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Potential of anogenital distance as a genetic selection trait in Canadian Holsteins

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ABSTRACT

Maintaining optimal fertility in dairy cattle herds is a global challenge that is typically addressed through the genetic selection of fertility indicator traits. However, many of the traits currently implemented in breeding programs are heavily influenced by environmental factors, resulting in a slow rate of genetic improvement. Anogenital distance (AGD) has recently emerged as a promising fertility indicator trait due to its association with favorable reproductive outcomes and its higher heritability estimates compared with currently evaluated traits. This study aimed to enhance the understanding of AGD's genetic potential by estimating its genetic parameters in Canadian Holsteins, assessing the reliability of breeding values, comparing pedigree BLUP to single-step genomic BLUP approaches, and estimating the correlation between AGD breeding values and those of currently evaluated traits. The dataset used in this study comprised 5,541 Canadian Holstein cows and heifers from 20 herds, collected between 2015 and 2020. The final dataset consisted of 4,988 animals with AGD phenotypes after filtering. The pedigree-based heritability estimate for AGD was 0.39 ± 0.04, whereas the incorporation of genomics resulted in a lower estimate of 0.37 ± 0.03. The reliability of estimated breeding values ranged from 0.49 ± 0.03 for phenotyped animals to 0.81 ± 0.05 for proven sires with at least 30 phenotyped daughters. The integration of genomic information improved the reliability of breeding values, with gains ranging from 0.01 gain for proven sires to 0.14 relative gain for unproven sires. High gain in observed reliability for females without records was demonstrated when genomic information was included, using both split forward validation (0.26) and 5-fold cross-validation (0.14). The AGD breeding values showed moderate unfavorable correlations with relative breeding values of age at first service and production traits including milk yield, fat yield, and protein yield. This suggests that AGD may influence reproductive maturity in heifers but could also have an unfavorable association with production traits, highlighting the need for balanced breeding strategies that consider both fertility and production outcomes. Future studies should aim to expand phenotype data across lifetimes and breeds and estimate genetic correlations with traditional reproduction and production traits using multitrait models.

Key words: genetic parameters, breeding value correlation, single-step genomic BLUP, fertility indicator trait

INTRODUCTION

Despite the importance of fertility to the productivity and profitability of the dairy industry, maintaining optimal fertility levels within dairy cattle herds remains a substantial challenge worldwide (Miglior et al., 2017; Fleming et al., 2019). Fertility encompasses various reproductive functions, such as conception, pregnancy maintenance, calving, and early resumption of postcalving cyclicity. Given the complexity of representing fertility through a single phenotype, the dairy industry uses a range of fertility indicator traits as proxies to address most of these aspects. In Canada, currently evaluated traits include calving to first service (**CTFS**), first service to conception (**FSTC**), 56-d nonreturn rate (**NRR**), and age at first service (**AFS**), which are routinely derived from insemination records (Fleming et al., 2019). However, many of these traits are heavily influenced by

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environmental conditions and management decisions resulting in low heritability estimates. Factors such as the timing of insemination and the methods used for estrus detection can skew the interpretation of fertility indicator traits, leading to an inaccurate representation of an animal's true fertility. Extended voluntary waiting periods and milking beyond the standard 305 d can prolong the time between pregnancies, affecting traits like CTFS and calving interval (Wall et al., 2003). Additionally, inadequate methods for estrus detection may result in the misclassification of highly fertile cows as having poor fertility (Reith and Hoy, 2018). Moreover, reproductive technologies like timed artificial insemination may obscure true reproductive performance and introduce bias into genetic evaluations (Goodling et al., 2005; Lynch et al., 2021; Oliveira et al., 2021).

Recognizing the limitations of current fertility indicator traits, both the scientific community and the dairy sector have called for biologically relevant indicators that are less influenced by environmental conditions (Miglior et al., 2017; Fleming et al., 2019; Martin et al., 2022). These traits hold promise for enhancing genetic progress by more accurately capturing the complexities of fertility. One such promising trait is anogenital distance (**AGD**), which is defined as the distance from the center of the anus to the base of the clitoris in female cattle (Gobikrushanth et al., 2017). The phenotypic variance observed in AGD is believed to be primarily influenced by increased exposure to androgens during fetal development, particularly during the critical reproductive programming window (MacLeod et al., 2010; Dean et al., 2012). Overexposure to androgens prenatally in females results in longer AGD, androgenization of the female reproductive system, and reduced postnatal fertility (Zehr et al., 2001, Bánszegi et al., 2012). Due to its foundation in reproductive physiology, AGD has become the focus of extensive research into its potential as a fertility indicator trait for dairy cattle.

