The expression of plasminogen activators (PA) and their inhibitors during different functional stages in the bovine ovary

Berisha B, Kliem H, Welter H, Pfaffl MW, *Pfarrer C, Schams D

Physiology Weihenstephan, Technische Universität München and
*Dept. of Obstetrics and Gynecology, Justus-Liebig-University, Giessen, Germany

Introduction

Members of the serine proteases, such as tissue-type (tPA), urokinase (uPA) plasminogen activator and its inhibitor (PAI), are known to be secreted by a large number of cell types that convert plasminogen to plasmin. uPA is secreted as an inactive single-chain molecule while tPA is secreted in an active form. PA not only play roles in fibrinolysis but also in various reproductive processes. In the ovary they are supposed to be important regulators of the ovulatory process and corpus luteum (CL) formation and function. Thereby extensive tissue remodelling accompanied by vascular changes as well as cellular proliferation and differentiation of theca and granulosa cells into luteal cells occurs.

Material and Methods

Tissue sampling, classification and preparation of follicles and CLs

Experiment 1: ovaries containing preovulatory follicles or new CL were collected by transvaginal ovariectomy at 0, 4, 10, 20 and 25h (follicles) and 60h (CL day 1-2) relative to injection of GnRH.
Experiment 2: CL were divided in following groups: Days 1-2, 3-4, 5-7 and 8-12 of the estrous cycle.
Experiment 3: cows in the mid-luteal phase (days 8-12) were injected with Cloprostenol (Estrumate) for induction of luteolysis and CL were collected at 0, 0.5, 2, 4, 12, 24, 48 and 64h after injection.

Messenger-RNA-Quantification

Follicles and CLs were dissected from the ovary. All follicle and CL samples were aliquoted, quickly frozen in liquid nitrogen and total RNA was isolated. Transcripts were amplified by RT-PCR and quantified relatively using real time PCR (Rotor-Gene 3000®).

Figure 1: Relative mRNA expression data for PA family members in periovulatory follicles collected at (I) 0, (II) 4, (III) 10, (IV) 20, (V) 25 (follicles) and (VI) 60h (new CL) relative to injection of GnRH to induce an LH surge (n=5/group). The changes in mRNA expression for the different groups were calculated by normalization to the UBQ and relative to the follicle group 0h as previously described by Livak and Schmittgen [2001], where $\Delta CT=CTtarget–CTUBQ$ and where $\Delta\Delta CT=\Delta CT(group\ I = control)–\Delta CT(group\ II-VI)$. Results are presented as expression changes ($\Delta\Delta CT\pm$ SEM) in the target gene expression, normalized to UBQ and relative to 0h (control). Different superscripts denote statistically different values (P < 0.05).

Results

Experiment 1: The mRNA expression of PA system members in follicles before and after LH surge and after ovulation

The tPA mRNA expression increased 4h after GnRH (during LH surge) and remained high during the whole experimental period. uPA transcripts did not change in follicle classes during periovulation but increased significantly only after ovulation. Both uPAR and PAI-1 mRNA expression increased in follicle group at 4h after GnRH, in order to increase again after ovulation.

Experiment 2: The mRNA expression of PA system members in luteal tissue during estrous cycle

uPA mRNA increased on days 8-12 of estrous cycle. In contrast, PAI-1 und PAI-2 mRNA were high on days 1-7 and decreased significantly on days 8-12 while uPAR and tPA mRNA did not change throughout the investigated periods.

Experiment 3: The mRNA expression of PA system members in luteal tissue after induced luteolysis

After induced luteolysis the PA system members (tPA, uPA, uPAR) and their inhibitors were upregulated from 2h till 64h, only tPA increased already after 0.5h.

Aim

The aim of this study was to evaluate the expression patterns of PA system members in follicles and CL during following functional stages in the bovine ovary:
Experiment 1: Follicle during periovulation;
Experiment 2: CL during estrous cycle;
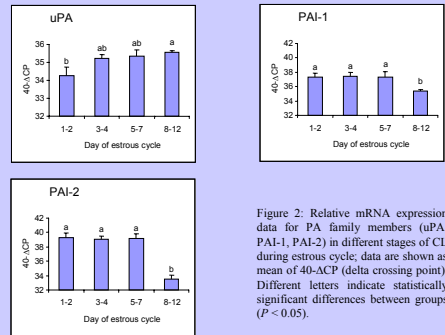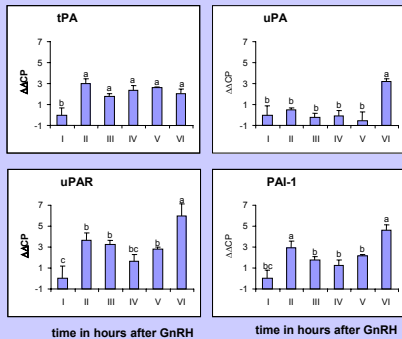*Experiment 3:* CL after induced luteolysis

Figure 2: Relative mRNA expression data for PA family members (uPA, PAI-1, PAI-2) in different stages of CL during estrous cycle; data are shown as mean of 40-ΔCP (delta crossing point). Different letters indicate statistically significant differences between groups (P < 0.05).
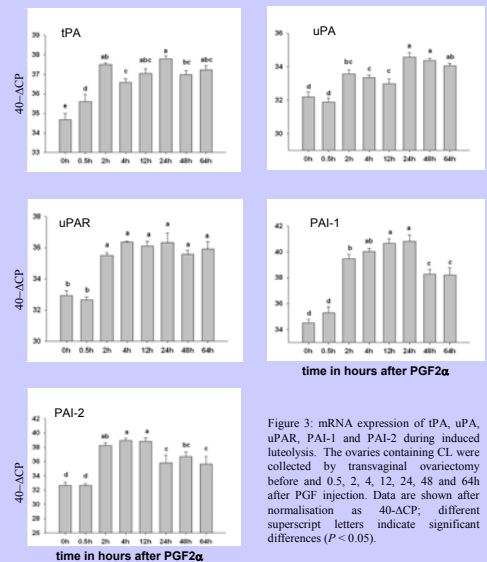
Figure 3: mRNA expression of tPA, uPA, uPAR, PAI-1 and PAI-2 during induced luteolysis. The ovaries containing CL were collected by transvaginal ovariectomy before and 0.5, 2, 4, 12, 24, 48 and 64h after PGF injection. Data are shown after normalisation as 40-ΔCP; different superscript letters indicate significant differences (P < 0.05).

Conclusion

These data demonstrate that several members of the PA-system are differently expressed during periovulation as well as during CL formation, function and regression. The strong upregulation of tPA, PAI-1 and uPAR during LH surge suggest them to be important mediators of LH dependent rupture of bovine follicle. In addition these data suggest, that members of the PA system play an important role during CL formation (Angiogenesis) and function as well as in degradation of extracellular matrix during induced luteolysis in cow.