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The most common reproductive disorders of cows in Holstein cattle breeding

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Abstract: The paper deals with the genomic evaluation of retained placenta (RP), metritis/endometritis (MET), and cystic ovarian disease (CYS) and their involvement in the selection programme of Holstein cattle. A procedure for the estimation of genetic parameters and genomic breeding values was proposed. The dataset included farmers' records of 127 000 lactations from 109 farms for RP, 185 000 lactations from 131 farms for MET and 115 000 lactations from 87 farms for CYS. The lactational incidence was 6.9% (RP), 12.8% (MET) and 9.2% (CYS). The single-trait animal model equation included fixed effects of herd-year-season of calving and parity of the cow, random direct additive genetic effect, random permanent environmental effect and residual effect. The single-step genomic prediction (ssGBLUP) was based on more than 50 000 SNP-genotyped individuals. Heritability for all traits did not exceed 2%. Favourable correlations were found between reproductive disorders resistance, fertility and longevity traits. The correlation between CYS and milk protein yield indicated a genetic predisposition to cystic ovarian disease in high-producing cows. All significant correlations between health traits (clinical mastitis, claw and feet disorders, reproductive disorders) were positive, indicating that the selection for improvement of one health trait would induce a favourable selection response in the others.

Keywords: dairy cattle; retained placenta; uterine inflammation; ovarian cysts; genetic parameters; single-step genomic prediction

Reproductive disorders are one of the most common causes of involuntary culling in dairy cows in the Czech Republic with a frequency of 19% (Syrucek et al. 2022). Their incidence can be reduced by improving environmental conditions (nutrition, management, prevention, treatment), and also by animal breeding and selection. This involves exploiting genetic variability in resistance to health disorders. A prerequisite for genetic selection and breeding is a long-term observation of the traits and knowledge of their expression in the population. Only a few countries have reported recording of health disorders/diseases in most of the dairy cattle population: the oldest system was established in the Nordic countries in the 1970s due to cooperation between breeding and veterinary associations (Osteras et al. 2007). Based on the same approach, a national health-

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monitoring system was developed and implemented in 2006 in Austria (Egger-Danner et al. 2012). Other countries have tested the usability of data recorded in on-farm management software programs, as reported by Zwald et al. (2004) in the USA and Koeck et al. (2012) in Canada. The consecutive genetic evaluation showed that heritabilities of health traits were low and, in most cases, did not exceed 10% (Heringstad 2010; Jamrozik et al. 2016; Gernand and Konig 2017).

Although as early as the late 1990s, Kelton et al. (1998) published the guidelines and standards for the recording and presentation of eight economically important diseases, including retained placenta, metritis and cystic ovarian diseases, reproductive disorders were not routinely evaluated in many countries due to a lack of data. Yet efforts to include them in selection programmes were growing (Zavadilova et al. 2021). For example, the Nordic Total Merit Index NTM combines not only productive and functional traits but also early and late reproductive disorders, metabolic disorders, and feet and leg diseases (Kargo et al. 2014). The US Net Merit Index NM$ includes health traits (retained placenta, metritis, milk fever, displaced abomasum, ketosis, clinical mastitis) with a total weight of approximately 2% (Parker Gaddis et al. 2020). Reproductive disorders are also included in the selection indices of German dairy breeds, with total costs per case of metritis ranging from 173 € to 182 € (Schmidtmann et al. 2021).

In the Czech Republic, voluntary recording and processing of the national health data have been developed through the collaboration of experts from the Czech-Moravian Breeders' Corporation in Hradistko (CMBC), the Veterinary Research Institute in Brno and the Institute of Animal Science in Prague. Since 2017, dairy farmers have been able to record the occurrence of diseases and health disorders through the Diary of Diseases and Treatments web application (Diary), which is located on the 'Internet for farmers' CMBC web page. The application allows the recording, storage and processing of data on the occurrence of diseases and health events in all categories of cattle and the administration of medicaments on farms. Based on the Diary records, methodological procedures for routine genomic evaluation of resistance to clinical mastitis and claw disorders have already been developed (Krupova et al. 2019), and the Holstein Cattle Health Index (SI-health) has been designed.

