J. Dairy Sci. 106:6316–6324
https://doi.org/10.3168/jds.2022-23007

Genetic parameters of pregnancy loss in dairy cows estimated from pregnancy-associated glycoproteins in milk

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ABSTRACT

This study examined the feasibility of using pregnancy-associated glycoproteins (PAG) in milk within breeding for pregnancy maintenance and assessed the genetic variation in pregnancy loss traits. A total of 374,206 PAG samples from 41,889 Swedish Red (SR) and 82,187 Swedish Holstein (SH) cows were collected at monthly test-day milkings in 1,119 Swedish herds. Pregnancy status was defined based on PAG levels and confirmed by data on artificial insemination (AI), calving, and culling from d 1 postinsemination to calving. Pregnancy loss traits were defined as embryonic loss (diagnosed 28 d to 41 d after AI), fetal loss (42 d after AI until calving), and total pregnancy loss. Least squares means (± standard error, %) and genetic parameters were estimated using mixed linear models. Heritability was estimated to be 0.02, 0.02, and 0.03 for embryonic loss, fetal loss, and total pregnancy loss, respectively. Cows with pregnancy loss had lower PAG concentrations than cows which successfully maintained pregnancy and calved. PAG recording was limited to monthly test-day milking, resulting in low estimated embryonic loss (17.5 ± 0.4 and 18.7 ± 0.4 in SR and SH, respectively) and higher fetal loss (32.8 ± 0.5 and 35.1 ± 0.5 in SR and SH, respectively). Pregnancy loss might have occurred earlier but remained undetected until the next test-day milking, when it was recorded as fetal loss rather than embryonic loss. Estimated genetic correlation between embryonic and fetal pregnancy loss traits and classical fertility traits were in general high. Identification of novel genetic traits from PAG data can be highly specific, as PAG are only secreted by the placenta. Thus, PAG could be useful indicators in selection to genetically improve pregnancy maintenance and reduce reproductive losses in milk production. Further studies are needed to clarify how these results could be applied in breeding programs concurrent with selection for classical fertility traits.

Key words: pregnancy-associated glycoprotein, pregnancy loss, heritability, genetic correlation

INTRODUCTION

Previous work on improving fertility in dairy cattle has focused on the genetic ability to resume cyclicity after calving, show signs of estrus, and become pregnant when inseminated (Muuttoranta et al., 2019; NAV, 2021). The Nordic countries have been selecting for fertility for decades, but extensive pregnancy losses (54–73%) are still being reported based on progesterone profiles (Nyman et al., 2018; Ask-Gullstrand et al., 2021), highlighting the importance of pregnancy maintenance. Impaired fertility is the most commonly reported reason for culling in Sweden, accounting for 17.8% of culled cows (Växa, 2021).

Accurate and early pregnancy diagnosis is a vital part of reproductive management in dairy herds. Pregnancy is generally confirmed by rectal palpation or transrectal ultrasonography, but alternative methods such as chemical pregnancy detection have been developed to improve efficiency in herd management (Lawson et al., 2014; Pohler et al., 2016). In addition to optimizing fertility and productivity in herds by refining reproductive management practices, increasing the genetic progress of these traits is an important step toward improving overall fertility. Classical fertility traits generally have low heritability, which hampers genetic progress (Berry et al., 2014; Muuttoranta et al., 2019). Endocrine fertility traits have been proposed as an alternative indicator for fertility (Friggens and Chagunda, 2005; Petersson et al., 2008; Tenghe et al., 2015). These traits have higher heritability, partly because they reflect the cow's reproductive physiology more directly and are less biased by management decisions than classical fertility traits, which are defined from conventional reproductive parameters such as calving and insemination (Tenghe et al., 2015).

Pregnancy diagnosis by analysis of pregnancy-associated glycoproteins (**PAG**) is routine in many

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Received November 9, 2022.
Accepted March 9, 2023.
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dairy herds. PAG are secreted in the placenta, by cells deriving from fetal membranes. PAG can be detected in plasma and milk from approximately 3 wk of gestation and concentrations remain elevated throughout gestation, and may serve as an early pregnancy-specific marker in ruminant species (Zoli et al., 1992; Lawson et al., 2014; Ricci et al., 2015). The physiological function of PAG is still uncertain, but their spatio-temporal gene expression and secretion patterns (Green et al., 2000; Garbayo et al., 2008) suggest that they are involved in key components of gestation, such as placental formation, embryonic growth and development (Patel et al., 2004; Mercadante et al., 2016), pregnancy maintenance (Santos et al., 2018), and preparing the uterine environment for parturition (Patel et al., 2004). In the event of embryonic or fetal loss, placental function (and thus secretion of PAG) is disturbed and PAG concentrations decline over time, returning to nonpregnant levels within 7–14 d (Ricci et al., 2015).

