

# Genomic evaluation of reproductive disorders in Czech Holstein cattle

*E. Kasna<sup>1</sup>, L. Zavadilova<sup>1</sup>, J. Kucera<sup>2</sup> and J. Splichal<sup>3</sup>*

<sup>1</sup> *Institute of Animal Science, Pratelstvi 815, 104 00 Prague, Czech Republic*

<sup>2</sup> *Czech Moravian Breeders' Corporation, a.s., Benesovska 123, 252 09 Hradistko, Czech Republic*

<sup>3</sup> *Plemdat, s.r.o., Benesovska 123, 252 09 Hradistko, Czech Republic*

Corresponding author: [kasna.eva@vuzv.cz](mailto:kasna.eva@vuzv.cz)

---

## Abstract

Genomic breeding values were estimated for reproductive disorders in Czech Holstein cattle. Evaluated traits were defined as binary with no case (0) or at least 1 case (1) of retained placenta (RP), metritis (MET) or cystic ovaries disease (CYS) during lactation. Dataset included 91 368 lactations of 57 788 Holstein cows calved between July 2017 and February 2022 in 95 herds for RP; 135 361 lactations of 83 564 Holstein cows calved in 116 herds for MET and 82 724 lactations of 49 130 Holstein cows calved in 71 herds for CYS. The lactation incidence was 5.46% for RP, 12.68% for MET and 9.16% for CYS. The model equation included the fixed effects of herd-year-season of calving, parity of the cow, sex of the calf (for RP and MET), the random additive genetic effect of the animal, the random permanent environment of the cow, and the random residual effects. Pedigree involved from 111 858 (CYS) to 185 874 (MET) animals. The reference population for the single-step GBLUP method was defined as genotyped (Illumina BovineSNP50 BeadChip) bulls and cows that were present in the pedigree of animals with reproductive disorders records. Estimated heritabilities were 0.02 for all traits. Resistance to RP and MET were moderately genetically correlated (0.61), while the genetic correlation of both traits with CYS did not statistically differ from 0. Estimated breeding values were normally distributed with a mean of 0.00 and SD ranging between 0.11 and 0.17. Their mean reliabilities were low due to data structure and a relatively short period of recording. The results were expressed as relative breeding values RBV (mean = 100, SD = 12, for base bulls) and reversed in the sign which means that higher RBV are connected with better resistance to partial disorder. The correlations of RBV with other routinely evaluated traits showed, that CYS and MET were favourably correlated with fertility and longevity traits. MET was also favourably correlated with milk, fat and protein yield. Correlations with type traits showed an association of MET with udder traits and CYS with locomotion, bone quality and stature.

**Key words:** dairy cattle, reproductive disorders, genetic parameters

---

## Introduction

Reproductive disorders are the most common cause of involuntary culling of dairy cows in the Czech Republic. According to the Yearbook of cattle breeding, around 20% of cows are culled due to fertility problems, and around 10% are culled due to severe calving (Bucek et al. 2021). The resistance to reproductive disorders is heritable (Guarini et al. 2019; Heringstad 2010; Koeck et al. 2010)

and can therefore be improved by breeding. In the Czech Republic, breeding on health traits was not possible until recently because of the lack of data on the prevalence of diseases and health disorders in the dairy population. As a solution, a web application called “Diary of Diseases and Medication” was designed. The application allows data entry and transfer from farmers/breeders to a central database, their storage, management and continuous evaluation. Diseases recording is based on

ICAR Central Health Key. The application was launched in 2017. It is available to all registered farmers/breeders free of charge.

Our aim was to evaluate the quality of farmers' records on the incidence of most common reproductive disorders stored in "The Diary" and to design their genomic evaluation and implementation in Czech Holstein cattle breeding.

## Materials and Methods

### Traits

The most common reproductive disorders with potential economic impact were selected for the evaluation. They included:

- retained placenta (**RP**) defined as non-repulsion of foetal membranes within 24 hours after calving,
- metritis, (**MET**) defined as inflammation of the uterus, and
- cystic ovarian disease (**CYS**) defined as the presence of a rounded structure with a diameter greater than 25 mm on one or both ovaries.

The health traits were defined as binary, with score 0 (no case) or 1 (at least one case) in the period from calving to 14 (RP), 150 (MET) or 305 (CYS) days in milk.

### Data

The evaluated data set included cows calved after 1 July 2017, when the official recording of health traits started. As not all farmers recorded all disorders, we created a separate data file for each of them. To include a herd in the data file, the minimum frequency of the disorder for herd and year was 1% otherwise it was discarded, as we assumed, that the herd did not record that disorder at all or at least during the year.