A shorter AGD in cattle is closely linked with favorable reproductive outcomes, including higher pregnancy per artificial insemination in cows (Gobikrushanth et al., 2017; Carrelli et al., 2022) and heifers (Carrelli et al., 2021; Vidal et al., 2024), reduced age at first conception, and increased probability of pregnancy up to 450 d of age in heifers (Carrelli et al., 2021; Vidal et al., 2024). These results suggest that selecting for shorter AGD could enhance reproductive efficiency in dairy cattle.

Although no research has been conducted on the repeatability of AGD across different lactations, initial findings indicate a notable degree of consistency across various physiological stages, including that of the estrous cycle, lactation, and gestation (Rajesh et al., 2022). It is important to recognize, however, that the sample size in that study was relatively small, with fewer than 100 animals analyzed per stage. Despite this limitation, the observed high repeatability across various physiological states suggests that AGD could be a reliable trait for genetic selection, offering consistent predictions and facilitating meaningful comparisons between animals.

Recent studies on AGD have reported moderate heritability estimates. For instance, Gobikrushanth et al. (2019) estimated the heritability of AGD to be 0.37 ± 0.08 in Irish Holstein-Friesians, whereas Stephen et al. (2023) estimated it to be 0.23 ± 0.03 in heifers and 0.29 ± 0.05 in primiparous cows within a New Zealand Holstein-Friesians population. These heritability estimates are markedly higher than the typical range of 0.01 to 0.10 observed for traditionally evaluated fertility traits (Wall et al., 2003; VanRaden et al., 2004; Jamrozik et al., 2005, Fleming et al., 2019; Oliveira et al., 2021). Furthermore, Stephen et al. (2023) reported a strong genetic correlation of 0.89 ± 0.05 between AGD measured in heifers and later in the same cows, supporting the possibility of making early selection decisions based on AGD. Collectively, these findings underscore the potential of AGD as a valuable trait for improving the reproductive efficiency of dairy herds.

Current research on the genetics of AGD, primarily from pasture-based (seasonal) systems with different reproductive priorities, remains insufficient for comprehensive understanding of the trait (Fleming et al., 2019; Meier et al., 2021). In these systems, selection focuses on fertility traits like submission rate and calving rates, which are less relevant in intensive dairy systems (Berry et al., 2013; Bowley et al., 2015). Additionally, previous studies have conducted genetic evaluations using only pedigree information. Incorporating genomic information into analysis is expected to improve the reliability of estimated breeding values and provide more accurate relationship information within the population (Daetwyler et al., 2007; Van Raden et al., 2009).

Building on these considerations, this study aimed to contribute to the ongoing exploration of AGD as a biologically relevant fertility indicator trait. The objectives of this study were to estimate genetic parameters for AGD in a Canadian Holstein population, assess the reliability of breeding values, evaluate the impact of incorporating genomic information, and estimate correlations between AGD breeding values and milk production and fertility traits.

MATERIALS AND METHODS

No approval by an Institutional Animal Care and Use Review Board was required because data were obtained from an existing database.

Data

Data comprised 5,541 Canadian Holstein cows and heifers from 20 herds, each with one AGD record, collected between August 2015 and October 2020. Subsets of this data were previously used by Gobikrushanth et al. (2017) and Carrelli et al. (2021, 2022). AGD phenotypes were collected as described by Gobikrushanth et al. (2017). Phenotypic outliers were identified as observations falling outside the range of 1.5 times the interquartile range below the first quartile and above the third quartile (outside of 89.5–165.5 mm). A dataset of 5,506 cows and heifers remained after these outliers (0.6%, n = 35) were removed.

Records were assigned to contemporary groups composed of herd, year, and season of birth (**HYS**). Seasons were defined as winter (January–March), spring (April–June), summer (July–September), and fall (October–December). Data were filtered on the condition that HYS contained a minimum of 5 animals per level, ensuring adequate representation across contemporary groups. This criterion removed 9.4% (n = 518) of the data and led to a final dataset of 4,988 cows and heifers.

