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Effects of an induced mammogenesis and lactogenesis in sheep on the mRNA expression levels of immunoglobulin receptors (FcRn; pIgR) and zona occludens proteins (OCLN; ZO1; ZO2; ZO3)

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INTRODUCTION

Tight junctions (TJ) form a narrow continuous seal that surrounds each alveolar epithelial cell at the apical border, and regulate all movement through the paracellular pathway *(Nguyen & Neville, 1998)*. In the mammary gland, TJ are impermeable during lactation, thus allowing milk to be stored in the lumen without any loss of milk components. However TJ are dynamic and can be regulated by various stimuli. Systemic and local factors, such as changing hormone concentration (prolactin, progesterone, glucocorticoides, and oxytocin) intramammary pressure and mastitis have been shown to regulate TJ permeability *(Stelwagen et al., 1999)*. During pregnancy TJ are leaky, undergoing a closure shortly before parturition to remain impermeable during lactation. TJ barrier, built by zona occludens proteins 1-3 (ZO1-3) and occludin (OCLN) appears as a network of anastomosing intramembranous fibrils *(Haskins et al., 1998; Nguyen & Neville, 1998)*. The appearance of the fibrils limit the movement of immunoactive leukocytes from blood circulation via the paracellular pathway into milk. The mammary immune response is mediated mainly through phagocytotic leukocytes but to some extent by secreted immune globulin A (IgA) and immunoglobulin G (IgG). Immunoglobulins, such as IgG and IgA/IgM are transported transcellularly into secretion by IgG receptor (FcRn) and polymeric Ig receptor (pIgR), respectively.

In this study the influence of estradiol (E_2), progesterone, dexamethason (DEX) and prolactin (PRL), to induce mammogenesis and lactogenesis, were investigated in sheep. Mammary tissue expression profile of zona occludens proteins and IG receptors were quantified by real-time RT-PCR.

ANIMAL EXPERIMENT

Induced mammogenesis (MG)

MG was induced by a frequent sex-steroid treatment (250 µg E_2-benzoat and 60 mg progesterone) during day 1-27 (figure 1). In one group PRL secretion was inhibited by multiple 50 mg bromocryptin treatments ($BR+_{MG}$, n=7), compared with bromocryptin untreated ($BR-_{MG}$, n=7) and a non treated control (NaCl; K_{MG}, n=6). Sheep were slaughtered at day 31 and mammary tissue was collected for total RNA extraction.

Induced lactogenesis (LG)

LG was induced by a frequent sex-steroid treatment (figure 2), and additionally with a 2x 10 mg DEX application and manual stimulation during day 30-37. In one group PRL secretion was inhibited by multiple bromocryptin treatments ($BR+_{LG}$, n=6), compared with bromocryptin untreated ($BR-_{LG}$, n=6) and only sex-steroid treated control (K_{LG}, n=7). Sheep were slaughtered at day 38 and mammary tissue was collected for total RNA extraction.

$$R = \frac{(E_{target})^{\Delta CP_{target}\,(control\ -\ sample)}}{(E_{ubiquitin})^{\Delta CP_{ref}\,(control-\ sample)}}$$

RELATIVE mRNA QUANTIFICATION METHOD

Expression studies were done in real-time RT-PCR and each sample was normalised to the internal ubiquitin expression *(= house keeping gene)*. Relative expression levels of bromocryptin treated groups (BR+) and untreated groups (BR-) were compared with the corresponding control group, which were set to 100% in each experiment (table 1 and 2). The relative expression ratio (**R**) was calculated in real-time RT-PCR from the PCR efficiencies (**E**) and the crossing point (**CP**) deviation (**ΔCP**) of the unknown sample group mean versus the control group mean *(Pfaffl, 2001)*.

STATISTICS

Statistics was performed with t-Test. Significant differences (table 1 & table 2) between control and treatment are written in the boxes. Significant differences between both treatments (BR+ compared to BR-) are written in *italics letter*.