The objective of this study was to set up a methodological procedure for genomic evaluation of the most common reproductive disorders in Holstein cows, to estimate their genetic parameters and breeding values, and to assess their associations with other traits that are subject to selection in Holstein cattle in the Czech Republic.

MATERIAL AND METHODS

Data

For this study, we selected the records of diagnoses and health interventions linked to cows with at least 75% of the Holstein breed that were entered into the Diary between July 2017 and December 2022. Their overview and numbers are given in Table 1. A total of 65 025 records were primarily available for 32 796 cows of 170 milk producers. The records were grouped into three traits (Table 1), namely retained placenta (RP) with 9 670 records, metritis/

Table 1. Health events in the Diary of Diseases and Treatments used to define the traits of reproductive disorders and the numbers of their records in Holstein cows

Diagnosis/treatment	Trait	Number of records
Retained placenta	RP	9 381
Partial removal of retained placenta	RP	289
Metritis – inflammation of the uterus up to 20 days in milk	MET	24 693
Metritis – purulent discharge	MET	2 883
Metritis – putrid discharge	MET	1 818
Endometritis – inflammation of the uterus after the 20th day in milk	MET	8 425
E1 – catarrhal endometritis	MET	179
E2 – mucopurulent endometritis	MET	383
E3 – purulent endometritis	MET	807
E4 – pyometra	MET	409
Ovarian cysts	CYS	1 508
Cyst on left ovary	CYS	1 266
Cyst on right ovary	CYS	1 940
Cystic ovarian disease	CYS	10 508
Follicular cyst	CYS	401
Luteal cyst	CYS	129
Rupture of the cyst	CYS	6

CYS = ovarian cysts; MET = metritis/endometritis; RP = retained placenta endometritis (MET) with 39 597 records and ovarian cysts (CYS) with 15 758 records. The definition of these traits corresponded to the definitions for retained placenta, metritis and cystic ovarian disease proposed by Kelton et al. (1998). The data were further edited so that only the first record of a given trait was retained for cow and lactation. The record had to meet the criterion of the occurrence between 0–14 days in milk (DIM) for RP and between 0–305 DIM for MET and CYS. The datasets were supplemented with 'healthy peers', i.e. cows that produced in the same herd and in the same period but without a record of the selected reproductive trait. The trait incidence was defined as its occurrence/absence in lactation and its frequency was expressed as lactational incidence LI, which represents the percentage of lactations affected by a given trait in the respective population. A minimum LI of 1% was applied for all traits to include the herd in the evaluation. In order to maintain as much data as possible and to achieve higher reliabilities of estimates, the traits were evaluated separately. The statistical program SAS v9.4 (SAS/STAT®; SAS Institute, Inc., Cary, NC, USA) was used for data editing and basic statistical evaluation. The basic characteristics of files entered in the genetic evaluation of each trait are shown in Table 2. For the genomic evaluation, data on SNP genotypes determined on the Illumina BovineSNP50 BeadChip were available for 54 239 animals (5 792 bulls, 48 447 cows and heifers).

Model

We used a single-trait animal model for estimating genetic parameters and genomic breeding values (GEBV). The model equation was the same for all three traits:

Table 2. Summary statistics of the evaluated dataset

Trait	Number of herds	Number of cows	Number of lactations	LI (%)
RP	109	76 246	127 468	6.9
MET	131	106 531	185 133	12.8
CYS	87	66 487	115 399	9.2

CYS = ovarian cysts; LI = lactational incidence, e.g. proportion of lactations affected by the reproductive disorder; MET = metritis/endometritis; RP = retained placenta

$$y_{ijklmn} = \mu + PA_i + HYS_j + pe_k + a_l + e_{ijklm} \qquad (1)$$

where:

y_{ijklmn} – incidence (1) or absence (0) of the trait;

μ – population mean;

PA_i – fixed effect of *i-th* parity (3 levels: 1^{st} parity, 2^{nd} parity, 3^{rd} and later parities);

HYS_j – fixed effect of *j-th* level of herd-year-season;

pe_k – random effect of the *k-th* cow's permanent environment;

a_l – random direct additive genetic effect of the animal *l* connected with the three-generations pedigree;

e_{ijklm} – random residual effect.