Records were assigned to parities (designated as 0, 1, 2, and 3+), recording technicians (designated as A, B, and C), age of the animal in days, and days since last calving for cows at the time of phenotype collection. Distribution of records across parities and technician is provided in Table 1. Animals ranged from 329 d of age to 3,680 d of age at recording, and cows ranged from 3 d since last calving to 1,154 d since last calving at recording. The relevant pedigree accompanying the dataset included 26,959 individuals, with a minimum depth of 8 generations from the phenotyped population and an average pedigree-based inbreeding coefficient of 0.038.

Genotype information was available for 6,631 animals in the pedigree and 1,520 of the animals with AGD phenotypes. Genotypes were obtained with the Illumina Bovine SNP50 BeadChip v.2 (50K; Illumina, San Diego, CA). Single nucleotide polymorphisms on autosomal chromosomes, heterozygous in more than a 0.15 frequency departure from Hardy–Weinberg equilibrium, those with a minor allele frequency of less than 5%, those with call rates below 90%, and those in linkage disequilibrium (based on squared allele correlation, r^2) with other SNPs higher than 0.80 were removed. After quality control, 34,444 SNPs remained for analysis.

Table 1. Distribution of anogenital distance phenotype records across parity groups and recording technicians of the phenotype

Recording technician	Parity group 0	1	2	3+	Total
A	173	467	355	344	1,339
B	691	908	671	407	2,677
C	0	363	205	404	972
Total	864	1,738	1,231	1,155	4,988

An additional dataset was compiled to estimate correlations among breeding values of AGD, fertility indicator traits, and milk production traits. This was used as a simple proxy of genetic correlations, given that proper estimation of genetic correlation with lowly heritable fertility traits would require a larger sample size for AGD. Fertility indicator traits included AFS, CTFS, FSTC in heifers (**FSTCh**) and cows (**FSTCc**), and NRR in heifers (**NRRh**) and cows (**NRRc**), and milk production traits included 305-d milk yield (**MY**), 305-d protein yield (**PY**), and 305-d fat yield (**FY**). Fertility and production breeding values were provided by Lactanet Canada (Guelph, ON, Canada) from the April 2024 genetic evaluation run for sires of daughters with AGD records.

Genetic and Genomic Analyses

Variance components were estimated using an average information restricted maximum likelihood algorithm first using only pedigree information and then incorporating genomic information through single-step methodology (Legarra et al., 2009; Christensen and Lund, 2010; Wang et al., 2012). The single trait animal model used in our analysis was defined as follows:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e},$$

where $\mathbf{y}$ is a vector of observations, $\mathbf{X}$ is the incidence matrix of the fixed effects in vector $\mathbf{b}$, $\mathbf{Z}$ is the incidence matrix of the additive genetic effects in vector $\mathbf{a}$, and $\mathbf{e}$ is a vector of residual effects. Fixed effects included recording technician, a quadratic regression on the age of the animal in days, regression on days since calving at measurement, and HYS contemporary group of the individual.

The random animal additive genetic effects in $\mathbf{a}$ and the random residual effects in $\mathbf{e}$ were assumed to be normally distributed with mean zero and variances, defined as follows:

$$var\begin{bmatrix}\mathbf{a}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{A}\sigma_a^2 & 0\\ 0 & \mathbf{I}\sigma_e^2\end{bmatrix},$$

where $\mathbf{A}$ is the numerator relationship matrix, σ_a^2 is the additive genetic variance, $\mathbf{I}$ is an identity matrix with dimensions equal to the number of animals with records, and σ_e^2 is the residual variance.

Genomic information was incorporated into analysis using the single-step GBLUP (**ssGBLUP**) which replaces the numerator relationship matrix (**A**) with a hybrid matrix containing pedigree and genomic information (**H**; Legarra et al., 2009). The inverse of matrix **H** is calculated as follows:

$$\mathbf{H}^{-1} = \mathbf{A}^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & \tau\left(\alpha\mathbf{G} + \beta\mathbf{A}_{22}\right)^{-1} - \omega\mathbf{A}_{22}{}^{-1} \end{bmatrix},$$

where $\mathbf{A}^{-1}$ is the inverse of the pedigree-based relationship matrix, created using all available pedigree and accounting for inbreeding, **G** is the genomic relationship matrix calculated using the first method presented in VanRaden (2008) and allele frequencies that were estimated from the genotypes, and $\mathbf{A}_{22}$ is the section of **A** related to genotyped animals. Blending factors α (0.95) and β (0.05) were used to make **G** invertible and account for polygenic effects, whereas the τ (1.0) and ω (1.0) parameters (i.e., scaling factors) were used to account for reduced genetic variance and different pedigree depths, respectively, to make **G** compatible with $\mathbf{A}_{22}$.

