# Foetal and maternal placenta cells respond differently to a deleterious foetal mutation

*K. Rutkowska[1], K. Flisikowski[2], M. Lukaszewicz[1]& J. Oprzadek[1]*

*[1]Department of Animal Improvement, Institute of Genetics and Animal Breeding of the Polish Academy of Sciences, 05-552 Jastrzebiec, Postepu 36a, Poland*

*[2]Lehrstuhl für Biotechnologie der Nutztiere, Technische Universität München, Freising, Germany*

*j.oprzadek@ighz.pl (corresponding author)*

Summary

The aim of this study was to analyse the expression of *DDX1*, *RB1CC1*, *MKI67*, *EMP2*, and *AHNAK* genes expressed in maternal and foetal placenta from 12 cows gestating *MIMT1*$^{Del/WT}$ foetuses and 12 cows gestating wild-type foetuses terminated on 94$^{th}$ ± 12 day of gestation. All foetuses were gestated in wild-type cows and all were fathered by the same *MIMT1*$^{Del/WT}$ sire. The relative gene expression difference between IUGR and wild-type in both tissues was calculated for each animal (ΔΔCt). All cDNA samples were assayed in triplicate and relative expression levels normalized to endogenous *GAPDH* expression. Two-way ANOVA was used to estimate the effect of interaction of the *MIMT1* genotype with placenta tissues. The study revealed increased *DDX1*, *RB1CC1*, *MKI67*, *AHNAK* gene expression in the maternal placenta of the *MIMT1*$^{Del/WT}$ foetuses. No expression differences in foetal placenta for the studied genes were found. Our study evidenced that maternal and foetal placental cells respond differently to the foetal IUGR genotype. Caution is therefore warranted when analysing gene expression changes in non-dissected samples of placenta.

*Keywords: placenta, cattle, gene expression, intrauterine growth restriction*

## Introduction

Foetal growth is a complex process that depends on balanced communication between foetus, placenta, and mother`s organism. Placental dysfunctions are one of the primary causes of the intrauterine growth restriction (IUGR) (Goldenberg *et al*., 1989). Among livestock species, stillbirth is mainly recorded in dairy cattle and is an important functional trait from both economic and animal welfare standpoints. For example, in US Holstein cattle ~7% of all calves are stillborn (Meyer *et al*., 2001).

In recent years, our knowledge about prenatal growth and development of mammals significantly broadened, although the aetiology of genetically conditioned IUGR is still poorly understood. It is possible to recognize foetal growth restriction during pregnancy, but more than 50% of IUGR is recognized only after birth (William *et al*., 2002).

In a previous study (Flisikowski *et al*., 2010), we identified and characterized a cattle-late-abortions-stillbirths-IUGR-causing microdeletion of the 3rd and 4th exons, that truncates the 3' end of the MER1 repeat containing imprinted transcript 1 (*MIMT1*) locus of the PEG3 domain. The aim of the research was to study the expression of genes involved in the foetal growth and development in cattle in the context of IUGR.

## Material and methods

The selected loci were *DDX1*, *RB1CC1*, *MKI67*, *EMP2*, and *AHNAK*. The genes were chosen based on the results of study Xu et. al. (2017). As the reference the *GAPDH* gene was used and all placenta samples, both maternal and foetal, were analysed in triplicate. The samples were collected from 24 cows gestating 12 $MIMT1^{Del/WT}$ and 12 $MIMT1^{WT/WT}$ foetuses, on the 94th ± 12 day of gestation. Quantitative real-time reverse transcriptase polymerase chain reaction (RT-PCR) was performed as described by Higucchi *et al.* (1992). The relative expression differences between the IUGR and wild-type placentas, in both parts of the placenta, were calculated for each foetus (ΔΔCt), following log-transformation of the expression levels. All the dams carried the wild-type *MIMT1* genotype and were mated with a single $MIMT1^{Del/WT}$ Ayrshire bull. Two-way ANOVA was used to estimate the effect of interaction of the *MIMT1* genotype with placenta tissues.

## Results and discussion

The study revealed increased *DDX1*, *RB1CC1*, *MKI67*, *AHNAK* gene expression in the maternal placenta of the $MIMT1^{Del/WT}$ foetuses (Figure 1). Specifically, the *EMP2* and *DDX1* showed 2.2- and 16- fold higher expression in the maternal than foetal placenta. No expression differences in foetal placenta for the studied genes were found.

**Figure 1**. Natural logarithms of relative expressions of 5 loci (*DDX1*, *RB1CC1*, *MKI67*, *EMP2*, *AHNAK*) expressed in foetal and maternal placenta of the IUGR or wild-type *MIMT1* genotype foetuses. The relative expression differences, within maternal or foetal placenta, between the foetal *MIMT1* genotypes denoted with the same letter differ significantly at: $p \leq 0.01$ $^{(A,A)}$ – upper case; $p \leq 0.05$ $^{(a,a)}$ – lower case letters.

Xu *et al.* (2017) analysed gene expression changes in the foetal and maternal transcriptome in the IUGR foetuses and showed that maternal placenta respond differentially than foetal placenta to the foetal deleterious mutation. The present study is consistent with our previous findings and show different gene expression pattern of the selected genes in both placenta tissues studied.

## Conclusions

Our study shows that caution is warranted when analysing gene expression changes in non-dissected samples of placenta. Our unique genetic IUGR model has also revealed that maternal placenta modulates the penetrance of mutations in the foetal genome.

The study was funded by the National Science Centre of Poland, research project no. 2016/23/N/NZ9/00232.

## List of References

Flisikowski, K., H. Venhoranta, J. Nowacka-Woszuk, S.D. McKay, A. Flyckt, J. Taponen, R. Schnabel, H. Schwarzenbacher, I. Szczerbal, H. Lohi, R. Fries, J.F. Taylor, M. Switonski & M. Andersson, 2010. A novel mutation in the maternally imprinted PEG3 domain results in a loss of MIMT1 expression and causes abortions and stillbirths in cattle (Bos taurus). PLoS One 5(11):e15116.

Goldenberg, R.L., G.R. Cutter, H.J. Hoffman, J.M. Foster, K.G. Nelson & J.C. Hauth, 1989. Intrauterine growth retardation: standards for diagnosis. Am J Obstet Gynecol 161(2): 271–277.

Higuchi, R., G. Dollinger, P.S. Walsh & R. Griffith, 1992. Simultaneous amplification and detection of specific DNA sequences. Biotechnology (N Y) 10(4):413–417.

Meyer, C.L., P.J. Berger, K.J. Koehler, J.R. Thompson & C.G. Sattler, 2001. Phenotypic trends in incidence of stillbirth for Holsteins in the United States. J Dairy Sci 84(2):515–523.

William, W.H., P.J. Thureen & M.S. Anderson, 2001. Intrauterine growth restriction. J Pediatr 2(6):129.

Xu, H., H. Pausch, H. Venhoranta, K. Rutkowska, C. Wurmser, B. Rieblinger, T. Flisikowska, D. Frishman, L. Zwierzchowski, R. Fries, M. Andersson, A. Kind, A.

Schnieke & K. Flisikowski, 2017. Maternal placenta modulates a deleterious fetal mutation. Biol Reprod. doi: 10.1093/biolre/iox064.