Enzyme-linked immunosorbent assays have been developed to detect PAG in milk and are easily incorporated into routine milk recording schemes, as a convenient early indicator and monitor of pregnancy status in dairy herds (Lawson et al., 2014; Ricci et al., 2015; Santos et al., 2018). The industry has been accumulating large quantities of PAG data from recording schemes since the commercial pregnancy tests became available. Most research has, however, focused on the accuracy and efficiency of measuring plasma and milk PAG, and the usefulness of routine milk recording samples for large-scale pregnancy diagnosis compared with conventional measurements (e.g., Lawson et al., 2014; Ricci et al., 2015; Mercadante et al., 2016). If PAG data are useful indicators of pregnancy maintenance, they could be a valuable tool in determining genetic variation in pregnancy loss traits, as a relevant complement in genetic or genomic evaluations seeking to improve dairy cattle fertility and reduce losses in production. Furthermore, no scientific publications on PAG in Swedish dairy cattle have been published to date, even though PAG-based pregnancy diagnosis has been in routine use in Swedish commercial dairy herds since 2014. The aims of this study were: i) to assess the potential for using PAG information from Swedish routine milk recording in breeding to improve pregnancy maintenance and ii) to examine factors associated with PAG levels in milk during gestation in Swedish Red (**SR**) and Swedish Holstein (**SH**) cows.

MATERIALS AND METHODS

This study used already-collected data from a cow database, and no handling of the already-recorded cows was required.

The PAG records for 1,119 Swedish dairy herds were extracted from the database maintained by Växa (Stockholm, Sweden). These PAG data were derived from milk samples collected in routine (monthly) milk recording in herds during 2014 to 2020. The PAG were analyzed using an enzyme-linked immunosorbent assay (IDEXX Laboratories Inc.). A total of 439,565 PAG observations from 241,780 lactations in 45,709 SR and 90,957 SH cows were used. Additional information, such as pedigree, calving, insemination, culling, and test-day milk records, was also extracted from the database. Mean milk yield per lactation at national level in Swedish milk-recorded herds in 2020 was 10,152 kg in SR and 11,064 kg in SH cows (Växa, 2021).

Trait Definitions

Pregnancy status at the monthly test-day milking was determined based on threshold values set by the commercial kit manufacturer, with the PAG value derived from optical density of the sample and corrected for reference wavelength of the sample and a negative control (Ricci et al., 2015). Pregnancy loss was defined based on PAG profiles, where an insemination was considered unsuccessful if the PAG value was less than 0.1, requiring re-check if the PAG value was in the range 0.1 to 0.25, and successful (pregnancy) if the PAG value was higher than 0.25 (Lawson et al., 2014; Ricci et al., 2015). Pregnancy loss was not dependent on a preceding high PAG value. Three definitions of pregnancy loss (embryonic, fetal, total) were used in the present study based on the nomenclature established by the Committee on Bovine Reproductive Nomenclature (1972), and were based on the pregnancy status derived from PAG sampling in the monthly milk recording scheme. The start of PAG recording in Swedish herds is set to 28 d after insemination at the earliest, embryonic loss was therefore defined as failure to maintain pregnancy during the first 28 to 41 d of pregnancy, whereas fetal loss was defined as pregnancy loss detected based on the PAG sampling from 42 d until calving. These definitions of pregnancy loss reflect the restrictions of PAG sampling on a monthly basis. According to previous studies, the majority of pregnancy losses occur during the embryonic period (Walsh et al., 2011; Nyman et al., 2018; Ask-Gullstrand et al., 2021), and an unknown proportion of embryonic loss will be detected as fetal loss when using monthly-recorded PAG data. Total pregnancy loss was defined as losses during the whole sampling period from 28 d after insemination to calving.

The 3 traits were compared with other direct or indirect information, such as manual pregnancy diagnosis, repeated inseminations, culling, and calving records to confirm gestation or pregnancy loss. The outcome of the pregnancy diagnosis was also dependent on whether the cows were culled due to poor fertility or for other reasons. If a cow was culled due to reproductive failure during gestation, the pregnancy outcome was included in all pregnancy loss traits. However, culling for reasons not relating to fertility was not included in total pregnancy loss, so as not to penalize the fertility of the individual cow. A total of 86,856 cows were culled during the study period, of which 22,182 (25.5%) were culled for fertility-related causes.

