EXPRESSION OF OESTROGEN AND PROGESTERONE RECEPTORS IN THE BOVINE MAMMARY GLAND DURING DIFFERENT DEVELOPMENTAL AND FUNCTIONAL STAGES

B Berisha, D Schams, S Kohlenberg, W Amselgruber[1], MW Pfaffl & F Sinowatz[2]

Institute of Physiology, Technical University Munich - Weihenstephan, D-85350 Freising,
[1]Dept of Anatomy and Physiology, University Hohenheim, D-70593 Stuttgart and
[2]Dept of Animal Anatomy II, LMU Munich, 80539 Munich, Germany

INTRODUCTION

It is now well established that oestrogen and progesterone are absolutely essential for mammary gland development. Lactation can be induced in non-pregnant animals by sex steroid hormone treatment. Most of genomic actions of oestrogens are mediated by two oestrogen receptors ERα and ERβ, and for gestagens in ruminants by the progesterone receptor (PR). The aim of the presented paper was to study expression of ERα, ERβ and PR by real-time RT-PCR and localisation of ER and PR by immunohistochemistry in the bovine mammary gland during different stages of development, function and involution.

MATERIAL AND METHODS

The mammary gland from German Fleckvieh and Holstein Frisian cows (n=53) was removed within 20 min after slaughter during defined stages. Small pieces (1–2 g) of mammary tissue were frozen in liquid nitrogen and stored at –80°C until the RNA isolation. For the histological investigations (immunohistochemistry), mammary tissue was fixed via immersion with Bouin's and methanol/glacial acid solution. The classification of the animals was established as follows. (i) Ductal growth (18 month old virgin heifers, n=4); lobulo-alveolar development during first pregnancy (only for immunohistochemistry) (ii) Days 194-213 of pregnancy (n=4); (iii) Days 252-272 of pregnancy (n=4); (iv) lactogenesis (onset of secretion during days 4-8 post partum (n=4); galactopoiesis (v) peak lactation (2-8 weeks post partum, n=5), (vi) midlactation (4-5 months, n=4), (vii) late lactation (8-12 months, n=4); involution (after dry off) (viii) 8 h (n=4), (ix) 24 h (n=4), (x) 48 h (n=4), (xi) 96-108 h (n=4), (xii) 14-28 d (n=6), and 2.5 years (n=1, only for immunohistochemistry).

RESULTS AND DISCUSSION

The quantitative mRNA expression (LightCycler RT-PCR) of the steroid hormone receptors ERα, ERβ and PR in the bovine mammary gland at different stages of development and function are shown in Figure 1 and Table 1. In general highest mRNA expression for ERα and PR in fg/µg total RNA range is found during early mammogenesis in non-pregnant heifers followed by a significant decrease to lower levels at the time of lactogenesis with remaining low concentrations during lactation and the first 4 weeks of involution. In contrast, expression of ERβ is about 1000-fold lower (ag/µg range) and shows no clear difference during the stages examined followed by a significant increase 2-4 weeks of involution.

Immunolocalisation with a monoclonal antibody for ERα (Fig. 2) revealed a strong positive staining in nuclei of lactocytes in non-pregnant heifers, became undetectable during pregnancy, lactogenesis, lactation, and was once again detectable 14-28 d after dry off. In contrast, the PR is localised (Fig. 3) in nuclei of lactocytes in mammary tissue of heifers, pronounced in nuclei of basal epithelial cells in primigravid animals. During lactogenesis less nuclei of epithelial cells were positive, but increased staining of cytoplasm of epithelial cells is obvious. The staining intensity and localisation is similar during peak and mid lactation followed by a change during late lactation and involution where staining is now observed again in nuclei of epithelial cells.

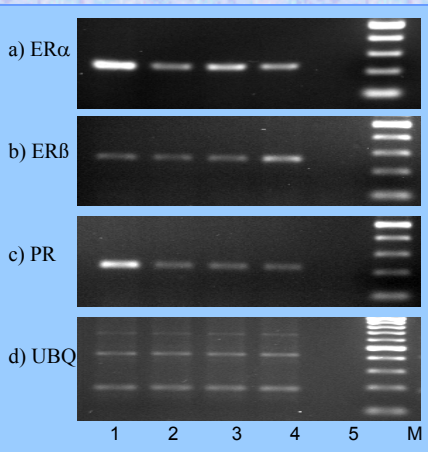

Figure 1: *Representative sample of specific RT-PCR products for (a) oestrogen receptor α (ERα, 234 bp); (b) oestrogen receptor β (ERβ, 262 bp); (c) progesterone receptor (PR, 227 bp) and (d) ubiquitin (UBQ, 198, 426, 654 bp) in bovine mammary gland during different phases. (1) mammogenesis (heifers); (2) lactogenesis; (3) galactopoiesis; (4) involution; (5) no template control; (M) DNA mass ladder (100-500 bp), separated by agarose gel electrophoresis.*

CONCLUSIONS

In conclusion, the mRNA expression and localisation data for ER and PR show clear regulatory changes suggesting involvement of these receptors in cow mammary gland development and remodelling.