Figure 1:

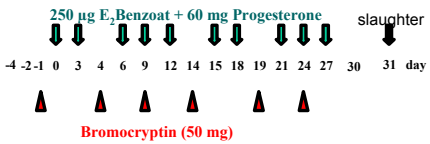


Figure 2:

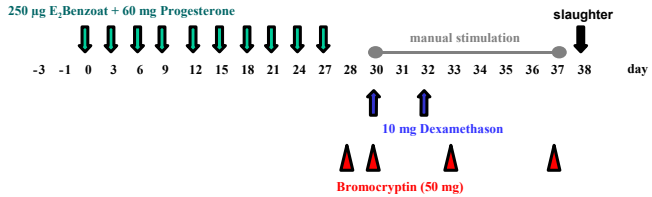


Figure 3:

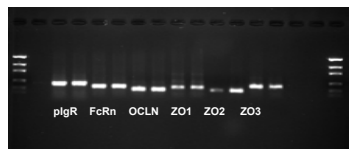


Table 1:

MG	FcRn	pIgR	OCLN	ZO1	ZO2	ZO3
$BR+_{MG}$ n=7	83.3% p=0.72	7,112.5% p<0.001	65.0% p<0.01	43.4% p<0.001	77.9% p=0.46	86.2% p=0.75
	p=0.56	*p=0.14*	*p=0.21*	*p<0.001*	*p=0.32*	*p=0.20*
$BR-_{MG}$ n=7	94.1% p=0.41	23,380% p<0.001	41.3% p<0.01	145.7% p=0.08	83.1% p<0.05	134.3% p=0.76
K_{MG} n=6	100%	100%	100%	100%	100%	100%

Table 2:

LG	FcRn	pIgR	OCLN	ZO1	ZO2	ZO3
$BR+_{LG}$ n=6	44.9% p=0.23	278.9% p<0.05	6.2% p<0.001	11.4% p<0.001	50.2% p=0.53	46.3% p=0.06
	p=0.59	*p<0.05*	*p=0.17*	*p<0.05*	*p<0.05*	*p=0.22*
$BR-_{LG}$ n=6	26.0% p=0.25	47.2% p=0.94	3.0% p<0.001	6.5% p<0.001	23.2% p<0.05	50.0% p=0.86
K_{LG} n=7	100%	100%	100%	100%	100%	100%

RESULT & DISCUSSION

MG could be successfully induced with the applied steroid treatment. LG could be induced by manual stimulation, multiple steroid and DEX treatment. Histology of mammary gland tissue showed in untreated groups an alveolar development filled with secret after the induced MG (figure 4A) and induced LG (figure 5A). The white secret could be retrieved by hand milking and physiological concentrations of fat, protein, lactose and somatic cells were measured. In comparison, the bromocryptin treatments (figure 4B & 5B) showed primarily multi layered epithelium, fat and stroma cells, and the milk ducts were not properly arranged.

Figure 4A: MG BR-

Figure 4B: MG BR+

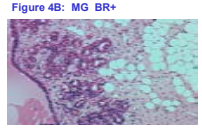


Figure 5A: LG BR-

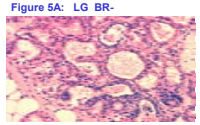
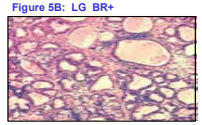


Figure 5B: LG BR+



PRL concentrations in blood were successfully suppressed in all bromocryptin treated groups. In MG prolactin could be suppressed from physiological concentrations 80.4±10.5 ng/ml ($BR-_{MG}$), 83.0±8.2 ng/ml (K_{MG}) down to 1.8±0.1 ng/ml ($BR+_{MG}$). In LG the PRL concentrations could be lowered from 24.0±8.3 ng/ml ($BR-_{LG}$), 80.4±10.5 ng/ml (K_{LG}) to 0.31±0.03 ng/ml ($BR+_{LG}$).

Relative expression data

The expression of all factors could be performed with high specificity in real-time RT-PCR (figure 3). No significant changes of ubiquitin (data not shown), FcRn and ZO3 expression were measured in MG and LG.

Induced mammogenesis

pIgR expression was extremely elevated and OCLN expression was reduced by sex-steroid treatment, but BR application had no effects. Low PRL concentrations (BR+) inhibited the ZO1 expression and high PRL concentration (BR-) trended to enhance the ZO1 expression. ZO2 was expressed in both treatment groups at lower levels as compared to control group.

Induced lactogenesis

pIgR expression was elevated in bromocryptin treated group. Up-regulation, influenced by sex-steroids and DEX, will result in a higher IgA/IgM concentration in colostrum milk. Tight junction protein expressions of OCLN, ZO1 and ZO2 were reduced under sex-steroid and DEX treatment. High PRL concentration resulted in a further down-regulation. Lower TJ protein expression in lactogenesis will decrease TJ formation and leads to increased concentration of phagocytotic leukocytes and a better immune response in the lumen.

References

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