Model assumptions were:

a ~ $N(0, \mathbf{H}var(a))$, where *var(a)* was the direct additive genetic variance and **H** was the pedigree-genotypes relationship matrix (Aguilar et al. 2010; Christensen and Lund 2010);

pe ~ $N(0, \mathbf{I}var(pe))$, where *var(pe)* was the variance due to permanent environment and **I** was the identity matrix;

e ~ $N(0;1)$.

Methods

Co-variance components were estimated using the residual maximum likelihood method with an average information algorithm, as implemented in AIREMLF90 (Misztal et al. 2022). Heritabilities of all traits were calculated as:

$$h^2 = \frac{var(a)}{var(a) + var(pe) + var(e)} \qquad (2)$$

Corresponding repeatabilities were calculated as:

$$r_p = \frac{var(a) + var(pe)}{var(a) + var(pe) + var(e)} \qquad (3)$$

Genomic breeding values (GEBV) were estimated with the single-step GBLUP method, as implemented in BLUP90IOD2 (Misztal et al. 2022).

Estimated GEBV were expressed as relative breeding values (RBV) with a mean of 100 and a standard deviation SD = 12 for base bulls born in 2010. RBV were reversed in sign so that the higher values were desirable expressing better resistance to the reproductive disorder. The reliabilities of GEBV were approximated with ACCF90GS (Misztal et al. 2013). Pearson correlations between the RBV of bulls with at least 100 daughters for health traits and for other routinely evaluated traits and indices were used to approximate the genetic associations between them.

RESULTS AND DISCUSSION

The frequency of reproductive disorder traits RP, MET and CYS expressed as LI is shown in Table 2. The highest incidence (LI = 12.8%) was found in MET, which was also recorded in the highest number of herds (*n* = 131). The incidence of all traits was comparable with other population studies conducted in Holstein cattle (Zwald et al. 2004; Jamrozik et al. 2016; Gernand and Konig 2017). The estimates of co-variance components (Table 3) resulted in low heritability for all three traits, characterized by the coefficient of heritability $h^2 \sim 0.02$. This is consistent with the results of other population studies that have used linear models to estimate genetic parameters (Koeck et al. 2012; Jamrozik et al. 2016). The repeatability of the traits, which indicates the similarity between successive observations of the same cow, showed a comparable value of approximately 0.02–0.03. Jamrozik et al. (2021) reported a higher permanent environmental effect (from 0.02 to 0.03), but they observed repeatability only between the second and subsequent parities. Higher estimates of genetic parameters are usually achieved using threshold models. For example, Vukasinovic et al. (2017) reported the heritabilities for RP and CYS as 0.07 and 0.06 with corresponding repeatabilities of 0.12 and 0.13.

The distribution of estimated GEBV was close to normal with a mean of 0 and genetic SD = 0.01 (RP, CYS) and SD = 0.02 (MET). More detailed characteristics of the standardized RBV are shown in Table 4. The average reliability of the GEBV estimates was low (0.08–0.12). This is partly due to the low heritability of the traits, partly due to the short period of recording, which has covered only one generation of animals so far, and partly due to the high proportion of animals in the third generation of the pedigree (ancestors) with a low relationship to the animals with phenotype, and thus minimal reliability of the estimate.

As expected, the reliability of estimates increased with an increasing amount of information per individual. GEBV of bulls with a higher number of daughters with phenotypes, GEBV of cows with their own phenotypes and GEBV of young genotyped animals were estimated with higher reliabilities. Much higher GEBV reliabilities in young genotyped animals (up to 50%) were achieved by Vukasinovic et al. (2017). Their result was due to both the use of a threshold model, which led to much higher estimated genetic parameters, and the multiplicatively higher number of phenotypes and genotypes entering the evaluation. Plotting RBV averages by year of birth (Figures 1–3) showed a more or less neutral genetic trend for RP resistance and a gradual improvement in genetically determined resistance to MET. In contrast, for CYS resistance, RBV averages remained below the mean of 100 over the long term, and the trend for both males and females showed a gradual deterioration in genetically determined resistance to CYS.