Editing Criteria

Gestation stage at PAG sampling was restricted to 28 to 302 d, i.e., the interval from insemination to first sample was limited to 28 d and the maximum time allowed for a given gestation period was 302 d. The mean interval from insemination to first PAG sample was 50 ± 24.3 d, from insemination to last PAG sample 78 ± 42.6 d, and from first to last PAG sample 28 ± 39.0 d. A maximum of 3 PAG samples (mean number of PAG samples per insemination ± 2SD for all cows) were included per insemination, a criterion that excluded 3,625 PAG samples from 656 inseminations. Furthermore, at least 5 PAG samples were required per contemporary group for inclusion in the final data set, affecting 5,622 PAG samples. On average, 1.5 ± 0.55 PAG samples were taken per gestation. Approximately 36% of all inseminations were never monitored by PAG sampling, and these inseminations were excluded from the analysis. On average, the interval between repeated inseminations was 51 ± 54.3 d in cows that returned to heat. Manual pregnancy diagnosis, with or without transrectal ultrasound, was performed in 14.3% of inseminations, with an overall interval from insemination to examination of 57 ± 47.4 d. Gestation period ranged from 260 to 302 d, with an average gestation length of 279 ± 7.9 d.

Data on manual pregnancy diagnosis, repeated inseminations (25%), calving (63% of gestations), and culling (68% of cows) were used to evaluate the pregnancy status of individual cows in each gestation. A total of 33,633 PAG samples (8.76%) were excluded because their records were open at the time of data extraction in the herds and therefore lacked the necessary information. A further 5,625 PAG samples were excluded because they lacked test-day milk yield records (these samples were taken solely for pregnancy diagnosis and no milk parameters were recorded). Lastly, 255 PAG samples were removed due to double insemination within the same cycle (within ≤ 6 d). The final data set comprised 374,206 PAG samples, which were linked to 264,009 inseminations from 214,134 lactations in 41,889 SR and 82,187 SH cows (Table 1).

Table 1. Number of pregnancy-associated glycoprotein (PAG) analysis records, inseminations, lactations, and Swedish Red (SR) and Swedish Holstein (SH) dairy cows for which data were available in this study

Item	SR and SH	SR	SH
Cows	124,076	41,889	82,187
Lactations	214,134	73,340	140,794
Inseminations	264,009	88,748	175,261
First parity	101,512	32,150	69,362
Second parity	76,810	25,063	51,747
≥Third parity	85,687	31,535	54,152
PAG records	374,206	125,824	248,382
First parity	144,458	45,674	98,784
Second parity	108,795	35,591	73,204
≥Third parity	120,953	44,559	76,394

Five classical fertility traits were also analyzed: interval from calving to first service (**CFS**), interval from calving to last service (**CLS**), interval between first and last service (**FLS**), calving interval (**CVI**), and number of inseminations per series (**NINS**). Thresholds were set to handle outliers (mean ± 2SD) in these traits, with CFS between 42 and 169 d, CLS between 42 and 278 d, and FLS of maximum 173 d permitted, and CVI greater than 536 d excluded.

Statistical Analysis

The accuracy of PAG analysis in measuring pregnancy status was estimated using 5 parameters: (1) sensitivity, i.e., percentage of samples from confirmed pregnant cows identified by the analysis as pregnant; (2) specificity, i.e., percentage of samples from confirmed open cows identified by the analysis as nonpregnant; (3) positive predictive value, i.e., percentage of samples identified by the analysis as pregnant that were from confirmed pregnant cows; (4) negative predictive value, i.e., percentage of samples identified by the analysis as not pregnant that were from confirmed open cows; and (5) accuracy, i.e., percentage of samples from confirmed open/pregnant cows accurately identified as open or pregnant by the analysis. The agreement between insemination records and individual cow pregnancy status according to PAG analysis was determined by calculating the kappa (κ) statistic, where $\kappa > 0.80$ indicates a high level of agreement.

Pregnancy loss traits were analyzed using mixed linear models in SAS (version 9.4, SAS Institute Inc., 2017) to estimate least squares means. Model 1 [1] was used for pregnancy status (pregnant or open), and model 2 [2] to analyze PAG value during gestation. The PAG values for cows with successful pregnancies were also analyzed to test the effect on PAG levels in milk of calving ease, calf survival, calf sex, and number of calves born. Analysis was performed across both breeds and within each breed separately (ignoring the breed effect in the model). Classical fertility traits were analyzed without the effect of insemination number, and were (natural) log-transformed. The heritability estimates were based on variance components estimated from univariate animal models using model 3 [3] in DMU (Madsen and Jensen, 2013), and standard error of heritability was computed based on Taylor series of approximation (Madsen and Jensen, 2013; McKinnon Edwards, 2017). Heritability was estimated as $\sigma_a^2/(\sigma_a^2+\sigma_{pe}^2+\sigma_e^2)$. The genetic correlations between traits were estimated using bivariate repeatability models, where correlations <0.4 were considered weak, 0.4–0.7 moderate, and >0.7 strong. The models were as follows:

$$y_{ijklm} = \mu + B_i + P_j + I_k + hys_l + c_m + e_{ijklm}, \quad [1]$$

$$y_{ijklmno} = \mu + B_i + P_j + I_k + MY_n + ISP_o + hys_l + c_m + e_{ijlkmno}, \quad [2]$$

$$y_{ijklm} = \mu + B_i + P_j + I_k + hys_l + a_m + pe_m + e_{ijklm}, \quad [3]$$

where y is the trait analyzed; μ is overall mean; B_i is the fixed effect of the *i*th breed (SR or SH); P_j is the fixed effect of the *j*th parity (lactation 1 to 7, grouped as 1, 2 and ≥3); I_k is the fixed effect of the *k*th insemination number (k = 1–4); MY is daily milk at monthly test-day milking, categorized in 10 levels based on deciles; ISP is the interval from service to when PAG sample was taken, categorized in 10 levels based on deciles; hys_l is the random effect of herd by insemination year and season (with 1,052 herds, 7 years (2014–2020), and 4 seasons (Dec–Feb, Mar–May, Jun–Aug, Sep–Nov) and $\sim N(0, \mathbf{I}\sigma_{hys}^2)$, where **I** is an identity matrix and σ_{hys}^2 is the random herd-year-season variance); c_m is the random effect of cow *m* ($c_m \sim N(0, \mathbf{I}\sigma_c^2)$, where σ_c^2 is the variance of the cow); and e is a random error term ($e \sim N(0, \mathbf{I}\sigma_e^2)$, where σ_e^2 is residual variance). Model 3 also included the random genetic effect of animal *m* ($a_m \sim N(0, \mathbf{A}\sigma_a^2)$, where **A** is the additive genetic relationship matrix and σ_a^2 is the additive genetic variance); and the permanent environmental effect of animal *m* to account for repeated inseminations within lactation ($pe_m \sim N(0, \mathbf{I}\sigma_{pe}^2)$, where σ_{pe}^2 is the permanent environmental variance).

RESULTS

For the 264,009 inseminations represented by the data, pregnancy loss was reported for 100,858 inseminations during gestation (Table 2). The overall sensitivity, specificity, positive predictive value, and negative predictive value of the PAG analysis was found to be 99%, 77%, 91%, and 97%, respectively, in both SR and SH cows. The accuracy of the assay was 93% in SR cows and 92% in SH cows. The overall κ value was 0.81 ± 0.001.

Around 70% of all pregnancy losses were detected within the first 70 d post-AI. Regardless of time period, pregnancy loss was significantly more frequent ($P < 0.0001$) in SH than in SR cows (Table 3). Pregnancy loss also increased significantly with parity ($P < 0.0001$) for all 3 traits (embryonic, fetal, total pregnancy loss). Overall, PAG levels were significantly lower in cows that suffered pregnancy loss than in cows that successfully maintained pregnancy and calved (Table 4). The PAG level increased with gestational stage, i.e., the longer the cow had been pregnant the higher the PAG level in the milk sample, resulting in average PAG level in pregnant cows varying from 0.77 to 1.41. In gestations with subsequent calving, PAG levels were significantly higher in younger animals than in multiparous cows, and in pregnant SR compared with pregnant SH (both $P < 0.0001$). The PAG value also varied depending on

Table 2. Number of inseminations (percentage in brackets) resulting in pregnancy losses in 41,889 Swedish Red (SR) and 82,187 Swedish Holstein (SH) cows, based on pregnancy-associated glycoprotein analysis records

Trait[1]	SR and SH	SR	SH
EL	33,168 (15.7)	10,909 (15.3)	22,259 (16.0)
FL	67,690 (29.4)	22,146 (28.6)	45,544 (29.9)
TPL	71,015 (30.5)	22,897 (29.3)	48,118 (31.1)

[1] EL = embryonic loss, 28–41 d after AI; FL = fetal loss, 42 d after AI until calving; TPL = total pregnancy loss, diagnosed 28 d after AI until calving, excluding inseminations from cows culled due to non-fertility–related causes.