Breeding values and variance components were estimated using AIREMLF90 from the BLUPF90 family of programs for the genetic and genomic analyses (Misztal et al., 2014).

Reliability of Breeding Values

Theoretical reliabilities of EBVs and GEBVs were estimated as described by Van Vleck (1993):

$$Rel_i = 1 - \frac{PEV_i}{\left(1 + F_i\right)\sigma_a^2},$$

where Rel_i is the reliability of animal i's EBV or GEBV, PEV_i is the predictor error variance of the ith EBV/GEBV, F_i is the inbreeding of animal i, and σ_a^2 is the additive genetic variance. Reliability EBVs were then averaged for animals with phenotype records, sires with at least 10 phenotyped daughters, and sires with at least 30 phenotyped daughters.

Correlation Between EBVs and GEBVs

To assess the potential reranking of animals between pedigree BLUP (**pBLUP**) and ssGBLUP, Spearman rank correlation coefficients were estimated between the rankings of sires of phenotyped daughters based on EBVs from pBLUP and GEBVs from ssGBLUP. Sires were grouped into subsets based on genotype status and Spearman rank correlation coefficients were estimated to evaluate the degree of reranking between the 2 methods. Additionally, Pearson correlations were estimated between EBVs and GEBVs for sires to quantify the level of agreement between the 2 methods. This analysis allowed for the assessment of the reranking of animals and the overall consistency and concordance in the EBVs between pedigree-based and genomic-based methods.

Validation

Two validations were conducted to estimate the observed reliability for unphenotyped cows and heifers, focusing on the utility of genomic information in the absence of phenotypic data: (1) k-fold cross-validation and (2) split forward validation. Although the cross-validation attempts to estimate the observed reliability of breeding values for unphenotyped cows in the population, the split forward validation elucidates the estimated observed reliability of breeding values for young unphenotyped heifers in the new generation.

A k-fold cross-validation with $k = 5$ was conducted using a subset of cows with both phenotypic and genotypic data, and that did not occur as dams of any other phenotyped animals (n = 1,513). These animals were then randomly sorted into 1 of 5 mutually exclusive groups. In each round of the cross-validation, phenotypes from one of the groups were masked from the dataset while the phenotypes of the 4 remaining groups were included in the dataset. For each round of the 5-fold cross-validation, pBLUP and ssGBLUP were run to estimate breeding values. Results were then compared between the full analysis, where all phenotypes were included, and the masked analyses. Observed reliabilities of breeding values for the masked animals were estimated using the square of the Pearson correlation between breeding values for both full and masked analyses. Theoretical reliabilities were estimated as described above. Observed and theoretical reliabilities were then averaged across the 5 analyses for both pBLUP and ssGBLUP methods.

Additionally, split forward validation was conducted using heifers with phenotype and genotype data born in the final year of the dataset (n = 429). These heifers were randomly divided into 2 groups for analysis. For each group, the process was conducted as follows:

1. The phenotypic information of the heifers in one group was masked from the dataset, but their pedigree and genotype information were retained for analysis.

2. The other group of heifers was completely excluded from the analysis, with pedigree, phenotype, and genotype data removed.

This process was then repeated by switching the roles of the 2 groups. Breeding values were estimated separately for each group using both the pBLUP and ssGBLUP methods. Observed and theoretical reliabilities were calculated in the same manner as in the 5-fold validation. Finally, the reliabilities for the genetic (pBLUP) and genomic (ssGBLUP) analyses were averaged across both groups to provide an overall measure of reliability.

Breeding Value Correlations

The relationships between production and fertility traits in sires with phenotyped daughters were analyzed using approximate genetic correlations. Data for this analysis were provided by Lactanet in the form of relative breeding values (**RBV**) or genomic RBV (**GRBV**), where values are standardized with an average of 100 and a standard deviation of 5, with higher values indicating more favorable outcomes for the trait. Production traits, however, were kept on the standard EBV and GEBV scales. Throughout this study, the terms proof breeding values (**PBV**) and genomic PBV (**GPBV**) are used to refer to these datasets. To ensure comparability between traits, AGD breeding values were standardized to the RBV scale.