Correlations with routinely evaluated traits and indices

Since the Czech Holstein population was not directly selected for the reproductive disorders, we could assume that the genetic trends were the result of selection for genetically correlated traits. We calculated the Pearson correlations between RBV for health traits and the routinely evaluated traits and indices to approximate their genetic as-

Table 3. Variance components, standard errors of their estimation (in parentheses), calculated heritability (h^2) and repeatability (r_p) of evaluated reproductive disorders

Trait	*var(a)*	*var(pe)*	*var(e)*	h^2	r_p
RP	0.001 0 (0.000 1)	0.000 3 (0.000 2)	0.045 0 (0.000 2)	0.02	0.02
MET	0.002 0 (0.000 2)	0.002 4 (0.000 3)	0.089 0 (0.000 4)	0.02	0.03
CYS	0.001 2 (0.000 2)	0.001 8 (0.000 3)	0.073 8 (0.000 4)	0.02	0.02

CYS = ovarian cysts; MET = metritis/endometritis; RP = retained placenta; *var(a)* = direct additive genetic variance; *var(e)* = residual variance; *var(pe)* = permanent environmental variance

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Table 4. Relative genomic breeding values (RBV) for resistance to reproductive disorders and the reliabilities of their estimates

Group of animals	*n*	Trait	RBV ± SD	r^2 ± SD
All animals	230 477	RP	100 ± 10	0.08 ± 0.074
	272 385	MET	100 ± 11	0.12 ± 0.095
	220 958	CYS	99 ± 10	0.10 ± 0.084
Genomic bulls	5 792	RP	99 ± 13	0.10 ± 0.104
		MET	97 ± 15	0.17 ± 0.136
		CYS	100 ± 12	0.12 ± 0.115
Bulls with >100 daughters	490	RP	99 ± 17	0.27 ± 0.183
	571	MET	100 ± 18	0.40 ± 0.204
	464	CYS	97 ± 16	0.32 ± 0.195
Genomic cows	48 447	RP	99 ± 13	0.15 ± 0.055
		MET	103 ± 13	0.23 ± 0.061
		CYS	97 ± 12	0.18 ± 0.060
Young genomic bulls	215	RP	97 ± 12	0.12 ± 0.038
		MET	105 ± 13	0.18 ± 0.042
		CYS	96 ± 13	0.13 ± 0.039
Genomic heifers	10 604	RP	100 ± 12	0.15 ± 0.041
		MET	106 ± 13	0.21 ± 0.047
		CYS	97 ± 11	0.17 ± 0.046

CYS = ovarian cysts; MET = metritis/endometritis; *n* = number of animals; r^2 = reliability of breeding value estimate; RP = retained placenta

sociations. The predictive ability of correlations between RBV depends on their reliability and tends to underestimate the true genetic correlations. As the overall reliability of RBV estimates was low, the results should be interpreted with caution as a general indication. Figure 4 shows the correlations between RBV for health traits and production and functional traits. All the correlations ranged from low to moderate and did not exceed 35% in either direction.

