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INTERPRETIVE SUMMARY

Genomic predictions of fertility related disorders. *Haugaard et al pages 000*. The aim was to examine whether including information from later lactations improves accuracy in prediction of genomic breeding values for fertility related disorders in Norwegian Red. Health records from >6 million lactations of 2.4 million cows were analyzed. Genomic breeding values for cystic ovaries, metritis, retained placenta and silent heat were predicted based on first lactation only and by using information from lactations 1-5. Including later lactations improved accuracy of genomic breeding values for cystic ovaries, retained placenta and silent heat, while no obvious advantage in accuracy was found for metritis.

10 **GENOMIC PREDICTIONS OF FERTILITY RELATED DISORDERS**

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12 **Information from later lactations improve accuracy of genomic predictions of fertility**

13 **related disorders in Norwegian Red**

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ABSTRACT

The aim was to investigate whether including information from later lactations improves accuracy of genomic breeding values for the 4 fertility related disorders cystic ovaries, retained placenta, metritis and silent heat. Data consisted of health records from 6,015,245 lactations from 2,480,976 Norwegian Red cows, recorded from 1979 to 2012. These were daughters of 3,675 AI-bulls. The mean frequency of these disorders for cows in lactation 1-5 ranged from 0.6% to 2.4% for cystic ovaries, 1.0% to 1.5% for metritis, 1.9% to 4.1% for retained placenta and 2.4% to 3.8% for silent heat. Genomic information was available for all sires, and the 312 youngest bulls were used for validation. After standard editing of a 25k/54k SNP dataset that was imputed both ways, a total of 48,249 SNP loci was available for genomic predictions. Genomic breeding values were predicted using univariate GBLUP for first lactation only (GEBV-1) and for the first 5 lactations (GEBV-S), and multivariate GBLUP with 5 lactations for each disorder was also used for genomic predictions (GEBV-M). Correlations between EBV for the 4 traits in 5 lactations with GEBV-1, GEBV-S and GEBV-M were compared. Accuracy ranged from 0.47 and 0.51 for cystic ovaries, 0.50 to 0.74 for retained placenta, 0.21 to 0.47 for metritis and 0.22 to 0.60 for silent heat. Including later lactations in a multitrait G-BLUP improved accuracy of GEBV for cystic ovaries, retained placenta and silent heat, while for metritis no obvious advantage in accuracy was found.

Keywords:

Fertility related disorders, genomic prediction, dairy cattle

INTRODUCTION

In a progeny testing scheme, only first lactation information from the daughters is available when the bulls get their first official proofs. The frequency of fertility related disorders such as cystic ovaries (**CO**), retained placenta (**RP**) and metritis (**MET**) however, often increases as the cow gets older (Haugaard and Heringstad, 2013). This implies that potentially valuable information is not yet available at the time when the elite sires are selected. With the introduction of genomic selection, information from later lactations may more easily be utilized as the reference population includes older bulls with information from daughters of all ages.

Some of the factors affecting the accuracy of genomic predictions are the size of the reference population, heritability of the trait, quality and number of available phenotypes, population structure and effective population size, and the density of the genomic markers (Hayes et al., 2009). Functional traits, such as fertility and health, have low heritability and show lower accuracy in genomic predictions compared to production traits (Luan et al., 2009; Zhou et al., 2014). Few populations record direct health traits, though recently several countries have started recording health traits as well as production traits in contract herds (Gernand et al., 2012) or in the main population (e.g. Koeck et al., 2012; Egger-Danner et al., 2012). In the Scandinavian countries, disease records have been collected for more than 30 years, and direct health traits (e.g. mastitis) are included in routine genetic evaluations. Fertility related disorders have so far not been included in the routine genetic evaluations in Norway, except RP which is included in "other diseases", a trait with 2% relative weight in the current total merit index for Norwegian Red. Fertility related disorders is a disease category that has increased somewhat in frequency the recent years. The number of cows treated for any fertility related disorders per

cow-year (incidence rate) increased from 6.6% in 2008 to 8.5% in 2013 (Norwegian Cattle Health Services, 2014) and inclusion in the breeding scheme may therefore become desired.