Supported by *DFG, Scha 257/13-3*

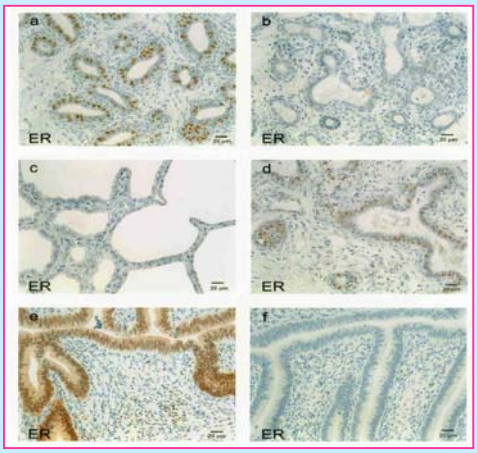

Figure 2: *ERα localisation in bovine mammary gland during development (a), first pregnancy (b), lactogenesis/galactopoesis (c), involution (d), positive control (e) bovine endometrium, and negative control (f) bovine endometrium. Positive staining was observed exclusively in epithelial nuclei and in glands (c) during involution. Myoepithelial and cells of the vascular system are consistently negative. Positive control sections of bovine uterus (e, epithelial cells and stromal cells exhibit a strong nuclear staining).*

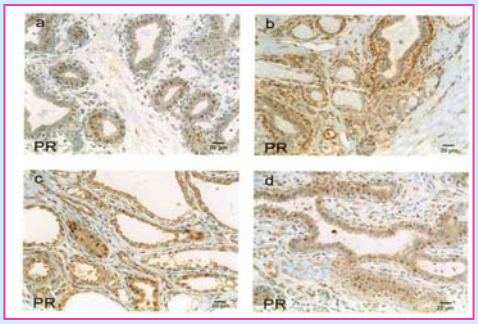

Figure 3: *PR localisation in bovine mammary tissue. PR was immunolocalised in the nuclei of gland cells, cells of the vascular system and in stromal cells. Strong staining of epithelial cells is observed in (a) virgin heifers (18 months old). In (b) primigravid animals (194 d of pregnancy) PR is predominantly and strongly expressed in nuclei of basal localised epithelial cells and during (c) lactation (13 month of lactation) where preferentially groups of epithelial nuclei are positive. Increased cytoplasmatic staining is observed in these tissue samples. During (d) involution (3 weeks dry off) a distinct labelling of epithelial nuclei is the dominant feature. Note that beside epithelial cells a strong and pronounced staining of stromal cells and vascular cells can also be observed in (b) pregnancy, (c) lactation and during (d) involution.*

Table 1: *Real-time RT-PCR mRNA expression of oestrogen receptor α (ERα), ERβ and progesterone receptor (PR) in bovine mammary gland tissue. Results (concentration of specific mRNA / µg total RNA) represent means ±SEM from n = 4 – 6 / group. (I) Mammogenesis (non-pregnant heifers); (II) lactogenesis (d 4-8 post partum); (III) galactopoiesis; (IIIa) early (2-8 weaks); (IIIb) middle (4-5 month); (IIIc) late (8-12 month) and (IVa-e) involution (8h, 24h, 48h, 96-108h and 14-28d after dry off of non pregnant cows)*

	I	II	IIIa	IIIb	IIIc	IVa 8h	IVb 24h	IVc 48h	IVd 96-108 h	IVe 14-28 d
ERα (fg/µg)	**284.7 *** ± 39.1	**17.2** ± 5.2	**25.2** ± 8.6	**75.3** ± 13.1	**129.9** ± 53.8	**69.1** ± 18.1	**126.1** ± 17.7	**49.7** ± 14.7	**31.2** ± 10.2	**69.1** ± 13.8
ERß (ag/µg)	**200.9** ± 61.3	**83.0** ± 44.3	**156.9** ± 71.2	**197.1** ± 56.7	**93.0** ± 42.8	**101.4** ± 18.9	**54.9** ± 16.3	**87.3** ± 15.5	**93.3** ± 10.2	**515.2 *** ± 91.7
PR (fg/µg)	**62.5 *** ± 12.7	**6.7** ± 0.7	**6.2** ± 1.2	**8.2** ± 1.4	**9.4** ± 2.7	**3.9** ± 1.3	**4.4** ± 1.0	**3.9** ± 0.7	**2.4** ± 0.9	**3.0** ± 0.6

Asterisk (*) indicates significant differences between treatment groups (P<0.05).