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# Reproductive tract size and position score: Estimation of genetic parameters for a novel fertility trait in dairy cows

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## ABSTRACT

The dairy industry is moving toward selecting animals with better fertility to decrease the economic losses linked to reproductive issues. The reproductive tract size and position score (SPS) was recently developed in physiological studies as an indicator of pregnancy rate and the number of services to conception. Cows are scored as SPS 1, 2, or 3 based on the size of their reproductive tract and its position in the pelvis, as determined by transrectal palpation. The objective of this study was to estimate genetic parameters for SPS to assess its potential as a novel fertility trait. Phenotypes were collected at the University of British Columbia's research herd from 2017 to 2020, consisting of 3,247 within- and across-lactation SPS records from 490 Holstein cows. A univariate animal model was used to estimate the variance components for SPS. Both threshold and linear models were fit under a Bayesian approach and the results were compared using the Spearman rank correlation (r) between the estimated breeding values. The 2 models ranked the animals very similarly (r = 0.99), and the linear model was selected for further analysis. Genetic correlations with other currently evaluated traits were estimated using a bivariate animal model. The posterior means  $(\pm \text{ posterior standard deviation})$  for heritability and repeatability within- and across-lactation were 0.113  $(\pm 0.013), 0.242 \ (\pm 0.012), and 0.134 \ (\pm 0.014), respec$ tively. The SPS showed null correlations with production traits and favorable correlations with traditional fertility traits, varying from -0.730 (nonreturn rate) to 0.931 (number of services). Although preliminary, these results are encouraging because SPS seems to be more heritable than and strongly genetically correlated with

number of services, nonreturn rate, and first service to conception, indicating potential for effective indirect selection response on these traits from SPS genetic selection. Therefore, further studies with larger data sets to validate these findings are warranted.

**Key words:** Holstein cow, variance component estimation, genetic correlation, reproductive tract score

## INTRODUCTION

Suboptimal reproductive performance is a major contributor to economic losses in the dairy industry worldwide. The main concern is decreased female fertility leading to longer lactation and an increased number of involuntary cullings (Giordano et al., 2011). In Canada, reproductive problems have been the main cause of removal from the herd in dairy cattle, representing more than 30% of involuntary cullings (Van Doormaal, 2009; OMAFRA, 2021).

Selecting cows with superior genetic merit for fertility is a long-term solution to counteract reproductive decline. Despite the efforts made toward improving reproduction, the results are still below the level needed (Miglior et al., 2017; Fleming et al., 2019). The most cited problem is the low heritability of current fertility traits (Fleming et al., 2019), often used as justification for the low weight assigned to them in breeding goals (Berry et al., 2016; Miglior et al., 2017). However, the main underlying concern is the negative genetic correlation between production and fertility leading to an economic impasse (Pryce et al., 2014; Miglior et al., 2017). Since the 1960s, this antagonistic correlation has been controversial because of the lack of corresponding evidence of a biological link between milk production and fertility (Miglior et al., 2017). The notion was that coupling intense selection for increased milk production with improvements in herd management would suffice to counterbalance the genetic deterioration of functional traits, whereas others have argued that overly intensive selection on yield was an important factor in reproduc-

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tive decline (Pryce et al., 2014; Butler and Moore, 2018). Since the negative effect of intensive selection for production traits on reproductive physiology became more commonly accepted, fertility traits have been included in national selection indices (Miglior et al., 2017). However, the underlying physiological factors affecting the reproductive system and their genetic background are still largely unknown, leading to difficulty in collecting accurate and high-quality phenotypes and preventing rapid progress with genetic selection (Fleming et al., 2019). Despite this, high-producing cows do not always exhibit poor fertility, and high milk production is not necessarily a feature of low fertility (Britt, 1992; Bello et al., 2013; LeBlanc, 2013).

Historically, fertility traits were chosen based on their simplicity and ease of measurement on a large scale (Berry et al., 2016; Fleming et al., 2019). Fertility is a complex phenotype and currently recorded traits are strongly affected by the environment and management practices. This explains why most evaluated fertility traits have low heritability but large genetic variation, which indicates good potential for selection (Miglior et al., 2017).

Recently, a novel fertility trait has been described, based on the morphology of the reproductive tract that directly relates to the fertility status of the animal (Young et al., 2017; Madureira et al., 2020). This trait consists of categorizing the female reproductive tract by transrectal palpation. As shown in Figure 1, animals are classified into 3 groups depending on the size and position score (**SPS**) of their reproductive tract, where SPS 1 describes cows with a small and compact uterus and uterine horns resting entirely on the pelvis; SPS 2 cows have a uterus of medium size with longer uterine horns resting partially outside of the pelvic cavity; and SPS 3 cows have a large reproductive tract mostly outside of the pelvic cavity.