Table 3. Number of cows (N) and LSM (%) ± SE of pregnancy loss traits estimated from monthly pregnancy-association glycoprotein analysis in Swedish Red (SR) and Swedish Holstein (SH) cows

		Trait[1]		
Effect	N	EL	FL	TPL
Breed				
SR	41,889	17.5 ± 0.4[a]	32.8 ± 0.5[a]	31.2 ± 0.5[a]
SH	82,187	18.7 ± 0.4[b]	35.1 ± 0.5[b]	34.4 ± 0.5[b]
Parity				
1	82,769	15.7 ± 0.4[a]	29.2 ± 0.5[a]	30.1 ± 0.5[a]
2	61,882	18.3 ± 0.4[b]	34.1 ± 0.5[b]	33.5 ± 0.5[b]
≥3	69,483	20.2 ± 0.4[c]	38.5 ± 0.5[c]	34.9 ± 0.5[c]

[a–c] Estimates with different superscripts are significantly different ($P \leq 0.05$).

[1] EL = embryonic loss, 28–41 d after AI; FL = fetal loss, 42 d after AI until calving; TPL = total pregnancy loss, diagnosed 28 d after AI until calving.

Table 4. Least squares means ± SE of pregnancy-associated glycoprotein levels in milk in Swedish Red (SR) and Swedish Holstein (SH) cows at first sample used to predict pregnancy status after insemination, in pregnant cows and cows with pregnancy loss (nonpregnant)

Effect	28–41 d[1]	42 d–calving	28 d–calving
Breed			
Pregnant[2]			
SR	1.10 ± 0.007[a]	1.14 ± 0.008[a]	1.14 ± 0.007[a]
SH	1.05 ± 0.007[b]	1.09 ± 0.007[a]	1.09 ± 0.007[b]
Nonpregnant			
SR	−0.16 ± 0.013[a]	0.39 ± 0.009[a]	0.14 ± 0.009[a]
SH	−0.11 ± 0.012[b]	0.41 ± 0.009[b]	0.18 ± 0.008[b]
Parity			
Pregnant			
1	1.14 ± 0.007[a]	1.18 ± 0.008[a]	1.18 ± 0.007[a]
2	1.08 ± 0.007[b]	1.12 ± 0.008[b]	1.11 ± 0.007[b]
3	1.01 ± 0.007[c]	1.05 ± 0.008[c]	1.04 ± 0.007[c]
Nonpregnant			
1	−0.18 ± 0.013[a]	0.36 ± 0.009[a]	0.14 ± 0.009[a]
2	−0.12 ± 0.013[b]	0.41 ± 0.009[b]	0.17 ± 0.009[b]
3	−0.11 ± 0.013[c]	0.43 ± 0.009[c]	0.18 ± 0.009[c]

[a–c] Estimates with different superscripts are significantly different ($P \leq 0.05$).

[1] 28–41 d = indicative of early embryonic loss during the first 41 d after AI; 42 d–calving = indicative of fetal loss from 42 d after AI until calving; 28 d–calving = indicative of pregnancy loss during the gestation period.

[2] Optical density readings (adjusted for background) were reported as an indication of the PAG in milk samples.

calf variables in successful pregnancies. Calf survival and calf sex had significant effects on milk PAG in SR cows ($P = 0.0232$ and $P = 0.010$, respectively), while number of calves influenced milk PAG level in both SR and SH cows, with higher PAG in twin births ($P < 0.0001$). Milk yield at monthly test-day recording also influenced milk PAG level, with higher milk yield associated with lower PAG level in samples. The PAG level in the lowest milk yield decile was 0.95 and decreased to 0.68 in the highest milk yield decile.

The heritability estimates of pregnancy loss traits were low, ranging between 0.02 and 0.05 (Table 5). The HYS variances ranged from 0.01 to 0.17. Embryonic loss and fetal loss both had a strong genetic correlation (0.80–0.99) with CLS, FLS, CVI and NINS, but a weak association (0.10–0.27) with CFS (Table 6).

There were significant differences (LSM ± SE) between the breeds in CFS (81 ± 0.2 d in SR, 84 ± 0.1 d in SH; $P < 0.001$), CLS (121 ± 0.3 d in SR, 129 ± 0.3 d in SH; $P < 0.001$), and FLS (35 ± 0.2 d in SR, 38 ± 0.2 d in SH; $P < 0.001$). CVI was significantly longer in SH than in SR, 396 ± 0.3 d compared with 390 ± 0.3 d. NINS was approximately 1.9 ± 0.005 in both SR and SH. CFS, CLS, FLS, and CVI were significantly longer ($P < 0.001$) in multiparous cows than in primiparous cows.