Detailed characteristic of the final data sets (number of records, cows, sires and animals in 3 generations of pedigree, number of herds) is given in Table 1. The incidence of reproductive disorders was expressed as lactational incidence  $Li$  (%), i.e. as the proportion of lactations with recorded disorder out of the total number of evaluated lactations.

**Table 1.** Data characteristic

Trait <sup>1</sup>	Records	Cows	Sires	Pedigree	Herds
RP	91 368	57 788	1 137	134 530	95
MET	135 361	83 564	1 339	185 874	116
CYS	82 724	49 130	1 069	111 858	71

<sup>1</sup>RP – retained placenta, MET – metritis, CYS – cystic ovarian disease

There are approximately 40 thousand Holstein animals genotyped for 54 609 SNP from the Illumina BovineSNP50K Bead Chip in the Czech Republic, which are used in routine genomic evaluation of traits included in the selection index. We selected of them the reference populations for each of the evaluated health traits. The reference population included cows with genotype and phenotype, and genotyped animals (males and females) that were present in their 3-generation pedigree. The sizes of reference populations for each health trait are given in Table 2.

**Table 2.** The size of reference populations

Trait <sup>1</sup>	Bulls	Cows
RP	2 779	7 359
MET	2 949	9 628
CYS	2 646	5 323

<sup>1</sup>RP – retained placenta, MET – metritis, CYS – cystic ovarian disease

### Model

We used single-trait animal models for evaluation with the model equation in matrix notation:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}_1\mathbf{u} + \mathbf{Z}_2\mathbf{pe} + \mathbf{e}, \quad (1)$$

where  $\mathbf{y}$  is a vector of phenotypic records,  $\mathbf{b}$  is a vector of fixed effects of contemporary group, sex of a calf (not included in CYS) and parity of a cow,  $\mathbf{u}$  is a vector of random additive direct genetic effect, where  $\mathbf{u} \sim N(0, \mathbf{H}\sigma_u^2)$ ,  $\sigma_u^2$  is the additive genetic variance and  $\mathbf{H}$  is the relationship matrix,  $\mathbf{pe}$  is a vector of random permanent environment effect with  $\mathbf{pe} \sim N(0, \mathbf{I}\sigma_{pe}^2)$ , where  $\mathbf{I}$  is an identity matrix and  $\sigma_{pe}^2$  is a variance due to permanent environment of a cow,  $\mathbf{e}$  is a vector of random residuals, where  $\mathbf{e} \sim N(0,1)$ .  $\mathbf{X}$ ,  $\mathbf{Z}_1$  and  $\mathbf{Z}_2$  are incidence matrices for  $\mathbf{b}$ ,  $\mathbf{u}$  and  $\mathbf{pe}$ , respectively.

## Methods

Co-variance components were estimated using AIREML algorithm as implemented in BLUPF90 family of programs (Misztal et al. 2018). We used model (1) as described in *Model* section. The estimation of co-variances between health traits was based on two-traits models and included only animals, that have phenotypic records for both traits (51 582 animal with 79 725 records for RP-MET, 29 789 animals with 47 206 records for RP-CYS and 41 701 animals with 66 322 records for MET-CYS).

The genomic breeding values estimation was performed with the ssGBLUP method (Aguilar et al. 2010; Christensen and Lund 2010) as implemented in the BLUP90IOD2 program (Misztal et al. 2018). In ssGBLUP, the inverse of relationship matrix  $\mathbf{A}^{-1}$  is replaced by matrix  $\mathbf{H}^{-1}$ , which is defined as:

$$\mathbf{H}^{-1} = \mathbf{A}^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & \mathbf{G}^{-1} - \mathbf{A}_{22}^{-1} \end{bmatrix}$$

where  $\mathbf{G}^{-1}$  is an inverse of a genomic relationship matrix and  $\mathbf{A}_{22}^{-1}$  is an inverse of the pedigree relationship matrix for genotyped animals. All computations used default options in BLUPF90IOD2. Groups for unknown parents were not included.

Estimated genomic breeding values were reversed in signs so that higher values reflect a better resistance to the disorder. All estimates were then expressed as Relative Genomic Breeding Values (**RBV**), with mean = 100 and SD = 12, for base bulls born in 2010.

The reliabilities of breeding values were approximated using the accf90GS program (Misztal et al. 2018).