These scores have been associated with common indicators of fertility, such as pregnancy rate and the number of services to pregnancy, in physiological studies in dairy cattle (Young et al., 2017; Madureira et al., 2020). Lower SPS scores are favorably associated with higher pregnancy rate, lower number of services per pregnancy, and lower pregnancy loss. Cows show substantial variation in the SPS score at breeding time, which is also observed across different parities (Young et al., 2017). Thus, SPS may provide a new fertility trait that has the advantage of being morphological, which is commonly accepted as being affected by fewer environmental factors and, therefore, having potentially higher heritability than current fertility traits. Our objectives were to estimate the genetic parameters of SPS using both threshold and linear models, and to estimate the genetic correlations between SPS and other economically important traits.

## **MATERIALS AND METHODS**

### Data

Phenotypes for SPS were collected at the University of British Columbia research herd (Agassiz, Canada), as part of a physiological study (Madureira et al., 2020). A total of 3,247 records within and across lactations on 490 lactating Holstein cows scored from 2017 to 2020 were included in this study. Cows were scored by transrectal palpation from calving to conception. Details on cow management and SPS measurement are provided in Madureira et al. (2020). The corresponding pedigree file was provided by Lactanet (Guelph, ON, Canada) and consisted of 23,275 animals with an average depth of 4.6 generations. The Lifetime Profit Index (LPI) and Pro\$ index values for the cows with SPS records were also provided by Lactanet. All phenotypic and



Figure 1. Assessment of the reproductive tract size and position score (SPS). Reproductive tracts positioned entirely within the pelvic cavity were designated SPS 1. Reproductive tracts in which the cervix was within the pelvic cavity, but uterine horns were outside the pelvic cavity, were designated SPS 2. Reproductive tracts in which the cervix and uterine horns lay outside the pelvic cavity were designated SPS 3 (from Young et al., 2017). C = cervix; P = pelvis; RT = reproductive tract; PB = pelvic brim; SPS = size and position score.

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**Table 1.** Number of records of the reproductive tract size and position score  $(SPS)^1$  by parity and stage of lactation from 490 Holstein cows over 3 yr in 1 herd

|        | CL C                   | Number of records |       |       |       |
|--------|------------------------|-------------------|-------|-------|-------|
| Parity | lactation <sup>2</sup> | SPS 1             | SPS 2 | SPS 3 | Total |
| 1      | 1                      | 10                | 33    | 22    | 65    |
|        | 2                      | 227               | 353   | 56    | 636   |
|        | 3                      | 35                | 116   | 10    | 161   |
|        | 4                      | 2                 | 13    | 3     | 18    |
| 2      | 1                      | 1                 | 25    | 26    | 52    |
|        | 2                      | 109               | 366   | 51    | 526   |
|        | 3                      | 33                | 163   | 44    | 240   |
|        | 4                      | 2                 | 21    | 14    | 37    |
| 3      | 1                      | 0                 | 15    | 13    | 28    |
|        | 2                      | 48                | 224   | 71    | 343   |
|        | 3                      | 16                | 106   | 33    | 155   |
|        | 4                      | 2                 | 8     | 8     | 18    |
| 4      | 1                      | 0                 | 5     | 12    | 17    |
|        | 2                      | 18                | 149   | 70    | 237   |
|        | 3                      | 4                 | 102   | 52    | 158   |
|        | 4                      | 0                 | 15    | 10    | 25    |
| 5+     | 1                      | 1                 | 15    | 19    | 35    |
|        | 2                      | 16                | 208   | 72    | 296   |
|        | 3                      | 7                 | 103   | 65    | 175   |
|        | 4                      | 3                 | 15    | 7     | 25    |
|        | Total                  | 534               | 2,055 | 658   | 3,247 |

<sup>1</sup>SPS 1 was attributed to cows with small reproductive tract positioned entirely within the pelvic cavity; SPS 2 designated cows with a reproductive tract in which the cervix was within the pelvic cavity, but uterine horns were outside the pelvic cavity; SPS 3 was attributed to cows with large reproductive tract lying outside the pelvic cavity. <sup>2</sup>Stage of lactation (1–4) was defined as 1 to 21, 22 to 120, 121 to 240, and >240 DIM, corresponding to uterine involution, early lactation, mid-lactation, and late lactation, respectively.

pedigree data used in this study were obtained from pre-existing databases. Therefore, no animal care committee approval was necessary for the purposes of this study.

Data editing and formatting were done with R software (R Core Team, 2018) using the "nadiv" package for pedigree handling (Wolak, 2012) and the "dplyr" package for data formatting (Wickham et al., 2021). The 120-d periods for the stages of lactation were adapted for our data set by adding a uterine involution period, when the size of the reproductive tract is naturally unstable. Based on the available measurements, the uterine involution, early lactation, mid-lactation, and late lactation periods were defined as 1 to 21, 22 to 120, 121 to 240, and >240 DIM, respectively. The seasons were based on the recording date by defining October to March as the cold season and April to September as the warm season.

