Validation of RT-qPCR measurements in primary bovine mammary gland epithelial cells (pbMEC)

Sorg D. , Potzel A., Meyer H.H.D., Viturro E., Kliem H.

Physiology Weihenstephan, Technische Universität München, Freising, Germany
E-mail: diana.sorg@wzw.tum.de

Introduction

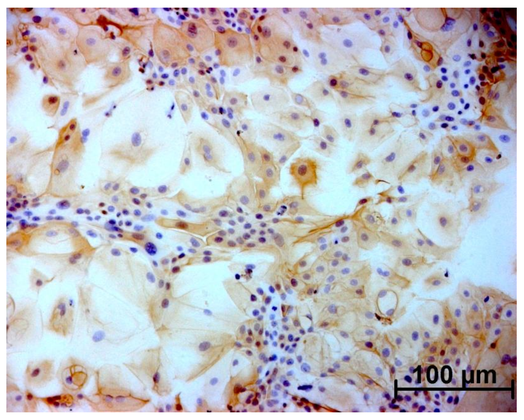

Figure 1: primary bovine mammary gland epithelial cells

The inner surface of the bovine mammary gland is covered by a layer of epithelial cells *(figure 1).* These mammary gland epithelial cells (MEC) secrete the milk constituents and form the first barrier against invading pathogens. Cell lines and primary cultures of these cells (pbMEC) are often used for *in vitro* studies of inflammation and metabolism in the udder. They are mostly taken from udder tissue, but can also be extracted from fresh milk.

Little is known, however, about the changes in gene expression of pbMEC in cell culture. In this study the aim was to measure the gene expression in pbMEC over the course of the first three passages and to compare cells taken from tissue (n=4) and from milk (n=3). In addition, the effect of cryopreservation in liquid nitrogen on gene expression was analysed.

Materials & Methods

- pbMEC were extracted from milk (n=3) and from udder tissue (n=4)
- cultured in DMEM/F-12 Ham with antibiotics and 5% FBS at 37°C and 5% CO_2
- grown in 25 cm^2 cell culture flasks
- at confluence they were detached with accutase solution, diluted and reseeded
- after each of these passages a sample of 100‘000 cells was reseeded into a well of a 6-well plate and allowed to grow for 5 more days
- after third detachment, one sample of each culture was frozen in liquid nitrogen before being reseeded in a 6-well plate *(figure 2).*

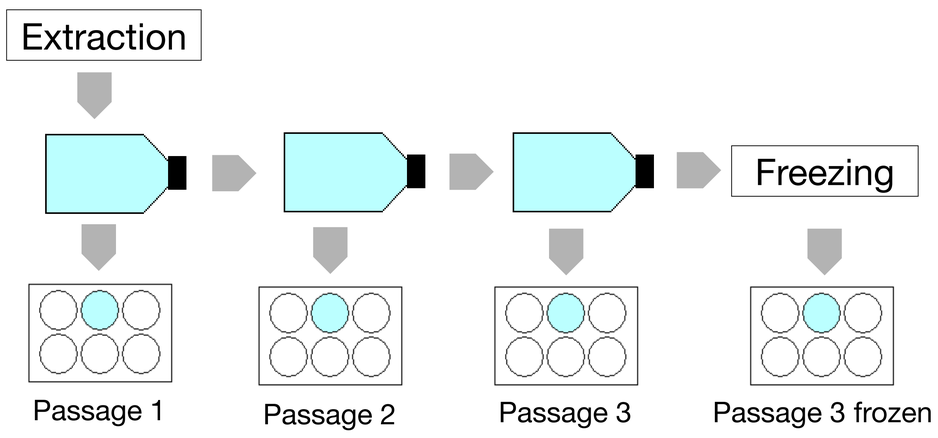

Figure 2: Cell culture of the pbMEC

Afterwards the mRNA was extracted. mRNA concentration and purity was measured (NanoDrop, Peqlab) and reverse transcription was done (M-MLV RT H(-), Promega). In the following real-time qPCR measurements on Qiagen Rotorgene these genes were analysed:

Reference Genes	Target Genes			
GAPDH	Caspase 3	Cytokeratin 8	HMGCR	IL1B
Histone	k-Casein	RANTES	SREBP2	TLR2

C_q values of the comparative quantification were used. Target gene C_q values were normalised against the mean of the reference genes‘ C_q values (= delta C_q). For better visualization, C_q values were subtracted from 20.

Results

- In none of the analysed genes a significant difference of expression could be observed ($p > 0.05$) .
- *Figure 3* shows the gene expression levels of histone for cell origin, passage and freezing comparison. GAPDH diagrams were similar
- *Figure 4* shows the normalised gene expression levels of TLR2 exemplarily. Diagrams of the other target genes were similar.

