Expression profile of genes and proteins involved in calcium signaling pathway during bovine oestrus cycle

Heidt, H., Salilew, D., Gad, A., Grosse-Brinkhaus, C., Tesfaye, D., Hoelker, M., Looft, C., Tholen, E. & Schellander, K.

Institute of Animal Science, Group of Animal Breeding and Genetic, University of Bonn, Endenicher Allee 15, 53115 Bonn, Germany ; e-mail:hhei@itw.uni-bonn.de

Introduction

Calcium signaling is believed to be a fundamental intracellular signal that mediates a variety of physiological functions including the resumption of meiotic maturation in oocytes. However, the role and expression level of key regulatory genes regarding the calcium signaling pathway during bovine estrous cycle is poorly understood. Therefore, the aim of the study was to investigate the expression profiling of genes involving in calcium signaling pathway during the bovine estrous cycle.

Materials & Methods

- 12 Simmentaler heifers were oestrus synchronized and slaughtered at day 0, 3, 7 and 14 of the oestrus cycle
- Uterine samples were taken at the ipsilateral and contralateral regions
- 11 genes: ADORA2B, ATP2B1, CALM3, HTR4, ITPR1, ITPR2, PLCD1, PLCE1, PLN, PRKCA and SLC8A1 (**Fig. 1**) were selected and quantified by using quantitative Real-Time PCR
- The statistical data analysis was performed by the software package SAS (version 9.2), Proc GLM:
  → Model: $y_{ij} = day_i + tissue_j + e_{ij}$
- Protein localization of ITPR1 was performed by immunohistochemistry

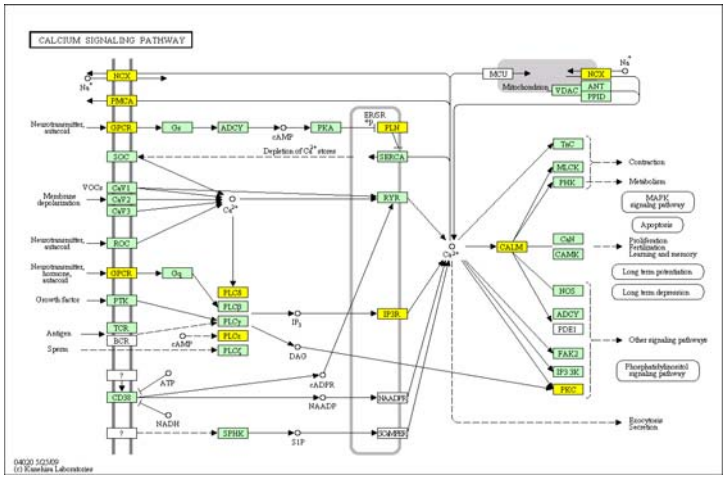

Fig. 1: Selected genes of the KEGG Calcium signaling pathway (http://www.genome.jp/kegg/pathway.html)

Results

Tab. 1: Means of the expression profiles at different days of the estrous cycle

Genes	Day 0	Day 3	Day 7	Day 14
ADORA2B	0.019^{b}	0.018^{b}	0.077^{a}	0.062^{a}
ATP2B1	0.295^{ab}	0.315^{ab}	0.367^{a}	0.229^{b}
CALM3	1.232^{c}	3.702^{b}	5.580^{a}	1.851^{c}
HTR4	0.007^{b}	0.019^{a}	0.012^{ab}	0.014^{a}
ITPR1	0.033^{c}	0.159^{b}	0.256^{a}	0.163^{b}
ITPR2	0.068^{c}	0.102^{bc}	0.217^{a}	0.151^{ab}
PLCD1	0.008^{c}	0.017^{b}	0.034^{a}	0.022^{b}
PLCE1	0.0002^{c}	0.0005^{ab}	0.0006^{a}	0.0002^{bc}
PLN	0.003^{b}	0.007^{b}	0.026^{a}	0.009^{b}
PRKCA	57.194^{c}	147.956^{bc}	282.605^{a}	212.697^{ab}
SLC8A1	0.004^{b}	0.008^{b}	0.026^{a}	0.010^{b}

Rows with different letters are significantly different ($p < 0.05$)

- Important differences were determined between the different days of the oestrus cycle.
- All genes except HTR4 were significantly higher at day7 of the estrous cycle.
- No differences between the different tissues of uterus were observed.
- The ITPR1 protein expression was higher at day 7 of the estrous cycle and more abundant in Myometrium

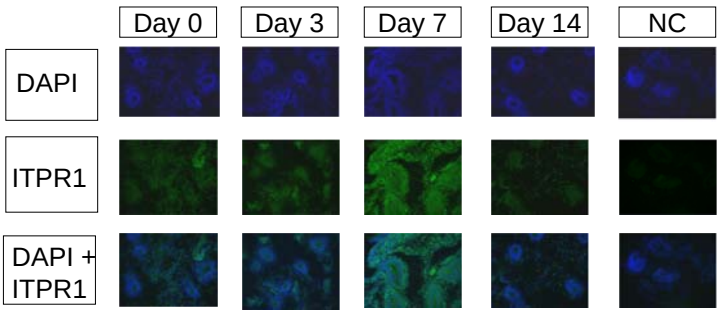

Fig. 2: Protein localization of ITPR1 in the glandular layer of the endometrium at different days of the estrous cycle

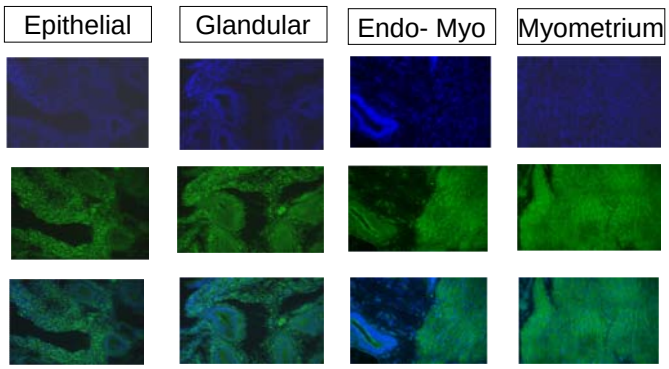

Fig. 3: Protein localization of ITPR1 in different layers of the uterus at day 7 of the estrous cycle (for staining see **Fig. 2**)

Conclusion

Based on this results, the regulatory genes of the calcium signaling pathway showed the highest expression level at the diestrus phase of the bovine estrous cycle.