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Milk biomarker discovery for detection of recombinant bovine somatotropin abuse

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Background

Recombinant bovine somatotropin (rbST) can be used to increase milk production in dairy cows, but treating animals with this hormone is illegal in the E.U. Direct detection of the hormone is difficult due to its short half-life time in circulation and its high similarity to the endogenous ST. Instead of direct detection, rbST-dependent biomarkers can be measured. Serum biomarkers, identified based on literature, were analysed before using immunoassay techniques [1]. Milk offers a good opportunity for analysis, because it is obtained by non-invasive sampling and it is taken from the cows anyway.

Objective

The objective of the research is the identification of proteins in milk, whose concentrations are significantly increased or decreased after treating dairy cows with rbST. These identified milk protein biomarkers will be the basis of a screening method to detect rbST doping in dairy cows.

Biomarkers

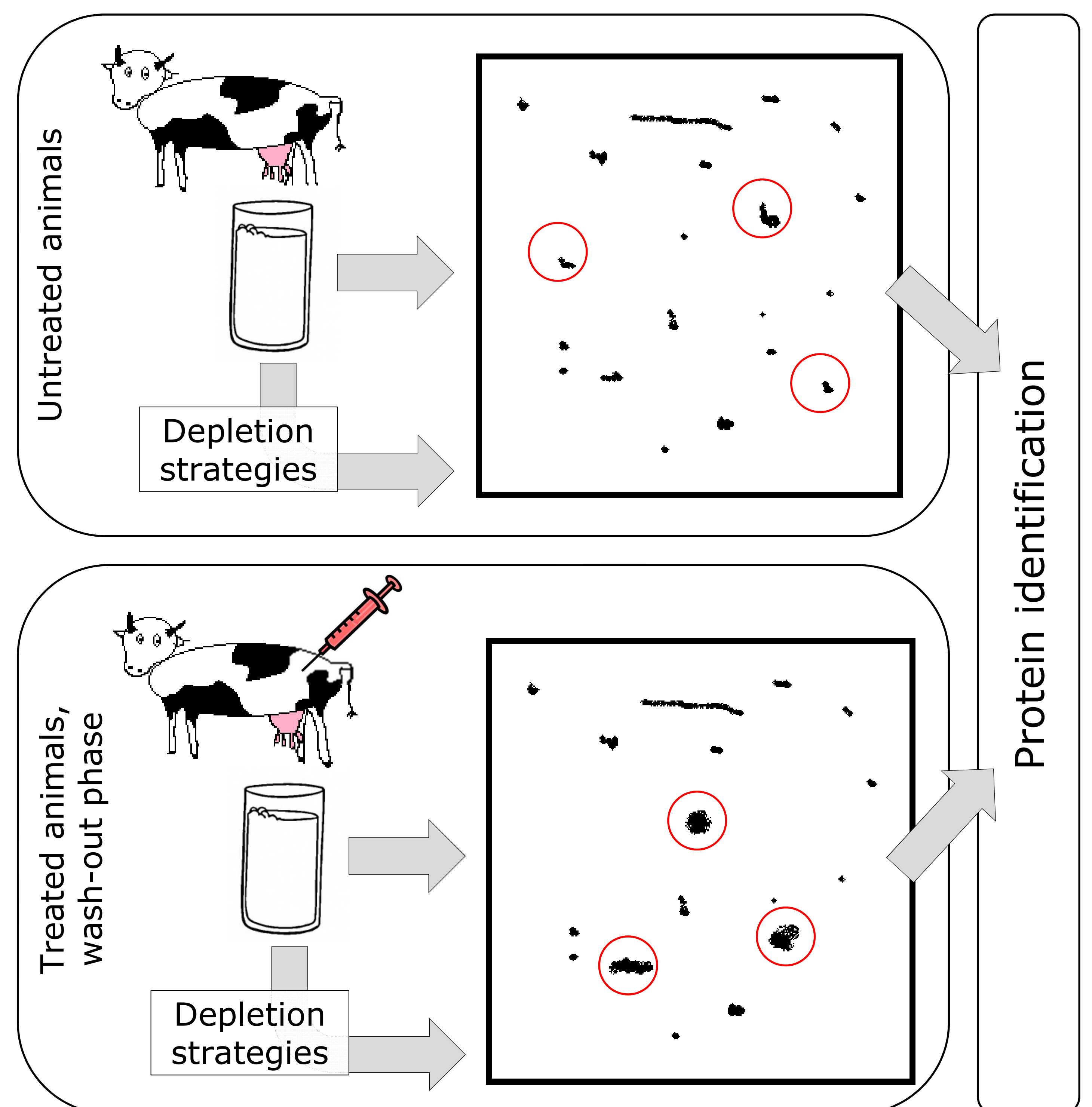
- Fast-responding biomarkers: helpful for abuse identification in the beginning of a treatment period
- Long-lasting biomarkers: prolong the window of detection even after the end of the treatment period
- Abundant milk proteins
- Non-abundant milk proteins: only accessible after depletion strategies

Animal study

Controlled rbST treatment animal studies were conducted including an adaptation period, a treatment period and a wash-out period. Cows were treated with rbST following a biweekly injection schedule according to manufacturers' suggestions. Milk samples were taken throughout the whole study, thus giving the opportunity to analyse fast-responding and long-lasting biomarkers in treated animals versus untreated control animals.

Proteomic techniques

- Depletion of milk from high abundant proteins
- 2D gel electrophoresis experiments for identification of up- or down-regulated spots after rbST treatment
 - depleted milk for detection of low abundant proteins
 - non-depleted milk for detection of abundant proteins
- Protein staining and gel imaging
- Multivariate assessment of robust differential spots between untreated, shortly treated and wash-out period
- In-gel digestion of selected spots
- LC-MS/MS identifications of proteins (candidate biomarkers) from selected spots



Expected outcome

- Detection of significantly up- or down-regulated protein spots
- Protein identification of selected spots
- Identification of fast-responding and long-lasting candidate biomarkers
- Identification of abundant and/or non-abundant proteins as candidate biomarkers

References

[1] Ludwig, S. K.; Smits, N. G.; van der Veer, G.; Bremer, M. G.; Nielen, M. W., Multiple Protein Biomarker Assessment for Recombinant Bovine Somatotropin (rbST) Abuse in Cattle. *PLoS ONE* **2012**, 7, 27.

Acknowledgements

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