DISCUSSION

Poor reproductive performance is a major concern in the dairy industry. Reports of high fertilization rate and low calving rates indicate high reproductive loss during gestation (Nyman et al., 2018), which increases the risk of premature culling and compromises herd profitability. Studies based on progesterone profiles have confirmed high pregnancy losses in Nordic dairy cattle (Nyman et al., 2018; Ask-Gullstrand et al., 2021). In this study, we evaluated the extent of pregnancy loss and estimated genetic parameters for pregnancy loss traits based on data for PAG pregnancy analysis from the monthly milk recording scheme on Swedish dairy herds. The data set provided valuable information for

Table 5. Estimated heritability (h^2), SE, and additive genetic variance (σ^2_a) of pregnancy loss traits in 41,889 Swedish Red (SR) and 82,189 Swedish Holstein (SH) dairy cows in 17,334 contemporary groups

	SR and SH			SR			SH		
Trait[1]	h^2	SE	σ^2_a	h^2	SE	σ^2_a	h^2	SE	σ^2_a
EL	0.02	0.002	0.002	0.02	0.004	0.003	0.02	0.003	0.002
FL	0.02	0.002	0.004	0.02	0.004	0.004	0.02	0.003	0.005
TPL	0.03	0.002	0.006	0.05	0.006	0.009	0.03	0.003	0.006

[1] EL = embryonic loss, 28–41 d after AI; FL = fetal loss, 42 d after AI until calving; TPL = total pregnancy loss, diagnosed 28 d after AI until calving.

Table 6. Estimated genetic (r_g) correlation, with SE (subscript), between pregnancy loss traits and classical fertility traits in Swedish Red and Swedish Holstein cows[1]

	Trait				
Trait	CFS	CLS	FLS	CVI	NINS
EL	$0.10_{0.056}$	$0.92_{0.019}$	$0.99_{0.018}$	$0.80_{0.043}$	$0.89_{0.033}$
FL	$0.27_{0.062}$	$0.98_{0.005}$	$0.97_{0.014}$	$0.89_{0.019}$	$0.89_{0.022}$

[1] EL = early embryonic loss, diagnosed 28–41 d after AI; FL = fetal loss, 42 d after AI to calving; CFS = interval from calving to first service, d; CLS = interval from calving to last service, d; FLS = interval from first to last service, d; CVI = calving interval, d; NINS = number of inseminations per AI period.

determining genetic variation in pregnancy loss and for assessing the utility of pregnancy loss traits based on PAG recordings. Pregnancy loss is an interesting alternative trait to be considered in genetic or genomic evaluations, to genetically improve cow fertility and reduce pregnancy loss in production.

Circulating Concentrations of PAG

The PAG are produced by the placenta during pregnancy and concentrations continue to increase in circulation as gestation proceeds (Green et al., 2000; Garbayo et al., 2008; Santos et al., 2018). Cows in this study that suffered embryonic or fetal loss had reduced PAG levels in milk during gestation, which suggests that PAG is a useful biomarker for placental function and prediction of pregnancy maintenance (e.g., Lawson et al., 2014; Mercadante et al., 2016; Pohler et al., 2016). Furthermore, Ricci et al. (2015) observed similar PAG levels in pregnant cows that successfully maintained pregnancy and calved and cows with subsequent pregnancy loss, and concluded that cows will test positive for PAG as long as there is a viable pregnancy. We also identified higher PAG levels in milk from primiparous cows than in milk from multiparous cows in successful pregnancies with subsequent calving ($P \leq 0.0001$), which is in line with previous findings (Ricci et al., 2015; Mercadante et al., 2016). We found that SR cows had higher PAG levels in their milk samples than SH cows.

The PAG have a long half-life and can still be detected in circulation for a couple of weeks after the latest AI (Green et al., 2000; Ricci et al., 2015), but with a decrease in circulating PAG between approximately 46 and 72 d post-AI (Lawson et al., 2014; Ricci et al., 2015). Using a PAG-based pregnancy test during this part of the gestation period might therefore increase the number of cows being classified as open or re-check, requiring additional sampling later in gestation for reliable determination of pregnancy status (Lawson et al., 2014, Ricci et al., 2015). In this study we identified 5.95% of the PAG records as re-check, which is similar to the proportion found in previous studies (LeBlanc, 2013; Lawson et al., 2014). It is also important that cows identified as pregnant or re-check early in the gestation period are re-tested later, due to the similarities between truly pregnant cows diagnosed as re-check or open during the 46–72 d period, and cows undergoing pregnancy loss but with the PAG level not fully receded. Failing to do so increases the risk of missing open cows, which also extends FLS, delays CVI, and adds to the production costs (Ricci et al., 2015).

Application in Future Breeding Strategies

The heritability estimates for pregnancy loss traits based on PAG records in this study were low, and similar to those of classical fertility traits (Muuttoranta et al., 2019; NAV, 2021). The low estimates are possibly due to infrequent recording of milk PAG levels (on which the pregnancy loss traits are based) and the traits did not fully capture the genetic variation underlying fertility traits (Berry et al., 2014; Muuttoranta et al., 2019). Santos et al. (2018) found moderate genetic control of milk PAG levels ($h^2 = 0.11–0.23$), but other estimates of genetic parameters of pregnancy loss traits based on PAG data are scarce in the literature. Similarly, studies based on progesterone data have obtained low heritability estimates for pregnancy loss traits (0.02–0.06; van Binsbergen et al., 2019; Ask-Gullstrand et al., 2021).