The main aim was to examine whether including information from later lactations would increase accuracy of genomic predictions for fertility related disorders in Norwegian Red. Accuracy of genomic predictions based on data from first lactation only vs. using lactations 1 to 5 was compared. More than 30 years of health recordings of the 4 most common fertility related disorders; CO, RP, MET and silent heat (**SH**) were used.

MATERIAL AND METHODS

Data

Records on calving and health (veterinary treatments of disease) from 2,480,976 cows calving from January 1979 through December 2012 and sired by Norwegian Red AI bulls were extracted from the Norwegian Dairy Herd Recording System. Information on CO, RP, MET and SH from the first 5 lactations were used. The four disorders were chosen as these are the most frequent fertility related disorders in Norway. Cows without first lactation records in the dataset were omitted, and the cows had to be 20 to 36 months old at first calving and have reasonable calving intervals (280-500 d) thereafter. The traits were defined as binary (0=healthy, 1=affected) for each disorder in each lactation. For RP the veterinary treatment had to occur within the first 5 days after calving, whereas for the other disorders all health records within a lactation were used. The overall mean frequency of each disorder in each lactation is presented in Table 1. The mean frequency varied from 0.6% (CO in 1st lactation) to 4.1% (RP in 5th lactation). Only daughters of bulls with at least 150 first lactation daughters were included in the dataset. There were a total of 26,858 animals in the pedigree file which consisted of the

3,675 bulls with daughters in the dataset and their dams and sires traced back as far as possible, back to the 1950's.

Genomic information was available for all 3,675 sires where 2,165 were genotyped with the Affymetrix25K SNP chip and 1,967 were genotyped with the Illumina55K SNP chip. 457 of the sires were genotyped with both. An imputed 25k/54k SNP dataset (imputed both ways, imputation procedure described in Solberg et al., 2011) after standard editing had 48,249 SNP loci that was used for genomic predictions.

Daughter-yield-deviations

Daughter-yield-deviations (**DYD**) of the reference population were used as response variable for the genomic predictions. These were estimated using a subset containing only records from lactations starting before January 1st 2008. The 3,363 bulls with at least 150 first lactation daughters in this sub-dataset were included in the reference population, while the youngest 312 bulls that by January 1st 2008 did not have 150 first lactation daughters was defined as the validation set. The mean number of first lactation daughters per sire in the reference population and validation set was 675 daughters (min 150; max 10,197) and 227 daughters (min 150; max 2,742) respectively.

Each of the 4 fertility related disorders was analyzed separately using linear sire models to predict parameters for calculation of DYD using the subdataset, and to predict EBV using the full dataset for comparison with GEBV from the genomic predictions. For MET and SH the 5 lactations were analyzed as genetically correlated traits in multivariate models. CO was analyzed treating the 5 lactations as repeated records in a univariate repeatability model. A repeatability model was also used for RP in lactation 2 to 5, while RP in the first lactation was

analyzed as a correlated trait in a bivariate model. The choice of models was based on Haugaard and Heringstad (2013). For CO the systematic effects were year-season of calving (132 levels, seasons defined as January-March, April-June, July-September and October-December) and age at calving in months (76 single month classes). For MET the systematic effects were year-season of calving and twinning, recorded as 1 (single calf) or 2 (two or more calves). For RP the systematic effects were year-season of calving, twinning and calving interval in the previous lactation, divided into 6 categories: 1) ≤325 days, 2) 325-340 days, 3) 341-355 days, 4) 356-370 days, 5) 371-400 days and 6) >400 days. For SH the systematic effects were year-season of calving and calving interval in the previous lactation. Herd and sire were treated as random effects in all models. Single-trait analyses of the first lactation for each disorder were also performed. Solutions from the linear models of the reduced datasets were then used to calculate DYD to be used as response variable in the genomic predictions described below, where DYD is the average performance of the bulls daughters subtracted for all systematic and non-genetic random effects. All linear analyses were done using the DMU4-program in DMU (Madsen and Jensen, 2007). Heritability for all traits used in the analyses is presented in Table 2.