The distribution of the trait records by parity and stage of lactation is presented in Table 1. Of 490 cows, 213 cows (43%) had multiple records within one lactation, 5 cows (1%) had multiple single records across

lactations, 243 cows (50%) had multiple records both within and across lactations, and 29 cows (6%) had a single record. On average, cows had 4 records, with a maximum of 24 records.

Genetic correlations between SPS and 13 currently evaluated traits, chosen based on their economic importance and biological relevance, were estimated. The chosen traits were categorized as follows: (1) production traits: milk, fat, and protein yields; (2) conformation traits: BCS, thurl placement, and rump angle; (3) fertility traits: age at first service, nonreturn rate at 56 d, first service to conception, calving to first service, days open, number of services; and (4) calving traits: calving ease. All trait phenotypes were provided by Lactanet and did not contain any missing records.

For conformation traits and age at first service, only one record per cow was available. For the other traits, only records from the corresponding lactation when SPS was recorded were used.

## **Models**

**Single-Trait Linear Model.** The following univariate linear repeatability animal model was used to estimate variance components of SPS:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}_{\mathbf{a}}\mathbf{a} + \mathbf{Z}_{\mathbf{p}\mathbf{e}_{\mathbf{w}}}\mathbf{p}\mathbf{e}_{\mathbf{w}} + \mathbf{Z}_{\mathbf{p}\mathbf{e}_{\mathbf{a}}}\mathbf{p}\mathbf{e}_{\mathbf{a}} + \mathbf{e},$$

where **y** is a vector of SPS phenotypes (3 scores); **b** is a vector of fixed effects including year-season (7 levels), stage of lactation (4 levels), and linear and quadratic regression on lactation number (1, 2, 3, 4, and 5+); **a** is a vector of random animal additive genetic effects;  $\mathbf{pe}_{\mathbf{w}}$ is a vector of random within-lactation permanent environmental effects;  $\mathbf{pe}_{\mathbf{a}}$  is a vector of across-lactation permanent environmental effects; and **e** is a vector of random residual effects; **X**,  $\mathbf{Z}_{\mathbf{a}}$ ,  $\mathbf{Z}_{\mathbf{pe}_{\mathbf{w}}}$ , and  $\mathbf{Z}_{\mathbf{pe}_{\mathbf{a}}}$  are corresponding incidence matrices. The random effects were assumed normally distributed as follows:

$$\begin{bmatrix} \mathbf{a} \\ \mathbf{p} \mathbf{e}_{\mathbf{w}} \\ \mathbf{p} \mathbf{e}_{\mathbf{a}} \\ \mathbf{e} \end{bmatrix} \sim N \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \mathbf{A} \sigma_{a}^{2} & 0 & 0 & 0 \\ 0 & \mathbf{I} \sigma_{pe_{w}}^{2} & 0 & 0 \\ 0 & 0 & \mathbf{I} \sigma_{pe_{a}}^{2} & 0 \\ 0 & 0 & 0 & \mathbf{I} \sigma_{e}^{2} \end{bmatrix}$$

where **A** is the numerator relationship matrix,  $\sigma_a^2$  is the additive genetic variance, **I** is an identity matrix,  $\sigma_{pe_w}^2$  is the within-lactation permanent environmental vari-

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ance,  $\sigma_{pe_a}^2$  is the across-lactations permanent environmental variance, and  $\sigma_e^2$  is the residual variance.

The final model was defined by back-selection of all fixed effects, keeping only the significant ones (P < 0.05). Calving score and the incidence of uterine disease were not included in the model due to the lack of this information for most cows with an SPS score, but they might contribute to the variation of SPS score and should be considered in future studies.

Single-Trait Threshold Model. Due to the novelty of the trait, threshold and linear models were both fitted for comparison. Theoretically, the threshold model is advantageous because it respects the categorical nature of the trait by fitting an appropriate non-Gaussian distribution. However, the linear model is usually preferred because it is both less complex and less computationally demanding (Meijering and Gianola, 1985). The literature also indicates that the model fit and animal ranking do not significantly differ when a linear model is used to analyze categorical data (e.g., Jamrozik et al., 2005; Negussie et al., 2008; Neuenschwander et al., 2012).

The observed phenotype is assigned to categories (1, 2, or 3) based on a latent trait called liability (1), which is assumed to be normally distributed (de Villemereuil, 2018). The following repeatability univariate threshold model was used:

$$\mathbf{l} = \mathbf{X}\mathbf{b} + \mathbf{Z}_{\mathbf{a}}\mathbf{a} + \mathbf{Z}_{\mathbf{p}\mathbf{e}_{\mathbf{w}}}\mathbf{p}\mathbf{e}_{\mathbf{w}} + \mathbf{Z}_{\mathbf{p}\mathbf{e}_{\mathbf{a}}}\mathbf{p}\mathbf{e}_{\mathbf{a}} + \mathbf{e},$$

where  $\mathbf{l}$  is a vector of underlying liabilities corresponding to the categorical observations in  $\mathbf{y}$  (1, 2, or 3), and the other terms are as previously defined.