Increases in CLS, FLS, CVI, and NINS were genetically associated with increased pregnancy loss. The correlation between pregnancy loss traits and classical fertility traits was generally much higher than that found in previous studies based on in-line progesterone measurements, with both van Binsbergen et al. (2019) and Ask-Gullstrand et al. (2021) reporting moderate genetic correlations (0.31–0.52) between embryonic loss and classical fertility traits. Ask-Gullstrand et al. (2021) also found a high genetic correlation (0.91 ± 0.12) between late embryonic loss and CVI. To our knowledge, no previous study has reported a genetic correlation between fetal loss and classical fertility traits, possibly due to data limitations. The estimates for fetal loss in this study were of the same order of magnitude as those for embryonic loss. The strong genetic correlation observed between pregnancy loss traits and several classical fertility traits suggests that cows with impaired fertility have difficulties supporting embryo and fetal development and survival. Endocrine traits may provide a better definition of fertility because they reflect the cow's reproductive physiology more closely and are less biased by management. However, using endocrine traits in genetic or genomic evaluations has long been inhibited by laborious techniques and high costs associated with data collection. Automatic in-line sampling methods, such as progesterone recording using the Herd Navigator (DeLaval International, Tumba, Sweden), offer high resolution of pregnancy status in early gestation and are less laborious, but are still associated with high running costs to gather sufficient data per lactation for correct recording of pregnancy maintenance (Tenghe et al., 2015). Furthermore, progesterone is an indirect indicator of pregnancy and is more accurate at finding open cows (i.e., cows with low progesterone concentration) and returning them to service, rather than confirming pregnancy, because cows can have high progesterone concentrations without being pregnant (Lawson et al., 2014; Tenghe et al., 2015). PAG analysis data from monthly milk recording schemes offer an alternative for cost-effective large-scale recording. Whereas PAG recording was primarily developed as a management tool to simplify pregnancy diagnosis in the herds, pregnancy loss traits based on PAG analysis could contribute to better trait definition because they are a direct marker of placental function (Zoli et al., 1992; Patel et al., 2004; Mercadante et al., 2016), and thus an indicator of a cow's ability to maintain pregnancy. However, the current sampling strategy for PAG (at monthly test-day milking) limits measurements to a couple of events per gestation, resulting in imprecise classification of the occurrence of pregnancy loss and risking delay in pregnancy diagnosis. It could be interesting to increase PAG sampling frequency to around the level used for progesterone sampling with in-line milking systems. The potential to develop such a system exists, but the cost and accuracy of higher-frequency of PAG sampling need to be determined.

The fertility index used in the Nordic breeding program focuses on the genetic ability of the dam to resume cyclicity after calving, show sign of estrus, and conceive after insemination (NAV, 2021). PAG records are currently being used as an indicator in calculating conception rate in breeding evaluation of fertility (Muuttoranta et al., 2019; NAV 2021), but this low-heritability trait focuses on the cow's ability to conceive, rather than actual pregnancy maintenance. Endocrine fertility traits could be useful in selecting for improved fertility, with PAG as a potential indicator to define novel fertility traits. An updated genomic evaluation for female fertility could then consider the ability of high-yielding cows to maintain pregnancy to full term. Of the 2,147 herds affiliated with the Swedish milk recording scheme (Växa, 2021), 1,119 herds use PAG analysis for pregnancy diagnosis. The amount of data collected from these herds is sufficient for traditional genetic evaluation of pregnancy loss traits, and it would also constitute a large population for genomic evaluation, where cows from subscribing herds could form the reference population. It would be possible to increase the size of the training population, thus improving genomic predictions, through cooperation within the Nordic countries (Lund et al., 2011; Tenghe et al., 2018; Muuttoranta et al., 2019), with Sweden, Denmark, and Finland contributing to the joint Nordic fertility evaluation (Lund et al., 2011; Muuttoranta et al., 2019). However, the added information in addition to that already available from, e.g., CLS, seems to be low, given the high genetic correlation. Collecting PAG data solely for the use in genomic evaluation would also yield more expensive trait recording compared with classical fertility traits, which are estimated based on calving and insemination data. However, PAG analysis carry the added benefit of replacing manual pregnancy diagnosis with or without ultrasound, which is laborious and therefore costlier than PAG sampling.