Accuracy of genomic predictions

Direct genomic breeding values (**GEBV**) were predicted in 3 different ways:

1) **GEBV-1** - single trait GBLUP using DYD from first lactation only as response variable,

2) **GEBV-S** - single trait GBLUP using DYD from each of the 5 lactations separately as response variable

3) **GEBV-M** - multi trait GBLUP using DYD from the 5 lactations simultaneously as response variable. For MET the 5-variat GBLUP analyses did not converge, so only the first 4 lactations was used in a 4-variat GBLUP.

These analyses were performed using DMUAI in DMU (Madsen and Jensen, 2010). In matrix notation, the model can be written as $\mathbf{y=1\mu + Zg + e}$, where **y** is the response variable DYD, **1** is a vector of ones, **μ** is the overall mean, **g** is a vector of genomic breeding values, **Z** is the incidence matrix of **g** and **e** is the residuals. It was assumed that var(**g**)=$\mathbf{G_0 \otimes G}$ and var(**e**)=$\mathbf{R \otimes D}$, where **G** is the genomic relationship matrix and **D** is a diagonal matrix containing weighting factors for the residuals. $\mathbf{G_0}$ and **R** is the corresponding scalar (GEBV-1 and GEBV-S) or 5x5 (GEBV-M) matrices, containing the genetic and residual (co)variance.

The genomic relationship matrix was calculated using the program Gmatrix (Su and Madsen, 2012), constructed by method 1 of Van Raden (2008). Accuracy of genomic predictions was calculated as the correlation between GEBV for the 312 sires in the validation set and their EBV obtained from the full dataset. In the GEBV-S and GEBV-M approach, EBV for each lactation was correlated with GEBV of the same lactation, whereas in the GEBV-1 approach, EBV for all five lactations was correlated to the GEBV from the first lactation. The correlations were based only on those bulls in the validation set with daughters in the respective lactation, meaning that the validation set for lactations 4 and 5 were smaller (265 and 169 bulls, respectively) than for lactations 1, 2 and 3 (312 bulls). Regression analyses were used to validate whether GEBV over- or underpredict the genetic merit for the fertility related disorder in each case.

RESULTS AND DISCUSSION

Accuracy

Accuracy of genomic predictions ranged from 0.22 to 0.54 for GEBV-1, from 0.21 to 0.74 in GEBV-S and 0.24 to 0.74 in GEBV-M (Table 3).These accuracies were in the upper range of

169 those previously reported for functional traits in Norwegian Red. Luan et al. (2009) and
170 Svendsen (pers.comm) found accuracies for various production and health traits in Norwegian
171 Red in the range 0.15 - 0.41 and 0.16 - 0.77, respectively. In both studies, health and fertility
172 traits showed lower accuracies than production traits, and the highest accuracies among
173 functional traits were 0.46 for mastitis (Svendsen, pers.comm) and 0.43 for calving ease (Luan
174 et al., 2009). Compared to the present study, Luan et al. (2009) analyzed fewer bulls (500) with
175 fewer SNP (25K). These are factors that affect the results of the genomic predictions, and can
176 explain the differences in the results. Ødegård et al. (2014) presented accuracies of GEBV for
177 claw health in Norwegian Red ranging from 0.29 to 0.32, which were lower than accuracies in
178 the present study. As claw health is a novel trait in Norwegian Red, with records available since
179 2004 (disease records from claw trimming), the limited size of the reference population and low
180 reliability of the response variable may be a reason for the lower accuracies of Ødegård et al.
181 (2014) compared to the present study.
182
183 The accuracy of CO was 0.47 and 0.51 for GEBV-1 and GEBV-S, respectively (Table 3),
184 indicating that using information from only the first lactation gave slightly lower accuracy than
185 using information from all 5 lactations. High genetic correlations, above 0.90, between CO in
186 the 5 first lactations (Haugaard and Heringstad, 2013) indicates that the disorder is genetically
187 the same across lactations. Therefore, it seems reasonable that using information from one
188 lactation to predict the others is a possibility.
189
190 For RP, the differences between GEBV-1 and GEBV-S were larger than for CO (Table 3). For
191 1st-lactation RP, the accuracy was the same in both scenarios (0.50), while for 2-5 lactation RP
192 the accuracy was lower in GEBV-1 than in GEBV-S (0.51 vs 0.74). The highest accuracies
193 were acquired from using the GEBV-M approach with 0.55 and 0.74 for 1st and 2-5 lactation,

