Effect of mechanical stress on the integrity, signalling mechanisms and function of bovine mammary epithelial cells

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Abstract

Mammary gland engorgement due to milk accumulation in late lactation leads to changes in cell morphology and has been recognised as a potential key initiator of involution and remodelling of the mammary gland. The physical distension of mammary epithelial cells (MEC), due to udder filling, is likely to result in mechanical tension on cell-cell and cell-matrix interactions. Cell-cell and cell-matrix junctions provide tissue integrity, promote cell polarity, guarantee sufficient communication between cells to ensure synchronised milk secretion and support cell survival. Their disruption may be one of the early initiators of the mammary gland remodelling process. As a consequence, the primary goal of this study was to determine the potential effects of MEC stretch on changes in cell sensing within the mechanical micro-environment in the initiation of bovine MEC involution. During this investigation, particular emphasis was put on three potential mechanosensors: tight junctions (TJ), focal adhesions (FA) and primary cilia (PC), and their regulation in the early stages of involution using *in vivo* and *in vitro* experimental approaches. Static, biaxial *in vitro* cell stretch and acute physical distension *in vivo* resulted in changes in TJ protein expression levels implying a potential disruption of cell-cell communication as well as communication with the cell's cytoskeleton. Furthermore, down-regulation of Akt and pAkt following different periods of mechanical strain applied *in vitro* and decreased levels of pAkt following acute physical distension *in vivo* indicated a disruption of β1-integrin-FAK survival signalling through the PI3K-Akt pathway downstream of FA interactions. Increased numbers of ciliated MEC following extended periods of non-milking indicated a dedifferentiation of MEC.

Furthermore, increased levels of STAT6 transcription (part of PC signalling following mechanical stimulation) factor indicates the initiation of macrophage accumulation and promotion of tissue remodelling of the bovine mammary gland. In conclusion, this study supports the hypothesis that local factors play an important role during bovine mammary gland involution and that mechanical stimulation may play a part in the initiation of this process.

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Contents

List of Figures

List of Tables

List of Abbreviations

1X	Once daily milking
2X	Twice daily milking
3X	Thrice daily milking
BM	Basement membrane
CTK18	Cytokeratin 18
DIM	Days in milk
ECM	Extracellular matrix
EGF	Epidermal growth factor
FA	Focal adhesion
FAC	Focal adhesion complex
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
IFT	Intraflagellar transport
JAM	Junctional adhesion molecule
MEC	Mammary epithelial cell
NFMP	Non-fat milk powder
PC	Primary cilium
PC-1	Polycystin-1
PC-2	Polycystin-2
PRL	Prolactin
PRLR	Prolactin receptor
qRT-PCR	Quantitative real time-polymerase chain reaction
RIN	RNA integrity number
RT	Room temperature
SCC	Somatic cell count
SMA	Smooth muscle actin
TJ	Tight junction
TRP	Transient receptor potential
ZO-1/2/3	Zonula occludens 1/2/3