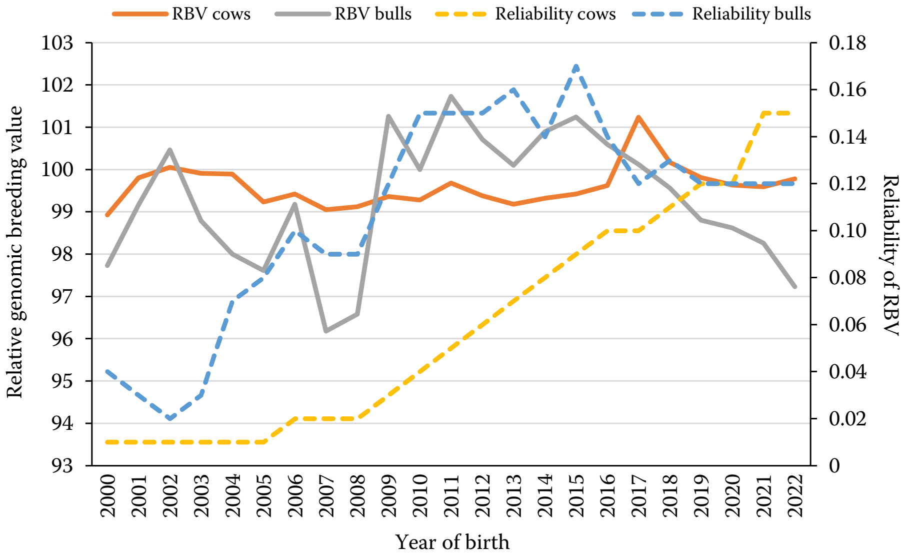

Figure 1. Genetic trend and reliability of genomic breeding values (RBV) for the resistance to retained placenta in Holstein cattle

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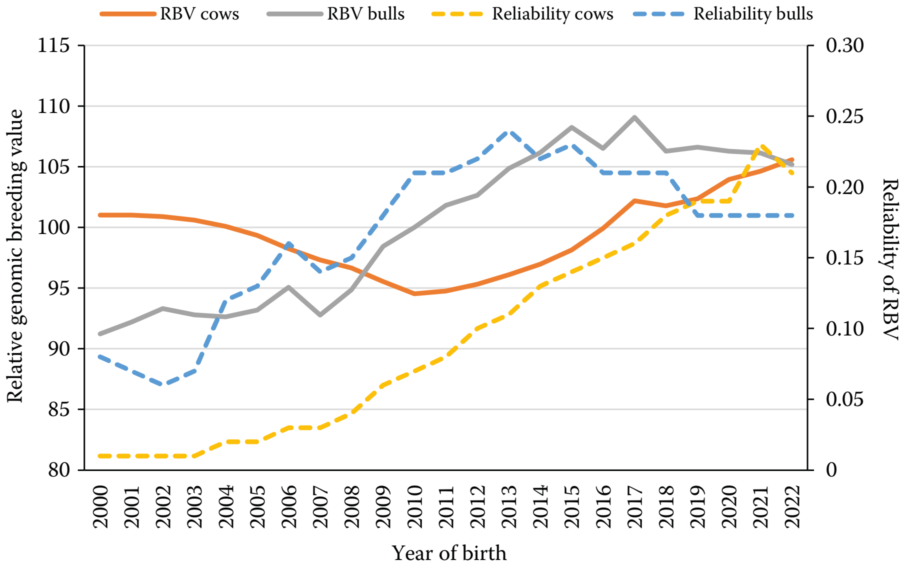

Figure 2. Genetic trend and reliability of genomic breeding values (RBV) for the resistance to metritis/endometritis in Holstein cattle