## **Genetic Parameters**

The (co)variance components were estimated using Gibbs sampling implemented in THRGIBBS1F90 software (Misztal et al., 2002). The analysis consisted of a single chain of 3,050,000 cycles, with the first 50,000 being discarded as a burn-in period. A long thinning interval of 3,000 cycles was used to guarantee minimization of the autocorrelation between consecutive samples. Convergence was assessed by visual inspection of the trace plots of each estimated variance component.

The Spearman's rank correlation between the EBV from linear and threshold models was used to determine whether the models would rank the animals similarly. For the threshold model, estimates from the underlying scale were used for ranking purposes.

The heritability  $(h^2)$  for SPS was calculated from the single-trait model as follows:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{pe_w}^2 + \sigma_{pe_a}^2 + \sigma_e^2},$$

where  $\sigma_a^2, \sigma_{pe_w}^2, \sigma_{pe_a}^2$ , and  $\sigma_e^2$  were as previously defined. Repeatability within lactation  $(\mathbf{r}_w)$  was calculated as follows:

$$r_w = \frac{\sigma_a^2 + \sigma_{pe_w}^2 + \sigma_{pe_a}^2}{\sigma_a^2 + \sigma_{pe_w}^2 + \sigma_{pe_a}^2 + \sigma_e^2}$$

and the repeatability across lactations  $(\mathbf{r}_{\mathbf{a}})$  as

$$r_a = \frac{\sigma_a^2 + \sigma_{pe_a}^2}{\sigma_a^2 + \sigma_{pe_w}^2 + \sigma_{pe_a}^2 + \sigma_e^2}$$

The expected heritability estimates  $(h_n^2)$  for the average values when considering a different number of records per animal (from 2 to 10) were calculated as follows:

$$h_n^2 = \frac{h^2 \times n}{1 + (n-1) \times r}$$

where n is the number of records per animal, and  $h^2$  and r are the heritability and repeatability (either across or within lactation) estimated from the single-trait model.

### **Genetic Correlations**

Bivariate animal models were used to estimate the genetic correlations between traits. The same previously defined linear model was used for SPS. For the other 13 chosen traits, the models were based on work by Oliveira Junior et al. (2021). The only modifications made were the removal of the herd effect and the addition of random across-lactation permanent environmental effects to fit the across-lactation repeated records of the production and reproduction traits. The model used for each trait is presented in Table 2.

A general description of the linear models used in the 2-trait analyses is as follows:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}_{\mathbf{a}}\mathbf{a} + \mathbf{Z}_{\mathbf{p}\mathbf{e}_{\mathbf{w}}}\mathbf{p}\mathbf{e}_{\mathbf{w}} + \mathbf{Z}_{\mathbf{p}\mathbf{e}_{\mathbf{a}}}\mathbf{p}\mathbf{e}_{\mathbf{a}} + \mathbf{e},$$

where  $\mathbf{y}$  is a vector of observations;  $\mathbf{b}$  is a vector of fixed effects;  $\mathbf{a}$  is a vector of random animal additive genetic effects;  $\mathbf{pe}_{\mathbf{w}}$  is a vector of random within-lactation permanent environmental effects;  $\mathbf{pe}_{\mathbf{a}}$  is a vector of across-lactation permanent environmental effects;  $\mathbf{e}$  is a

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vector of random residual effects; and  $\mathbf{X}$ ,  $\mathbf{Z}_{a}$ ,  $\mathbf{Z}_{pe_{w}}$ , and  $\mathbf{Z}_{pe_{a}}$  are corresponding incidence matrices.

For traits without any repeated records (conformation traits and age at first service), permanent environmental effects were not included in the model. For traits with only repeated records across lactation (production and other fertility traits), the within-lactation permanent environmental effect was not included in the model.

The variance-covariance matrices were as follows:

$$\begin{bmatrix} \mathbf{a} \\ \mathbf{pe}_{\mathbf{w}} \\ \mathbf{pe}_{\mathbf{a}} \\ \mathbf{e} \end{bmatrix} \sim MVN \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \mathbf{G} \otimes \mathbf{A} & 0 & 0 & 0 \\ 0 & \mathbf{P}_{\mathbf{w}} \otimes \mathbf{I} & 0 & 0 \\ 0 & 0 & \mathbf{P}_{\mathbf{a}} \otimes \mathbf{I} & 0 \\ 0 & 0 & \mathbf{O} & \mathbf{R} \otimes \mathbf{I} \end{bmatrix}$$

where **G** is the covariance matrix between traits for random additive genetic effects,  $\mathbf{P}_{\mathbf{w}}$  is the withinlactation permanent environmental covariance matrix,  $\mathbf{P}_{\mathbf{a}}$  is the across-lactation permanent environmental covariance matrix, **R** is the residual covariance matrix between traits, **A** is the additive relationship matrix, **I** is an identity matrix, and MVN indicates multivariate normal distribution.