## Results & Discussion

The lactational incidence  $Li$  of health traits and their genetic parameters (Table 3) were comparable with results found in the literature (Jamrozik et al. 2016; Koeck et al. 2010; Vukasinovic et al. 2017). However, the farmers tended to record only medicated cases, and their overall incidence may thus be underestimated. Genetic correlations showed a moderate association between RP and MET (Table 3).

Genetic correlation between CYS and other health traits did not statistically differ from 0 and suggested, that these traits were not genetically associated. Similar result was reported by Heringstad (2010) who estimated a negative genetic correlation between RP and CYS, that did not statistically differ from 0. Guarini et al. (2019) found a low positive genetic correlation between CYS and both other disorders. On the other hand, Gernand and König (2017) reported the association between RP and ovarian cysts was favourable (0.55). Gernand and König (2017) explained this broad spectrum of estimates by possible breed differences, by the effects of selection or by the environmental effects on gene expressions.

**Table 3.** Lactational incidence ( $Li$ ) and genetic parameters of health traits

<sup>1</sup> Trait	<sup>2</sup> RP	MET	CYS	$Li$ %
RP	0.02 (0.004)	0.61 (0.121)	-0.21 (0.135)	5.5
MET		0.02 (0.002)	0.14 (0.133)	12.7
CYS			0.02 (0.003)	9.2

<sup>1</sup>RP – retained placenta, MET – metritis, CYS – cystic ovarian disease

<sup>2</sup>heritabilities on the diagonal, genetic correlations above the diagonal, SE in brackets

Guarini et al. (2019) identified biological pathways and associated genes underlying RP, MET and CYS, and supported the polygenic nature of the traits with many regions across the genome having small contributions to the total genetic variation. The biological pathways and genes that were associated with RP, MET or CYS susceptibility, were related to processes such as oxidative stress in mammals, in modulation of immune responses, establishment and maintenance of pregnancy, mediation of inflammatory responses, cellular proliferation, cell movement, the cell cycle and apoptosis in the bovine endometrium.

Relative genomic breeding values RBV for all health traits had a mean close to 100 with SD fluctuating around 9, as shown in Table 4.

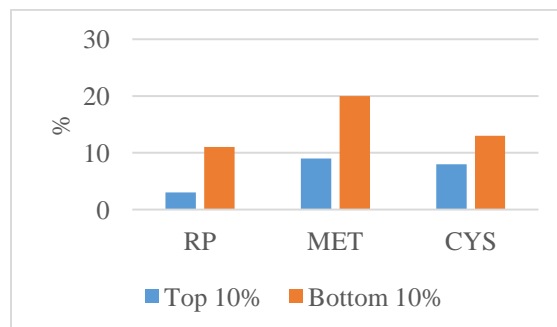
**Table 4.** Relative genomic breeding values for health traits

Trait <sup>1</sup>	Mean	SD	Min.	Max.
RP	99	9	30	160
MET	98	10	35	147
CYS	100	9	42	161

<sup>1</sup>RP – retained placenta, MET – metritis, CYS – cystic ovarian disease

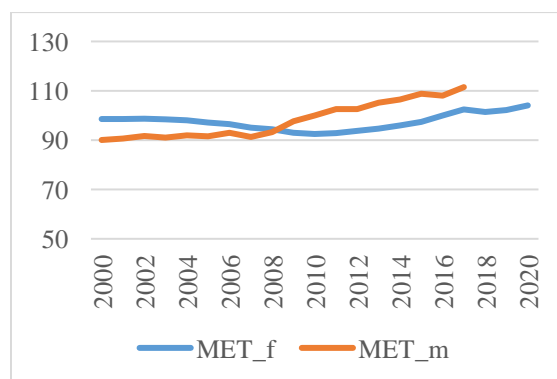
A reliability of RBV estimates was low on average (up to 20%), which reflects the data structure and a relatively short period of recording. It was higher for bulls – up to 35% on average – where more information was available, and sires with larger number of daughters and genomic information attained sufficient reliabilities of RBV. Overall, an improvement of reliabilities is necessary before using breeding values in selection. This can be achieved by increasing the amount of phenotypic data, by adding more genomic information (Lourenco et al. 2015; Vukasinovic et al. 2017), or by joint evaluation of all three health traits with a multiple-trait model (Guarini et al. 2019). In our study, we preferred a single trait model to take advantage of all the data recorded for each trait so far. As the volume of phenotypic records increases, a transition to a multiple-trait model would be possible.