Genetic correlations were estimated following the method described by Calo et al. (1973), which approximates genetic relationships based on reliabilities and observed correlations. Traits analyzed included production traits (MY, FY, and PY) and fertility traits (AFS, NRR, FSTC, and CTFS). The calculation formula was as follows:

$$r_{gA,T} = \frac{\sqrt{\sum_{i=1}^{n} RL_{A_i} \times \sum_{i=1}^{n} RL_{T_i}}}{\sum_{i=1}^{n}\left(RL_{A_i} \times RL_{T_i}\right)} \times r_{A,T}$$

Where, $r_{gA,T}$ represents the approximate genetic correlation between AGD and each trait, RL_{A_i} and RL_{T_i} are the individual reliabilities for AGD and each trait, n represents the number of individuals with records, and $r_{A,T}$ is the Pearson correlation coefficient between breeding values for AGD and each trait. The SE of the genetic correlations was calculated using the following formula:

$$SE = \sqrt{\frac{1 - {r_{gA,T}}^{2}}{n-2}}.$$

RESULTS AND DISCUSSION

Descriptive Statistics of AGD

The AGD measurements were normally distributed, with a mean value of 126.57 ± 13.79 mm (Figure 1). The distribution and variability in the phenotype were similar to previous estimates in Canadian Holstein cows (Gobikrushanth et al., 2017: 131.0 ± 12.2 mm; Carrelli et al., 2022: 132.0 ± 12.0 mm), though higher than those reported in other studies from Ireland (Gobikrushanth et al., 2019: 119.2 ± 11.6 mm), New Zealand (Grala et al., 2021: 98.9 ± 9.4 mm), and Spain (Vidal et al., 2024: 117.4 ± 14.6 mm). The lower mean AGD observed in Carrelli et al. (2021: 107.3 ± 10.5 mm), despite being from a Canadian population, likely reflects that the study was conducted exclusively on heifers. The higher average phenotypic values in the Canadian population relative to populations in Europe and New Zealand can likely be attributed to the larger average size of the Holstein breed in North America. In Ireland, stature has continually decreased over the past 2 decades, reflecting selection of shorter animals in breeding programs (Berry et al., 2022). In contrast, the Canadian Holstein population has increased in stature over time (Oliveira et al., 2021).

The average AGD observed within parity groups varied and increased with each parity, ranging from 110.95 ± 9.69 mm (heifers) to 132.77 ± 12.19 mm (3+ parity; Table 2). Significant differences ($P < 0.01$) were found between the means of all parity pairs except for the comparison of second-parity and third or higher parity cows. Positive relationships between AGD length and parity have been previously established in North American populations (Gobikrushanth et al., 2017; Carrelli et al., 2022). This finding suggests that AGD may elongate with increased number of parturitions, similar to growth and conformation traits.

Genetic Analysis

Pedigree-based heritability for AGD was estimated to be 0.39 ± 0.04 (Table 3). To the authors' knowledge, this is the first estimation of heritability for AGD in Canadian Holsteins and the first in any intensive dairy system. The heritability estimated in this study is consistent with previous estimates reported for pasture-based systems in Ireland and is marginally higher than what was found in New Zealand. In a population of Irish Holstein-Friesian cows, Gobikrushanth et al. (2019) reported a heritability of 0.37 ± 0.08, whereas Stephen et al. (2023) estimated lower heritability values of 0.23 ± 0.03 for heifers and 0.29 ± 0.05 for primiparous cows in New Zealand Holstein-Friesians. The consistency of heritability estimates across diverse populations and management systems