The additive genetic correlations  $(\mathbf{r}_g)$  were calculated as follows:

$$r_{g} = \frac{cov_{a}\left(t_{1},t_{2}\right)}{\sqrt{\sigma_{a_{1}}^{2}\times\sigma_{a_{2}}^{2}}}$$

where  $\text{cov}_{a}$  is the additive genetic covariance between trait 1 and trait 2 (t<sub>1</sub>, t<sub>2</sub>), and  $\sigma_{a_1}^2$  and  $\sigma_{a_2}^2$  are the additive genetic variances for trait 1 and trait 2, respectively.

The phenotypic correlations  $(\mathbf{r}_{\mathbf{p}})$  were calculated as follows:

$$r_{p} = r_{g}\sqrt{h_{1}^{2}h_{2}^{2}} + r_{pe_{a}}\sqrt{pe_{a_{1}}^{2}pe_{a_{2}}^{2}} + r_{e}\sqrt{e_{1}^{2}e_{2}^{2}},$$

where  $r_g$ ,  $r_{pe_a}$ , and  $r_e$  are additive genetic, across-lactation permanent environmental, and residual correlations between traits, respectively. For trait i,  $h_i^2 = \sigma_{a_i}^2 / \sigma_{p_i}^2$ ,  $pe_{a_i}^2 = \sigma_{pe_{a_i}}^2 / \sigma_{p_i}^2$ , and  $e_i^2 = \sigma_{e_i}^2 / \sigma_{p_i}^2$ , where  $\sigma_{a_i}^2$  is the additive genetic variance,  $\sigma_{pe_{a_i}}^2$  is the across-lactation permanent environmental variance,  $\sigma_{e_i}^2$  is the residual variance, and  $\sigma_{p_i}^2$  is the phenotypic variance, estimated as  $\sigma_{a_i}^2 + \sigma_{pe_{w_i}}^2 + \sigma_{pe_{a_i}}^2 + \sigma_{e_i}^2$ . Either or both  $\sigma_{pe_{w_i}}^2$  and  $\sigma_{pe_{a_i}}^2$  were excluded in the calculation of  $\sigma_{p_i}^2$  for traits without repeated records within or across lactation, respectively. As only SPS had repeated records

Table 2. Single-trait animal models<sup>1</sup> for currently selected traits used in the bivariate analyses for the estimation of the correlations between these traits and the reproductive tract size and position score

| $\mathrm{Trait}^2$          |                      | Fixed $effect^3$                                |                        | Random $effect^4$          |
|-----------------------------|----------------------|---|------------------------|----------------------------|
| Conformation                | Round classifier     | Age calving-stage lactation-                    |                        |                            |
|                             |                      | round   |                        |                            |
| BCS                         | Х                    | Х   |                        |                            |
| Thurl placement             | Х                    | Х   |                        |                            |
| Rump angle                  | Х                    | Х   |                        |                            |
| Production                  | Year-season calving  | Age calving                                     |                        | $Pe_a$                     |
| Milk yield                  | X                    | X   |                        | X                          |
| Protein yield               | Х                    | Х   |                        | Х                          |
| Fat yield                   | Х                    | Х   |                        | Х                          |
| Fertility                   | Year born-month born | Age prev calving-month                          | Age prev calving-month | $Pe_a$                     |
|                             |                      | first service                                   | prev calving           |                            |
| Age at first service        | Х                    |   |                        |                            |
| Nonreturn rate at 56 d      | Х                    | Х   |                        | Х                          |
| First service to conception | Х                    |   | Х                      | Х                          |
| Calving to first service    | Х                    |   | Х                      | Х                          |
| Days open                   | Х                    |   |                        | Х                          |
| Number of services          | Х                    | Х   |                        | Х                          |
| Calving                     | Year born-month born | Age curr calving-month<br>curr calving-calf sex | Calf sire              | $\mathrm{Pe}_{\mathrm{a}}$ |
| Calving ease                | Х                    | X   | Х                      | Х                          |

<sup>1</sup>Models adapted from Oliveira Junior et al. (2021).

<sup>2</sup>All traits are cow-related, except age at first service, which is a heifer trait.

<sup>3</sup>Prev = previous; curr = current.

 ${}^{4}\mathrm{Pe}_{\mathrm{a}} = \mathrm{across-lactation}$  permanent environmental effect.