The PAG Pregnancy Assay

In this study, estimated embryonic loss (diagnosed from 28 to 41 d after AI) ranged between 15.7 and 20.2% and estimated fetal loss (42 d post-AI onwards) between 29.2 and 38.5%. In a previous study based on PAG data, Pohler et al. (2016) found 12–20% pregnancy loss during 31–59 d post-AI, and 19% from 59 d to parturition. However, Mercadante et al. (2016) reported much lower incidence of embryonic loss of 4.3% at 32–46 d post-AI, 5.8% loss from 46 d to 74 d, and 6.4% loss from 74 d onward. Those results are similar to those of van Binsbergen et al. (2019), who found pregnancy loss ranging from 8 to 23% based on in-line progesterone recording. The differences between studies could be explained by sampling method and frequency, trait definition, and data editing.

In contrast to Mercadante et al. (2016), we found a significant effect of parity on all pregnancy loss traits ($P < 0.0001$), with higher incidence of pregnancy loss with increasing parity, suggesting that age of dam increases the likelihood of pregnancy loss.

The PAG pregnancy assay performed well and test parameters were comparable with previous findings (LeBlanc, 2013; Lawson et al., 2014; Ricci et al., 2015). The high negative predictive value reported for the assay (range 81–100% in various studies) indicates that it is efficient in finding open cows that should be returned to service. Reported positive predictive values are somewhat lower (range 79–91% in various studies), indicating that a few cows are still at risk of losing their pregnancy later in term. The κ value of pregnancy outcomes based on insemination records and the PAG analysis was 0.81, which is similar to the 0.77 reported by Ricci et al. (2015), but somewhat lower than the

0.98 reported by Lawson et al. (2014). Apart from being used as an indicator of pregnancy, PAG pregnancy analysis offers a reliable alternative for dairy herds that have limited access to skilled technicians or veterinarians who can perform manual pregnancy diagnosis (Lawson et al., 2014; Pohler et al., 2016). Moreover, the milk pregnancy test minimizes the amount of handling of animals compared with nonchemical pregnancy diagnostic methods, as sampling is done concurrently with the monthly test-day milk recording (LeBlanc, 2013).

Herds subscribing to Växa's milk PAG pregnancy analysis in Sweden are offered the following sampling strategies, with the aim of detecting open cows as early as possible and re-inseminating them: 1) one sample after 28 d post-AI, 2) one sample after 28 d post-AI and a second sample after 60 d post-AI to confirm an initial positive sample (recommended routine), 3) one sample in preparation for drying off, and 4) an additional individual sample at some point during gestation. The infrequent recording, at monthly test-day milking, might explain why so few embryonic losses were observed in the present study. If a cow is, for example, scheduled for a PAG sample at 28 d post AI but misses it due to a herd test-day at e.g., 24 d post AI, the sample will be rescheduled for the next month's test-day. This would result in a longer interval and no recording for embryonic loss unless individual samples are performed outside of the test-day. In the event of a pregnancy loss, the loss might in fact have occurred earlier, but the long sampling interval meant that the pregnancy loss could not be detected until the next monthly test-day milking, thus contributing to the high estimated fetal loss in this study. One way to overcome this issue would be to perform additional PAG sampling (outside the monthly test-day milk recording) in early pregnancy or to use other diagnostic tools, such as heat detection and manual pregnancy diagnosis. This also highlights the need for optimized reproductive management strategies in individual herds.

CONCLUSIONS

Using PAG data to define novel pregnancy loss traits in dairy cattle is an interesting prospect because these proteins are only produced and secreted by the placenta during gestation. Consequently, they are a more direct reflection of the cow's reproductive physiology in terms of pregnancy maintenance than the classical fertility traits currently used in the Nordic breeding program. Assessing the quality of PAG analysis and the genetic variation in novel pregnancy loss traits was a first step in determining whether these data can help increase genetic progress in cow fertility. Due to low heritabilities and strong genetic correlation to classical fertility traits, the potential usefulness of these pregnancy loss traits in selection is probably limited given the current sampling strategies. The cost of PAG sampling must also be taken into consideration as PAG derived traits will be more expensive than classical fertility traits in the current breeding program. Further research is needed to identify candidate genomic regions associated with pregnancy loss, and determining the accuracy of PAG derived pregnancy traits to ascertain their usability in genomic prediction of fertility. Selection for reduced losses would increase herd reproductive performance without excessive management interventions, allowing for increased longevity and better herd profitability.

ACKNOWLEDGMENTS

This project was funded by the Swedish Farmers' Foundation for Agricultural Research (project ID O-19-20-305; O-15-20-587). We gratefully acknowledge the herd owners in Sweden who contributed data from their herds, and Växa for providing us with data from the Swedish cow database. The authors have not stated any conflicts of interest.

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