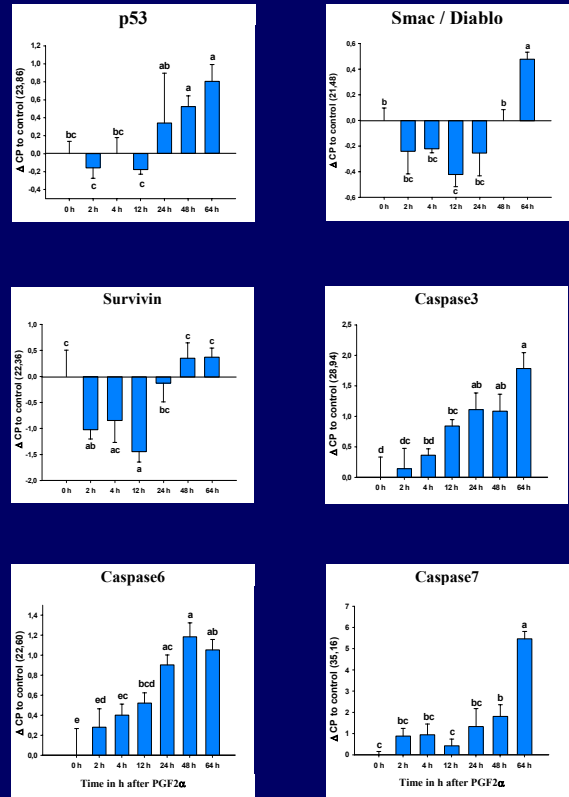


Fig. 2: mRNA expression data for p53, Smac/Diablo, Survivin, Caspases3, -6, -7 during induced luteolysis; data are shown as mean of crossing point difference (Δ CP) \pm SEM between control group (0h) and the following times in hours after PGF2 α administration (n=3-5/stage). Different superscript letters indicate significant differences ($P < 0,05$).

RESULTS AND DISCUSSION

The FAS mRNA expression showed a constant up-regulation to control from 2h on with the highest expression at 64h. For FAS-L all time points were significantly increased to control level by being constantly expressed. TNF-R1 showed no significant regulation during induced luteolysis, whereas the expression of TNF α increased at all time points to control with the highest level at 2h after PGF2 α . BAX showed a down-regulation at 2h and then increased steadily to an up-regulated level from 24h to 64h. BCL-X_L continued in the same way like BAX, however an up-regulation was first seen at 48h. An increase in expression level for p53 was revealed from 48h to 64h. Smac/Diablo was down-regulated at 12h and showed an increase to control level at 64h. A decreased expression level of Survivin was recognised at 2h and 12h, whereas all other time points were not significantly regulated in relation to control group. Expressions of Caspase3 and Caspase6 indicated a constant up-regulation, which was significant from 12h on with the highest expression at 64h. Caspase7 was increased from 48h to 64h with a strong up-regulation at 64h.

CONCLUSIONS

These results suggest, that apoptotic and anti-apoptotic factors may play an important role in functional and structural luteolysis in the bovine CL. The first apoptotic signal seems to be received through the extrinsic pathway and may trigger the activation of the Caspases. The intrinsic pathway joins these activation 24h later when the structural regression initiates.