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**Table 3.** Posterior mean, posterior standard deviation (PSD), and highest posterior density (HPD) interval of the genetic parameters resulting from the linear animal model for the reproductive size and position score, estimated on records from 490 Holstein cows over 3 yr in 1 herd

| Parameter <sup>1</sup> | Mean  | PSD   | Low HPD | High HPD |
|------------------------|-------|-------|---------|----------|
| $\sigma_{\rm p}^2$     | 0.323 | 0.004 | 0.316   | 0.330    |
| $\sigma_{\rm a}^2$     | 0.037 | 0.004 | 0.029   | 0.043    |
| $\sigma_{\rm e}^2$     | 0.245 | 0.004 | 0.238   | 0.251    |
| $\sigma_{pe_w}^2$      | 0.035 | 0.004 | 0.000   | 0.028    |
| $\sigma_{pe_a}^2$      | 0.007 | 0.002 | 0.000   | 0.003    |
| $h^2$                  | 0.113 | 0.013 | 0.093   | 0.135    |
| r <sub>w</sub>         | 0.242 | 0.012 | 0.223   | 0.262    |
| r <sub>a</sub>         | 0.134 | 0.014 | 0.109   | 0.156    |

<sup>1</sup>Where  $\sigma_p^2$  = phenotypic variance;  $\sigma_a^2$  = additive genetic variance;  $\sigma_e^2$  = residual variance;  $\sigma_{pe_w}^2$  = within-lactation permanent environmental variance;  $\sigma_{pe_a}^2$  = across-lactation permanent environmental variance;  $h^2$  = heritability;  $r_w$  = within-lactation repeatability;  $r_a$  = across-lactation repeatability.

within lactation, the within-lactation permanent environmental correlation  $(r_{pe_w})$  did not contribute to any  $r_p$ .

For all parameters, the posterior mean, posterior standard deviation (**PSD**), and 95% highest posterior density were calculated within the R software (R Core Team, 2018) based on the output of the THRG-IBBS1F90 software (Misztal et al., 2002). All parameters were calculated within each of the 1,000 samples and then averaged to obtain the final estimates.

Pearson correlations between EBV for SPS and both LPI and Pro\$ were used to evaluate the association of the novel trait with the current selection indices.

## **RESULTS AND DISCUSSION**

## Heritability and Repeatability Estimates

The difference between linear and threshold models is expected to increase for a combination of factors: (1) heritability is low, (2) there is a small number of phenotypic categories, and (3) there is a small number of records (Meijering and Gianola, 1985; Mrode, 2014). The Spearman rank correlation between the EBV from the 2 models was close to 1 (0.99). Based on this very similar ranking, the linear model was used for further analyses. The estimated variances, heritability, and repeatabilities from the linear model are presented in Table 3, and the same estimates from the threshold model are given in Supplemental Table S1 (https:// zenodo.org/record/6925896#.YuKfsXbMJhE; Martin et al., 2022). Only estimates from the linear model will be presented and discussed hereafter. The estimated heritability, within-lactation repeatability, and acrosslactation repeatability were 0.113, 0.242, and 0.134, respectively. The estimated heritability for SPS was, therefore, considerably higher than that of any fertility traits currently evaluated in dairy cattle in Canada (see Oliveira Junior et al., 2021).

These results are preliminary, as they are based on a small sample of cows from one research herd. The current data set was created to study the association of SPS with cow fertility over time and, to this end, there was no specific time window for SPS recording. The phenotypes were recorded from calving until the confirmation of conception, which could happen late in lactation for some cows. This large time window of collection, combined with the sparse repeated records, might explain the low estimated repeatability of SPS (Table 3). A more precisely defined phenotyping protocol is needed to improve SPS repeatability. In further studies, phenotypes should be assessed after uterine involution, which generally ends around 30 d after calving, and before the establishment of the next pregnancy. By doing so, the natural—but here undesirable—variation due to uterine involution could be removed.

Another point for improvement could be the definition of the trait itself. The size of the reproductive tract is a continuous trait that has been assigned to 3 ordinal categories (scores). The small number of categories may affect the repeatability of the score. A cow with a score close to the threshold between 2 categories could oscillate between 2 scores without a meaningful difference in the actual size and position of the reproductive tract. With only 3 categories, these small changes may represent a large variation between 2 records within the genetic analysis. Therefore, the repeatability of both across- and within-lactation records observed in this study may have been reduced by the long period of collection and the small number of categories for scoring SPS. Adding more categories to SPS could be beneficial to its genetic estimation. However, it would slightly increase the difficulty and possibly the precision of recording of this trait.

The repeatability of the trait indicates the upper limit of the heritability and the number of records per animal necessary to reach it (Falconer and Mackay, 1996). The expected increase in the heritability of the average from different numbers of repeated records across and within lactation for SPS is shown in Figure 2. Measuring an animal twice, either within or across lactations, would potentially double the heritability of the average SPS. With 5 records per animal, most of the potential increase would be captured but that may be unrealistic in practice.