We calculated the percentage of affected daughters of the 10% best and worst sires according to their RBV (Figure 1). The difference between Top and Bottom sires was the highest in the resistance of their daughters to MET: while only 9 of 100 daughters of Top bulls developed MET, the incidence was up to 20% in the daughters of the worst bulls. These differences suggest that despite low heritability there is a sufficient genetic variability to be used in breeding programmes aimed at improving health traits in dairy cows (also Guarini et al. 2019).



**Figure 1.** Percentage of daughters with retained placenta (RP), metritis (MET) or cystic ovarian disease (CYS) record in 10% of best (Top 10) and worst (Bottom 10) sires according to their RBV.

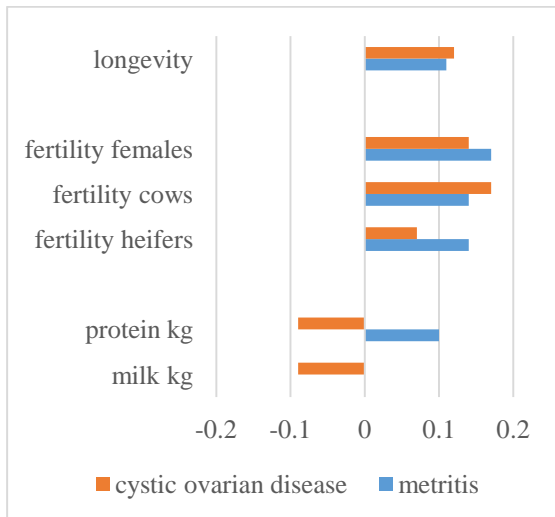
No genetic trend was evident in RP, and the RBV for CYS also fluctuated around the average. However, we observed gradual improvement in MET resistance (Figure 2). This trend might correspond with the inclusion of fertility traits to the selection index of Czech Holstein cattle in 2004.



**Figure 2.** Genetic trends for metritis resistance in male (MET\_m) and female (MET\_f) population

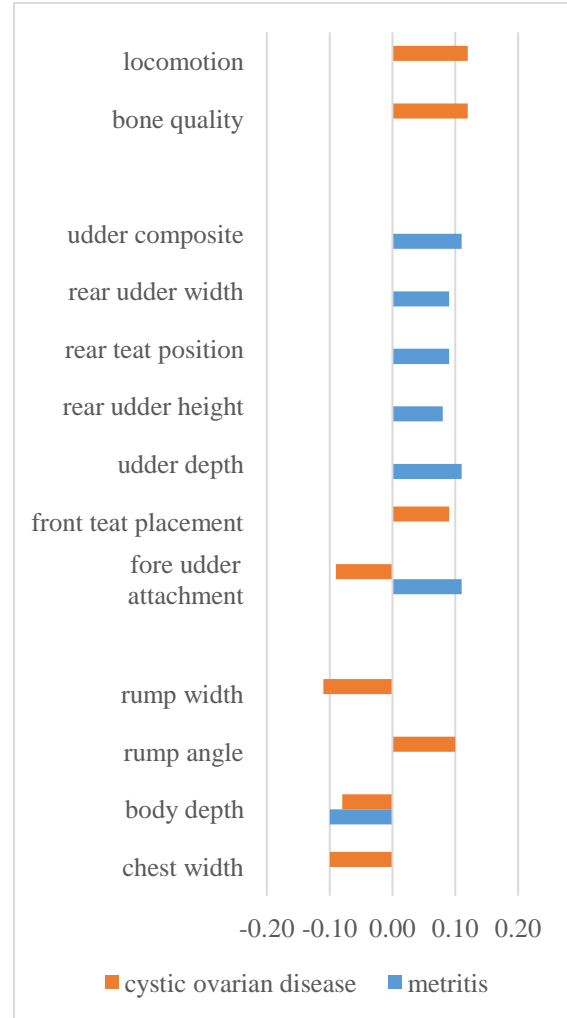
To approximate the associations between health traits and other routinely evaluated traits, we calculated the Pearson correlations between breeding values of sires with at least 50 daughters. The correlations did not exceed 20% in both directions, and the correlations of RP to other traits were not significant. As shown in Figure 3, the resistance to MET and CYS was favourably related to better longevity and fertility traits. Moderate to high correlations between reproductive disorders and fertility traits were reported also by Gernand and König, (2017), Koeck et al. (2010) or Vukasinovic et al. (2017). Koeck et al. (2010) and Vukasinovic et al. (2017) also reported a significant Pearson or genetic correlations between longevity and

disease traits suggesting that selection for disease resistance would improve longevity as well. Genetic predisposition to CYS susceptibility was associated with higher breeding values for milk and protein yield (also Gernand and König, 2017), which corresponds with expected antagonistic relationship between production and health traits. However, higher resistance to MET was related to higher protein yield. On the other hand, Vukasinovic et al. (2017) obtained low genetic correlations between most wellness traits (among them RP and MET) and milk yield and suggested, that high milk production may not necessarily be genetically strongly associated with poor health.