194 respectively. In all three approaches, the accuracy for RP was lower in first lactation than in
195 second to fifth lactation.
196
197 For MET, the lowest accuracy was obtained from the GEBV-M approach, and accuracies
198 decreased with increasing lactation number, from 0.47 in the first lactation to 0.21 in the fifth
199 lactation (Table 3). The accuracies from the GEBV-S approach was similar but slightly higher
200 than for the GEBV-M approach, while the overall highest accuracies was obtained with the
201 GEBV-1 approach, ranging from 0.39 to 0.47. Among the 4 fertility related disorders, MET
202 had the lowest mean frequency and the same level across lactations (Table 1). Estimates of
203 variance components and EBV for MET were therefore less accurate, especially in the later
204 lactations where information was sparse (Haugaard and Heringstad, 2013). However, the
205 genetic correlations between MET in first lactation and MET in later lactations were moderate
206 (0.51-0.67) (Haugaard and Heringstad, 2013). It was therefore not expected that first lactation
207 was as good a predictor of later lactations as that lactations itself (GEBV-1 vs GEBV-S/GEBV-
208 -M, Table 3).
209
210 For SH the accuracy was approximately the same in all 3 approaches for first lactation (Table
211 3), but while the accuracies decreased with increasing parity for the GEBV-1 and GEBV-S
212 approaches, the accuracies increased slightly with increasing parity for the GEBV-M approach.
213 SH had the highest mean frequency among the 4 disorders (Table 1), but decreasing with
214 increasing parity.
215
216 In general, accuracy of genomic predictions increased when using information from all
217 lactations, and the highest accuracies were obtained by using the GEBV-M approach for most
218 of the fertility related disorders. The exception was MET, where the highest accuracy was

obtained by using the first lactation to predict all lactations (GEBV-1). For SH, the GEBV-M approach gave higher accuracies, but it varied between lactations which was better of the GEBV-1 and GEBV-S approach. Among the fertility related disorders, the highest accuracy was obtained for RP. This may be due to the structure of the model (repeatability model), but also because this is the disorder that probably has few false negatives as it is easy to discover and demands veterinary attention. MET and SH are more troublesome disorders that can be difficult to discover and therefore probably have more false negatives. Regarding SH, cows may be culled instead of being treated or the disorder is unnoticed and therefore not treated, and many false negatives may occur.

Regression analyses were used to validate whether GEBV over- or underpredict the genetic merit. The regression coefficients with their standard errors are given in Table 3. Regression coefficients larger than 1 indicate that genetic merit is underpredicted by GEBV, while b-values lower than 1 indicate overprediction. Table 3 shows large variation between traits and approaches, with b-values ranging from 0.18 to 2.63. Indications of serious underprediction (b-values>2) were found for MET2 and MET4 when using single trait GBLUP (GEBV-S). Using GEBV based on first lactation to predict later lactations (GEBV-1) tended to overpredict genetic merit (b-values<1) for MET and SH. Traits analyzed by a repeatability model, CO and RP2-5, tended to underpredict genetic merit, The exception was CO in the GEBV-S approach which b-value, in addition to the regression coefficients for SH1 and RP1 in all three approaches, was closest to 1.

Assumptions and limitations

The present study used a relatively small validation set, containing approximately 10% of the total number of genotyped bulls. The validation bulls were the youngest, and some of them did

not have daughters in the latest lactations, which reduced the validation set further. It would be possible to include more bulls in the validation set, but then the reference population would be reduced.

In the present study the youngest bulls were defined as the validation set and the oldest bulls were in the reference population. This is how genomic selection would appear in practice. For the reference population all data after a cut-off date was removed, pretending they had not yet happened. In this scheme no sires would be predicted by their sons, as would happen in a full cross validation study.