A strategy for large-scale SPS phenotyping would be to measure SPS at the time of insemination. Insemina-

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Genetic correlation Posterior mean ( $\pm$  PSD) Trait Low HPD High HPD Conformation BCS  $0.632 \ (\pm \ 0.084)$ 0.4940.772Thurl placement -0.263 (± 0.118) -0.072-0.450Rump angle  $-0.739 \ (\pm \ 0.072)$ -0.866-0.636Production  $0.047 \ (\pm \ 0.074)$ -0.0790.167Milk yield  $-0.026 (\pm 0.088)$ -0.171Protein yield 0.115 $0.042 (\pm 0.091)$ Fat vield -0.1060.187Fertility  $0.444 \ (\pm \ 0.164)$ 0.1580.693 Age at first service  $-0.730(\pm 0.093)$ -0.876-0.582Nonreturn rate at 56 d  $0.694 (\pm 0.115)$ 0.528 0.897 First service to conception  $-0.371 (\pm 0.206)$ -0.724-0.066Calving to first service Days open  $0.435 (\pm 0.246)$ 0.0350.805Number of services  $0.931 (\pm 0.029)$ 0.9760.889Calving Calving ease  $0.061 \ (\pm \ 0.233)$ -0.3150.437

**Table 4.** Posterior mean, posterior standard deviation (PSD), and highest posterior density (HPD) interval of the genetic correlations resulting from the bivariate animal model between reproductive tract size and position score (SPS) and currently selected traits, estimated on records from 490 Holstein cows over 3 yr in 1 herd

tion technicians could be trained to record SPS during their daily work. This would give access to reliable and large data sets without the need for large investments. However, this strategy may be biased if repeated records are used. The SPS phenotype would be repeated within lactation for animals requiring multiple breedings to reach conception, leading to a biased sample. Multiple SPS records per cow would be advantageous, but the definition of the time window for collection needs to be investigated further.

## Genetic and Phenotypic Correlations

Knowledge of the genetic correlation between SPS and other economically important traits is important to assess the potential effect of selecting for SPS. These



**Figure 2.** Change in expected heritability of reproductive tract size and position score with an increased number of repeated measurements per cow across and within lactation.

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correlations are presented in Table 4. All estimates had a relatively high PSD due to the small size of the data set, but the point estimates were promising, as they were favorable in magnitude and direction. For instance, the correlations of SPS with milk, fat, and protein yields were close to zero and statistically not different from zero, meaning SPS could be selected without affecting production traits. This is direct contrast with currently evaluated fertility traits, which largely present unfavorable correlations with production (Oliveira Junior et al., 2021).

The high genetic correlation between SPS and BCS (0.632) is noteworthy. Even though BCS is known as an indicator of fertility, the underlying mechanism is not clearly defined (Berry et al., 2016; Miglior et al., 2017; Lucy, 2019). Interestingly, Madureira et al. (2020) reported no association between SPS and BCS at the phenotypic level. We reached the same conclusion, with a nonsignificant phenotypic correlation between the 2 traits (see Table 5). Moreover, Baez et al. (2016) found that cows with a smaller uterine volume, a trait similar to SPS, had a greater pregnancy per insemination than those with a larger uterine volume, regardless of BCS. When Baez et al. (2016) compared the uterine volume within each category of the BCS scale, the relationship appeared to be stronger in thinner cows. This reflects the complex relationship between SPS and BCS, which may not be properly captured by a linear correlation, as BCS is an intermediate optimum trait; a quadratic correlation may be more relevant to study this relationship. A possible explanation for the positive genetic correlation between SPS and BCS could be the number and size of the adipocytes around and within the

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Table 5. Posterior mean, posterior standard deviation (PSD), and highest posterior density (HPD) interval of the phenotypic correlations resulting from the bivariate animal model between reproductive tract size and position score (SPS) and currently selected traits, estimated on records from 490 Holstein cows over 3 yr in 1 herd