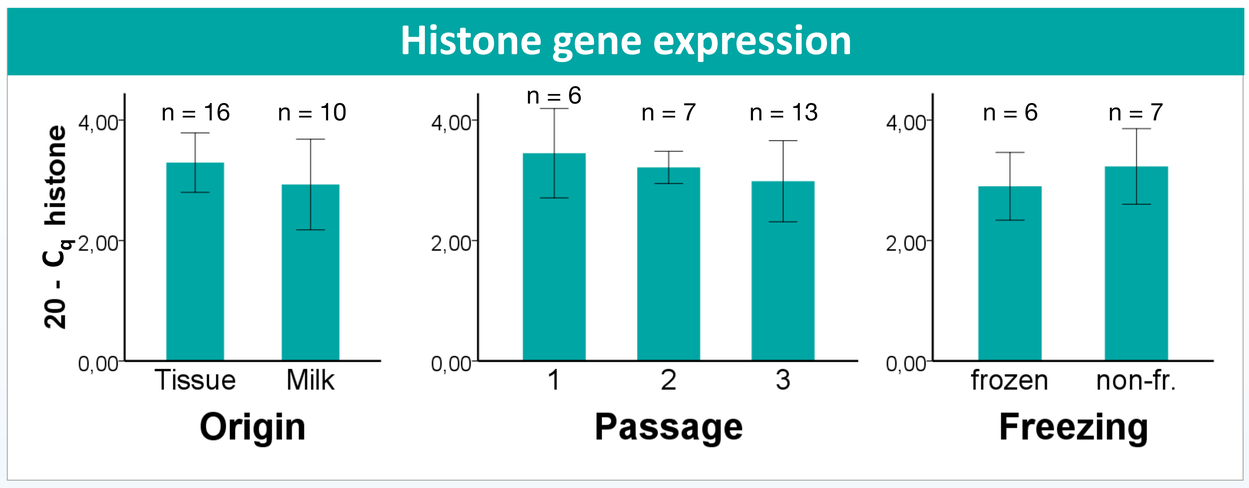

Figure 3: Histone gene expression. Bars show 20 - mean values +/- SD.

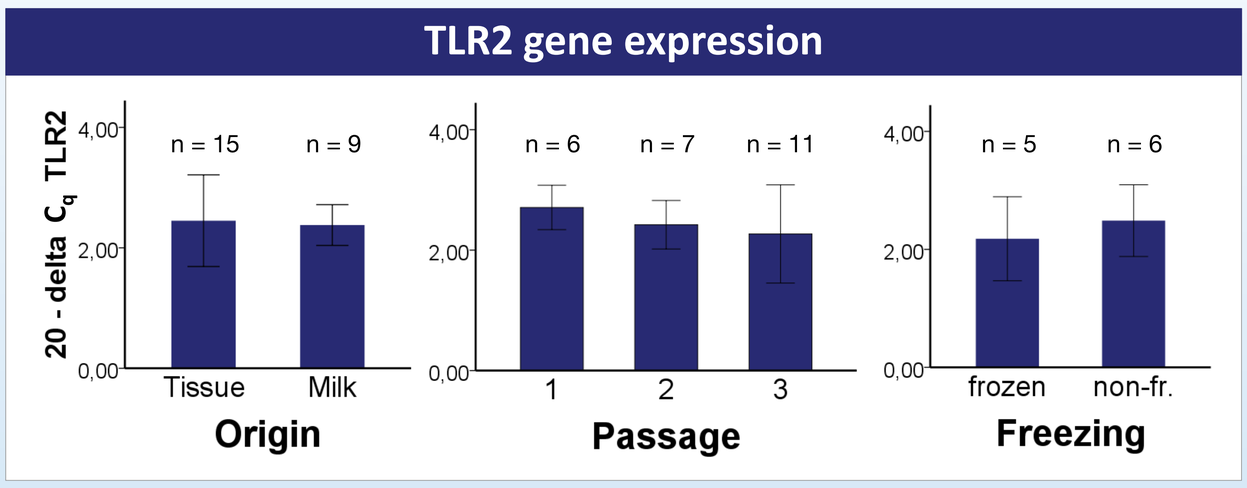

Figure 4: normalised TLR2 gene expression shown as 20-normalised mean value (delta C_q) +/- SD.

Conclusion

- These cells can be equally taken from both origins (tissue and milk) without different effects on gene expression in cell culture.
- They can be used during the first three passages without significant alteration of the gene expression pattern
- Cryopreservation in liquid nitrogen is often necessary preparing an *in vitro* experiment. This can also be done without influencing gene expression of the pbMEC.

qPCR 2011.net 28th March - 1st April 2011
Symposium & Exhibition & Workshops