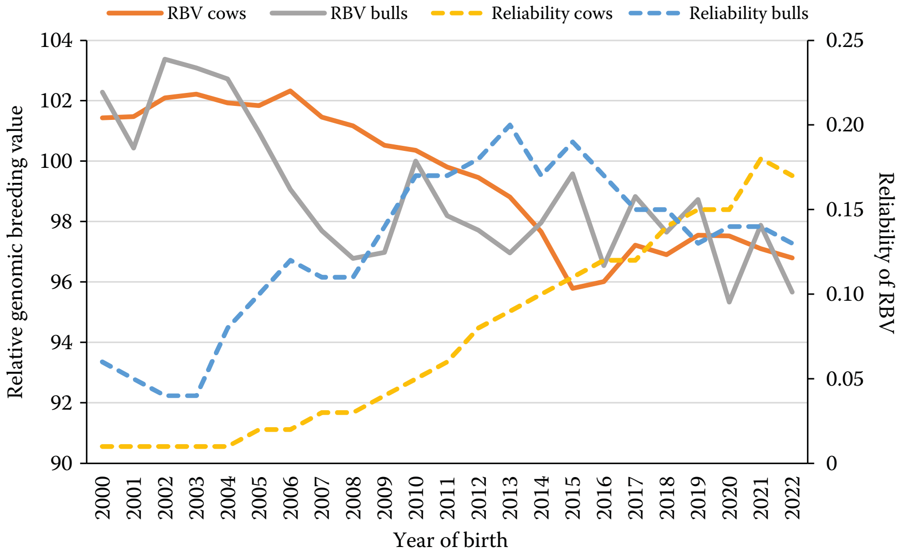

Figure 3. Genetic trend and reliability of genomic breeding values (RBV) for the resistance to ovarian cysts in Holstein cattle

The correlations of RBV-RP and RBV-MET were similar in direction, but RBV-MET correlations were stronger. The strongest favourable associations were found with the fertility of daughters and longevity, which was comparable with previous studies by Koeck et al. 2012; Vukasinovic et al. (2017) and Jamrozik et al. (2021). Based on these relationships, we can summarise that direct selection for better resistance to RP, MET, and CYS would favour dairy cows' fertility and longevity, and vice versa, selection on better fertility and longevity would positively affect the resistance to reproductive disorders. Jamrozik et al. (2021) found favourable correlations between RBV-MET and calving traits and with calf survival. Our study similarly showed a positive correlation of RP and MET resistance with maternal calving ease, which corresponded to a lower incidence of reproductive disorders in cows that gave birth easily (as reported also by Koeck et al. 2012). On the contrary, the correlation between RBV-MET and direct calving ease was negative, suggesting that easy-born females were genetically more susceptible to uterine diseases.

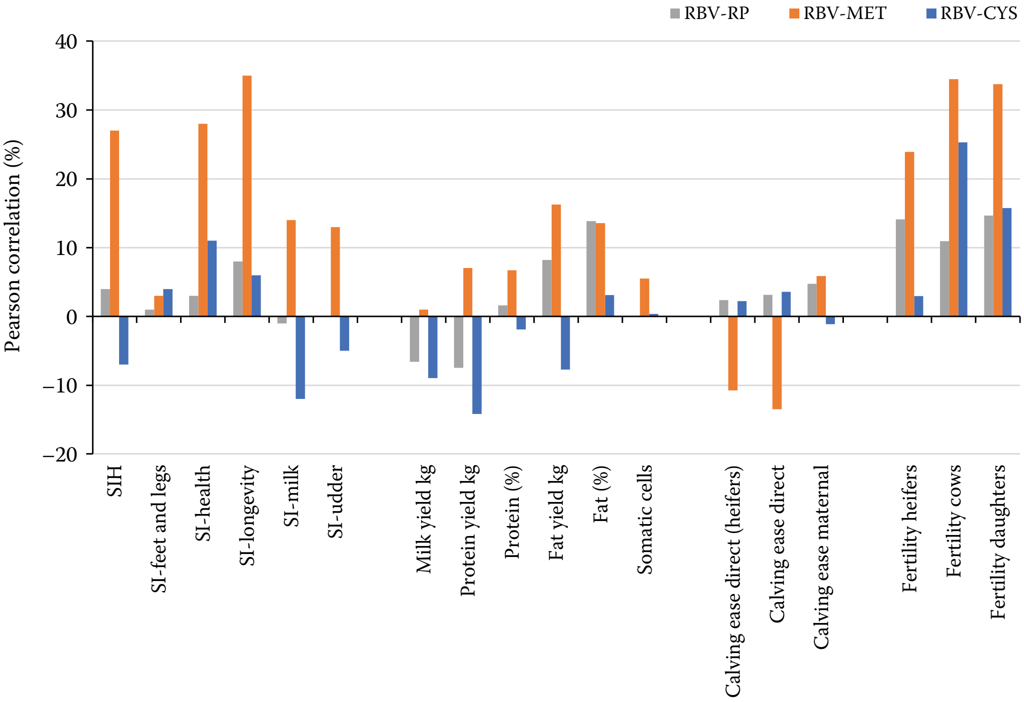

Figure 4. Pearson correlations between relative breeding values (RBV) of sires with at least 100 daughters for resistance to retained placenta RBV-RP, inflammation of uterus RBV-MET and ovarian cysts RBV-CYS and other routinely evaluated traits and indices