Another issue is the precision of EBV for the 4th and 5th lactation in the validation set. As some of the bulls did not have 5th lactation daughters, the validation set was smaller for these traits. Also, the number of daughters per bull was lower in the later lactations. Consequently, the precision of the bulls EBV is less accurate than in the 1st lactation. The accuracy in the 4th and 5th lactation is therefore based on a smaller validation set with less precise EBV. A solution could be to set a limit on a minimum of e.g. 150 daughters in the 5th lactation in the validation set, but this would decrease the validation set drastically.

An important question is which EBV and GEBV to compare. In the present study, EBV for each lactation and disorder was correlated with the GEBV of the same lactation or with GEBV for the first lactation. The latter is a measure of how well 1st lactation GEBV predict the later lactations. Another approach could be to use GEBVs from the 5 first lactations (together or separately) to predict the 1st and perhaps the 2nd lactation of the disorder. Which method to choose depends on the aim of the scheme; to reduce susceptibility to fertility related disorders

in the 1st and 2nd lactations, or to breed for a cow with reduced susceptibility to fertility related disorders over many lactations?

Implementation

Health data, including veterinary treatment of fertility related disorders, from more than 30 years are available. Haugaard and Heringstad (2013) estimated heritabilities on the underlying scale of CO, MET, RP and SH between 0.03 and 0.14. The present study shows accuracies of GEBV in the upper range of what was previously reported for traits with similar heritabilities for Norwegian Red. Reliability of GEBV for these fertility related disorders are expected to be higher than the reliability of parent average EBV (at the time of birth of the bull calf) and lower than the reliability of EBV after progeny testing. Genetic evaluation of these fertility related disorders can therefore be implemented in the breeding scheme for Norwegian Red with at least as precise evaluations as other health traits.

CONCLUSIONS

Accuracy of genomic predictions for fertility related disorders were in the upper range of those previously reported for functional traits in Norwegian Red. Including later lactations improved accuracy of GEBV for CO, RP and SH, while no obvious advantage in terms of accuracy was found for MET.

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353 **Table 1. Number of records and mean frequency of cystic ovaries (CO), retained placenta**

354 **(RP), metritis (MET) and silent heat (SH) in lactation 1-5 in the full dataset.**

355

		Frequency[1], %			
Lactation number	No of records	CO %	RP %	MET %	SH %
1	2,480,976	0.6	1.9	1.5	3.8
2	1,645,094	1.4	2.5	1.0	2.9
3	1,021,604	2.0	3.1	1.1	2.8
4	576,709	2.3	3.6	1.2	2.6
5	290,862	2.4	4.1	1.2	2.4
Overall	6,015,245				

356 [1] Frequency of at least one veterinary treatment

Table 2: Heritability of fertility related disorders used for prediction of EBV.

Trait[1]	Heritability[2]
CO1-5	0.009
RP1	0.008
RP2-5	0.010
MET1	0.002
MET2	0.001
MET3	0.001
MET4	0.001
MET5	0.002
SH1	0.005
SH2	0.002
SH3	0.002
SH4	0.002
SH5	0.002

[1]CO was analyzed with the 5 lactations as repeated records in a univariate repeatability model. RP in lactation 2 to 5 were analyzed as repeated records in a repeatability model, together with RP in the first lactation as a correlated trait in a bivariate model. MET and SH were analyzed with 5 lactations as genetically correlated traits in multivariate models.

[2] $h^2 = (4*\sigma^2_{sire})/(\sigma^2_{sire} + \sigma^2_{herd} + \sigma^2_{residual})$, where h^2 is the heritability, and σ^2_{sire}, σ^2_{herd}, $\sigma^2_{residual}$ is the sire variance, herd variance and residual variance, respectively. Estimated variance

components were larger than their standard errors, and standard error for σ^2_{sire} was <0.00001 for all traits.