|                             | Phenotypic correlation   |         |          |  |
|-----------------------------|--------------------------|---------|----------|--|
| Trait                       | Posterior mean (± PSD)   | Low HPD | High HPD |  |
| Conformation                |                          |         |          |  |
| BCS                         | $0.180 \ (\pm \ 0.023)$  | 0.001   | 0.145    |  |
| Thurl placement             | $-0.101(\pm 0.025)$      | -0.088  | -0.220   |  |
| Rump angle                  | $-0.119(\pm 0.024)$      | -0.155  | -0.079   |  |
| Production                  |                          |         |          |  |
| Milk yield                  | $-0.006 \ (\pm \ 0.018)$ | -0.034  | 0.024    |  |
| Protein yield               | $-0.018(\pm 0.018)$      | -0.046  | -0.013   |  |
| Fat yield                   | $-0.000(\pm 0.018)$      | -0.030  | 0.028    |  |
| Fertility                   |                          |         |          |  |
| Age at first service        | $0.045~(\pm 0.024)$      | 0.006   | 0.085    |  |
| Nonreturn rate at 56 d      | $-0.049(\pm 0.019)$      | -0.078  | -0.017   |  |
| First service to conception | $0.058 (\pm 0.030)$      | 0.012   | 0.107    |  |
| Calving to first service    | $0.009(\pm 0.021)$       | -0.027  | 0.041    |  |
| Days open                   | $0.005(\pm 0.019)$       | -0.030  | 0.033    |  |
| Number of services          | $0.120 (\pm 0.019)$      | 0.090   | 0.153    |  |
| Calving                     |                          |         |          |  |
| Calving ease                | $0.014~(\pm~0.021)$      | -0.019  | 0.046    |  |

reproductive tract (Crociati et al., 2018). The SPS phenotype is measured by transrectal palpation, which can be affected by fat accumulation around the tract. Accordingly, higher BCS cows could have a higher SPS measurement.

This association should be further investigated to assess the real relationship between BCS and SPS and evaluate the need to adjust for BCS when genetically evaluating cows for SPS. In our preliminary analyses, BCS was significant as an explanatory variable in the model, but it explained less than 1% of the variation in SPS. Moreover, the variance component estimates from models with and without BCS as a covariate were similar. As BCS is already a trait included in the LPI (albeit with a small contribution) and given the high genetic correlation between SPS and BCS (0.632), BCS was excluded from the univariate animal model for the final analyses.

For other conformation traits, the estimated genetic correlations were -0.263 (thurl placement) and -0.739 (rump angle). Selection for SPS should aim for lower scores to improve fertility, whereas thurl placement and rump angle optimum values are intermediate, making this negative linear association difficult to interpret.

For traits related to reproduction, the significant genetic correlations were desirable; that is, low SPS scores were related to better fertility, with the exception of calving to first service (-0.371). Age at first service, days open, first service to conception, nonreturn rate at 56 d, and number of services all showed moderate to strong estimated genetic correlations (-0.730 to 0.931), whereas calving ease had an estimated genetic correla-

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tion with SPS close to 0 (0.061), which was not statistically significant. However, it is important to note that only number of services had a relatively small PSD. The highest genetic correlation was estimated between SPS and number of services (0.931), which was expected because SPS was initially proposed as an indicator trait for number of services.

Supplemental Table S2 (https://zenodo.org/record/ 6925896#.YuKfsXbMJhE; Martin et al., 2022) presents the heritability estimates from the bivariate analyses for all other reproductive traits, which used only animals with SPS records available. The heritability estimates for the reproductive traits that are strongly genetically correlated with SPS were higher than those from Oliveira Junior et al. (2021). This indicates that these traits benefited from the additional information provided by SPS through their strong genetic correlation with SPS, and ended up with a significantly higher heritability estimates compared with those from Oliveira Junior et al. (2021), who used large data sets for their analyses. For comparison, heritability estimates  $(\pm PSD)$  from the univariate analyses for first service to conception, nonreturn rate at 56 d, and number of services were  $0.090 (\pm 0.080), 0.041 (\pm 0.035), and 0.081$  $(\pm 0.061)$ , respectively, using only cows with an SPS record, which were within the range of the estimates from Oliveira Junior et al. (2021).

The efficiency of indirectly selecting for other reproduction traits based on selecting for SPS could be assessed using the estimated genetic correlations and heritability of the traits. Assuming the same selection intensity and using heritability estimates from the uni-

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variate analyses for nonreturn rate at 56 d and number of services, selection based on a single measurement of SPS would lead to an indirect selection response on nonreturn rate at 56 d and number of services that would be 1.21 and 1.10 times greater, respectively, than the direct selection response on these traits. More interestingly, there would not be an antagonistic indirect selection response on production traits. In addition, the Pearson correlations between SPS EBV and the Canadian index values LPI and Pro\$ were -0.232 and -0.226, respectively, which are favorable in both cases. Therefore, SPS showed encouraging results for selection for fertility, with favorable indirect selection response on production traits.

### CONCLUSIONS

The SPS is a new fertility trait, based on transrectal palpation of the reproductive tract, that has been developed as an indicator of pregnancy rate, number of services per pregnancy, and pregnancy loss. This novel trait had no genetic correlation with production traits and had favorable genetic correlations with fertility traits, varying from -0.730 (nonreturn rate at 56 d) to 0.931 (number of services). Although preliminary, these results are encouraging, because SPS seems to be more heritable and highly genetically correlated with number of services to conception and strongly correlated with nonreturn rate at 56 d and first service to conception, indicating potential for effective indirect selection response on these traits. Further studies with larger data sets are warranted to validate these findings.

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