Calving ease direct (heifers) = RBV for the ability of the calf to be born easily, evaluated in heifers; Calving ease direct = RBV for the ability of the calf to be born easily, evaluated in heifers and cows; Calving ease maternal = RBV for the ability of the cow to give birth easily; Fertility of cows = RBV for the fertility of bull's daughters – cows; Fertility of daughters = RBV for the fertility of bull's daughters – heifers and cows; Fertility of heifers = RBV for the fertility of bull's daughters – heifers; Higher RBV are desirable in all health traits; higher values of RBV are desirable in all calving traits; RBV-CYS = RBV for resistance to ovarian cysts; RBV-MET = RBV for resistance to metritis/endometritis; RBV-RP = RBV for resistance to the retained placenta; SI-feet and legs = sub-index combining RBV for feet and legs total score, foot angle, rear legs from the rear view and locomotion; SIH = selection index for Czech Holstein sires consisting of RBV for fat yield and content, protein yield and content, somatic cells score, feet and legs total score, foot angle, rear legs from the rear view, locomotion, udder depth, front udder attachment, central ligament, rear teats placement, rear udder height, teat length and SI-longevity; SI-health = sub-index combining RBV for the resistance to clinical mastitis, infectious feet and claw diseases, non-infectious feet and claw disorders and overall feet and claw disorders; SI-longevity = sub-index combining RBV for the sire's direct longevity, udder depth, body depth, feet and legs total score, somatic cells score and fertility of daughters; SI-milk = sub-index combining RBV for fat yield and content, protein yield and content; SI-udder = sub-index combining RBV for udder depth, fore udder attachment, central ligament, rear teats placement, rear udder height and teat length

Pearson correlations > 10% were significant ($P < 0.05$)

The association with production traits was not explicit: while RBV-RP was correlated with lower milk and protein yield, the RBV-MET correlations with both traits were positive, as well as its correlations with fat yield and fat content (Koeck et al. 2012). As a result, MET resistance was favourably associated with higher selection index for Czech Holstein Sires (SIH) and SI-milk. Only weak negative and some positive Pearson correlations between reproductive health traits and production traits suggested that high performance might not necessarily be strongly genetically associated with poor health as reported also by Vukasinovic et al. (2017).

RBV-CYS was correlated with better cows and female fertility, and also the correlations with longevity and direct calving ease were favourable but weak. RBV-CYS was negatively correlated with yield traits and dairy cows with higher production tend to be less resistant to CYS. Breeding for higher production, which has the highest partial weight in the SIH (Krupova et al. 2019), may be one of the reasons for the observed unfavourable genetic trend for CYS resistance in the Czech Holstein population. Negative correlations between CYS resistance and productive traits are often mentioned in the literature (Koeck et al. 2012; Gernand and Konig 2017; Jamrozik et al. 2021). For example, Stadnik et al. (2022) found that ovarian response depended on the metabolic status of the cow and reflected the competition for energy and nutrients between production and reproduction. Hooijer et al. (2001) estimated that in the Dutch Black and White cattle population, the selection for milk yield would increase the incidence of CYS by 1.5% per 500 kg increase in milk.

Pearson correlations of all three reproductive health traits with conformation traits were mostly low and insignificant, so we do not report them in detail. The highest correlations (up to 25%) were found between RBV-MET, udder depth, total udder score, and total conformation score. For RBV-CYS, the highest correlations (up to 13%) were found for fore udder attachment, rear udder attachment width and body depth. The correlations between RBV-RP and conformation traits were weak and did not exceed 7% (fore teat placement). The results available in the literature were comparable with ours. Koeck et al. (2012) reported weak and positive correlations between RP resistance and total scores for udder and conformation. Jamrozik et al. (2021) found unfavourable correlations between RBV-MET and rear leg from a side view, teat length and body depth and unfavourable correlations between RBV-CYS and fore udder attachment, rear udder attachment width and mammary system.