**Figure 3.** Correlations of sires' RBV for health traits with other traits in selection index

MET and CYS breeding values were also correlated with some exterior traits (Figure 4). Significant correlations were calculated between MET resistance and udder traits, mainly those related to the udder support apparatus, with a preference for a flat, well-attached udder. Resistance to CYS was correlated with good locomotion, bone quality (fine, flat bones), a rather narrow chest and rump and sloping rump.



**Figure 4.** Correlations of sires' RBV for health traits with exterior traits

## Conclusions

Our study showed that farm-collected data on reproductive disorders can be used in routine genetic evaluation. Relative genomic breeding values could be incorporated into cows' health index, which already includes breeding values for mastitis and claw health traits. Another possibility may be to create a common fertility sub-index, as fertility traits and fertility disorders are significantly associated. Genetic selection for better health would help to improve the health status of Czech herds and provide a more complete tool for breeding the most profitable animals for sustainable dairy industry.

## Acknowledgements

All Czech farmers and dairy producers recording health data and the Holstein Cattle Breeders' Association in the Czech Republic are gratefully acknowledged. The research was supported by the Ministry of Agriculture of the Czech Republic, project No. QK1810253 and the institutional support MZE-RO0718.

## References

- Aguilar, I. Misztal, I., Johnson, D.L., Legarra, A., Tsuruta, S., Lawlor, J. 2010. Hot topic: A unified approach to utilize phenotypic, full pedigree and genomic information for genetic evaluation of Holstein final score. *J. Dairy Sci.* 93, (2), 743-752.
- Bucek, P., Kucera, J., Syrucek J. 2021. Yearbook of cattle breeding in The Czech Republic. CMSCH, a.s., Hradistko. 43 p.
- Christensen, O.F. and Lund, M.S. 2010. Genomic prediction when some animals are not genotyped. *Genet. Sel. Evol.* 42, 2.
- Gernand, E. and König, S. 2017. Genetic relationships among female fertility disorders, female fertility traits and productivity of Holstein dairy cows in the early lactation period. *J. Anim. Breed. Genet.* 134, (5), 353-363.
- Guarini, A.R., Lourenco, D.A.L., Brito, L.F., Sargolzaei, M., Baes, C.F., Miglior, F., Misztal, I., Schenkel, F.S. 2019. Genetics and genomics of reproductive disorders in Canadian Holstein cattle. *J. Dairy Sci.* 102, (2), 1341-1353.
- Heringstad, B. 2010. Genetic analysis of fertility-related diseases and disorders in Norwegian Red cows. *J. Dairy Sci.* 93 (6), 2751-2756.
- Jamrozik, J., Koeck, A., Kistemaker, G.J., Miglior, F. 2016. Multiple-trait estimates of genetic parameters for metabolic disease traits, fertility disorders, and their predictors in Canadian Holsteins. *J. Dairy Sci.* 99, (3), 1990-1998.
- Koeck, A., Egger-Danner, C., Fuerst, C., Obritzhauser, W., Fuerst-Waltl, B. 2010. Genetic analysis of reproductive disorders and their relationship to fertility and milk yield in Austrian Fleckvieh dual-purpose cows. *J. Dairy Sci.* 93, (5), 2185-2194.
- Lourenco, D.A.L., Tsuruta, S., Fragomeni, B.O., Masuda, Y., Aguilar, I., Legarra, A., Bertrand, J.K., Amen, T.S., Wang, L., Moser, D.W., Misztal, I. 2015. Genetic evaluation using single-step genomic best linear unbiased predictor in American Angus. *J. Anim. Sci.* 93, (6), 2653-2662.
- Misztal, I., Tsuruta, S., Lourenco, D.A.L., Masuda, Y., Aguilar, I., Legarra, A., Vitezica, Z. 2018. Manual for BLUPF90 family programs. University of Georgia. <http://nce.ads.uga.edu/wiki/doku.php?id=documentation>. (Accessed 20 July 2022)
- Vukasinovic, N., Bacciu, N., Przybyla, C.A., Boddhireddy, P., DeNise, S.K. 2017. Development of genetic and genomic evaluation for wellness traits in US Holstein cows. *J. Dairy Sci.* 100, (1), 428-438.