Table 3: Correlation (SE[1]) between EBV for cystic ovaries (CO), retained placenta (RP), metritis (MET) and silent heat (SH) in lactations 1-5, with genomic predictions based on first lactations only (GEBV-1) or 5 lactations (GEBV-S and GEBV-M)

		Genomic predictions	
Trait[2]	**GEBV-1[3]**	**GEBV-S [4]**	**GEBV-M[5]**
CO1-5	0.47 (0.05)	0.51 (0.05)	---
RP1	0.50 (0.05)	0.50 (0.05)	0.55 (0.05)
RP2-5	0.51 (0.05)	0.74 (0.04)	0.74 (0.04)
MET1	0.47 (0.05)	0.47 (0.05)	0.45 (0.05)
MET2	0.41 (0.05)	0.46 (0.05)	0.39 (0.05)
MET3	0.43 (0.05)	0.23 (0.06)	0.30 (0.05)
MET4	0.39 (0.05)	0.28 (0.05)	0.24 (0.06)
MET5	0.46 (0.05)	0.21 (0.06)	---
SH1	0.54 (0.05)	0.54 (0.05)	0.54 (0.05)
SH2	0.40 (0.05)	0.50 (0.05)	0.57 (0.05)
SH3	0.34 (0.05)	0.51 (0.05)	0.58 (0.05)
SH4	0.22 (0.06)	0.57 (0.05)	0.60 (0.05)
SH5	0.35 (0.05)	0.22 (0.06)	0.60 (0.05)

[1]SE was calculated as $\sqrt{(1-r^2)/(n-2)}$, where r is the correlation and n is the number of individuals.

[2]CO was analyzed with the 5 lactations as repeated records in a univariate repeatability model. RP in lactation 2 to 5 were analyzed as repeated records in a repeatability model, together with

RP in the first lactation as a correlated trait in a bivariate model. MET and SH were analyzed with 5 lactations as genetically correlated traits in multivariate models.

[3]EBV for the five lactations of a disorder correlated to the GEBV of the first lactation of the same disorder, GEBV estimated from a single trait GBLUP

[4]EBV correlated with GEBV of the same lactation for the same disorder, GEBV estimated from a single trait GBLUP

[5]EBV correlated with GEBV of the same lactation for the same disorder, GEBV estimated from a multitrait GBLUP

382 **Table 4:** Predicted b-values (SE) from regression analyses of EBV and GEBV

Trait[1]	**GEBV-1**[2]	**GEBV-S** [3]	**GEBV-M**[4]
CO1-5	1.70 (0.18)	0.91 (0.09)	-
RP1	0.87 (0.09)	0.87 (0.09)	0.83 (0.07)
RP2-5	1.81 (0.17)	1.50 (0.08)	1.43 (0.07)
MET1	0.53 (0.06)	0.52 (0.06)	0.56 (0.06)
MET2	0.27 (0.03)	2.63 (0.28)	0.36 (0.05)
MET3	0.38 (0.04)	0.41 (0.10)	0.32 (0.06)
MET4	0.36 (0.05)	2.66 (0.52)	0.24 (0.06)
MET5	0.68 (0.08)	0.69 (0.18)	---
SH1	0.91 (0.08)	0.91 (0.08)	0.88 (0.08)
SH2	0.41 (0.05)	0.87 (0.09)	0.71 (0.06)
SH3	0.32 (0.05)	0.74 (0.07)	0.66 (0.05)
SH4	0.18 (0.05)	0.84 (0.07)	0.58 (0.04)
SH5	0.23 (0.04)	0.99 (0.25)	0.56 (0.04)

383 [1]CO was analyzed with the 5 lactations as repeated records in a univariate repeatability model.
384 RP in lactation 2 to 5 were analyzed as repeated records in a repeatability model, together with
385 RP in the first lactation as a correlated trait in a bivariate model. MET and SH were analyzed
386 with 5 lactations as genetically correlated traits in multivariate models.
387 [2]b-value from regression between EBV for the five lactations of a disorder and GEBV of the
388 first lactation of the same disorder, GEBV estimated from a single trait GBLUP

[3]b-value (SE) from regression between EBV and GEBV of the same lactation for the same disorder, GEBV estimated from a single trait GBLUP

[4] b-value from regression between EBV and GEBV of the same lactation for the same disorder, GEBV estimated from a multitrait GBLUP