Pearson correlations of health traits

To incorporate reproductive health traits into the breeding system, it is important to balance them in relation to other health traits that are already part of the SI-health (Table 5). Pearson correlations between RBV for health traits were from low to moderate. All significant correlations were positive, which according to Heringstad et al. (2005) may be related to the existence of a genetically determined general immune response that corresponds to the major histocompatibility complex. A moderate correlation was found in accordance with the literature (Heringstad 2010; Koeck et al. 2012; Parker Gaddis et al. 2020) between RBV-RP and RBV-MET. The correlations between RBV-CYS and the other two reproductive health traits were weak and at the limit of statistical significance. The literature is divided on the genetic association of CYS to RP and MET with estimates ranging from weak negative (Heringstad 2010) to medium positive values (Gernand and Konig 2017). Gernand and Konig (2017) explained this broad spectrum of genetic associations by possible breed differences, due to the effects of selection or due to environmental effects on gene expression.

Table 5. Pearson correlations (%) between relative genomic breeding values for health traits in Holstein cattle

Trait	RP	MET	CYS
Number of sires	490	571	464
RP	–	43^{***}	8^{ns}
MET	–	–	11^{*}
CM	1^{ns}	14^{***}	12^{***}
CI	8^{ns}	14^{***}	12^{***}
CNI	-4^{ns}	29^{***}	-1^{ns}
OCD	4^{ns}	24^{***}	5^{ns}

CI = infectious feet and claw diseases; CM = clinical mastitis; CNI = non-infectious feet and claw disorders; CYS = ovarian cysts; MET = metritis/endometritis; OCD = overall feet and claw disorders; RP = retained placenta

$^{*}P < 0.05$; $^{***}P < 0.001$

Other assessed traits were the components of the SI-health, which combines GEBV for resistance to clinical mastitis (CM), infectious feet and claw diseases (CI), non-infectious feet and claw disorders (CNI) and overall feet and claw disorders (OCD) which is the sum of the two previous groups plus laminitis, oedema and tyloma. Correlations between RBV-RP and these traits were insignificant and close to zero in our study. Heringstad et al. (2005) and Koeck et al. (2012) found a positive correlation between RP and CM. Similar to our study, Koeck et al. (2012) reported positive correlations between MET and CM, CYS and CM, and in addition to that a weak positive correlation between RP, CYS and lameness or negative correlation between MET and lameness. In contrast, all correlations between RBV-MET and feet and claw disorders were positive in our study. In addition, RBV-CYS was positively correlated with CI. These positive correlations between reproductive and feet and claw disorders are consistent with a clinical study by Tsousis et al. (2022), who observed behavioural changes, stress and inflammatory responses in lame cows that affected the function of the hypothalamic-pituitary-ovarian axis, resulting in reduced reproduction. Lame cows were more susceptible to uterine infections post-partum, suffered more from a negative energy balance, resumed ovarian cyclicity to a lower proportion during the puerperium, and showed a decreased potential to conceive and higher pregnancy losses.

CONCLUSION

Based on the data recorded by farmers in the Diary of Diseases and Treatments web application, genomic breeding values of Holstein cattle for resistance to the three most common reproductive disorders (retained placenta, metritis/endometritis, and cystic ovarian disease) can be estimated with sufficient reliability. Indirect selection against these reproductive disorders is already underway through genetically correlated traits, in particular fertility and longevity traits. Genetic trends are favourable for retained placenta and metritis/endometritis, while unfavourable for cystic ovarian disease. Despite their low heritability, the resistance to all three disorders could benefit from their direct selection and inclusion in the current SI-health. The resulting genetic gain would depend on the accuracy of selection, i.e. reliability of breeding values and differentiation of genetically divergent animals. This can be achieved not only by recording a large volume of phenotypes but also by incorporating genomic information and predicting genomic breeding values.

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Conflict of interest

The authors declare no conflict of interest.

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