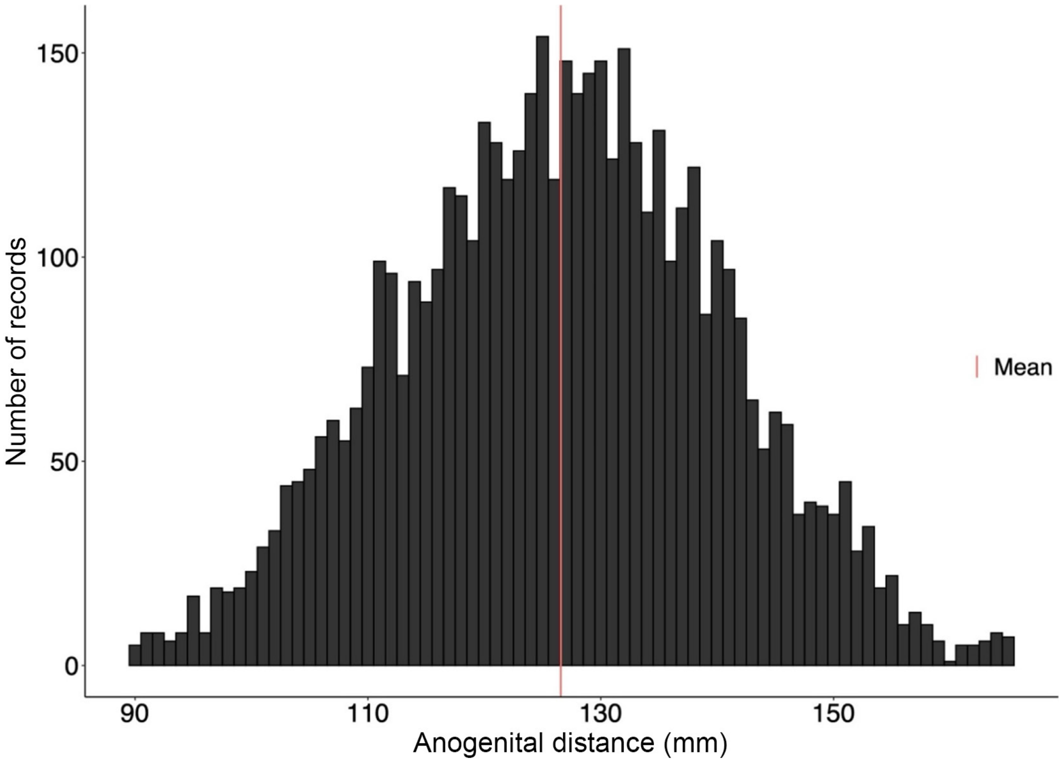

Figure 1. Distribution of anogenital distance phenotype records with a mean (± SD) observation of 126.57 ± 13.79 mm.

underscores the robustness of AGD as a heritable trait. Furthermore, the reliability of breeding values for all animals in the pedigree ranged from 0.00 to 0.91 for pBLUP, with averages ranging from 0.49 ± 0.03 for phenotyped cows to 0.81 ± 0.05 for proven sires (Table 4). Moderate to high reliability of breeding values for AGD supports its potential in selection programs.

The heritability estimated for AGD in this study (0.39 ± 0.04) surpasses that of several key fertility traits currently evaluated in breeding programs, which are often less than 0.10. Oliveira et al. (2021) reported heritability estimates ranging from 0.01 ± 0.001 for NRR to 0.06 ± 0.001 for CTFS in Canadian Holstein cows and from 0.01 ± 0.001 for FSTC, NRR, and NS to 0.05 ± 0.001 for AFS in heifers. Similarly, in the UK population, heritability estimates for fertility traits ranged from 0.02 ± 0.001 for NRR to 0.03 ± 0.001 for calving interval (Wall et al., 2003). In the US Holsteins, VanRaden et al. (2004) reported estimates of heritability range from 0.01 ± 0.001 for NRR at 70 d to 0.07 ± 0.003 for CTFS. These comparisons highlight the higher heritability of AGD compared with traditional fertility traits, further emphasizing its potential as a robust and valuable trait for inclusion in breeding programs

Genomic Analysis

The incorporation of genomic information resulted in a slight decrease in the estimated heritability for AGD, from 0.39 ± 0.04 without genomics to 0.37 ± 0.03 with genomics (Table 3). Slight shifts in heritability through ssGBLUP are expected due to the higher accuracy and

Table 2. Mean and standard deviation of anogenital distance (mm) phenotype records across parity groups in Canadian dairy cows

Parity group	Mean	SD	Group size
0	110.95[a]	9.69	864
1	126.57[b]	11.72	1,738
2	131.71[c]	11.73	1,231
3+	132.77[c]	12.19	1,155

[a–c]Means followed by different letters are significantly different at 1% probability level by Tukey's honest significant difference test.

Table 3. Variance components of anogenital distance (mm) estimated using pedigree (pBLUP) and genomic (ssBLUP) models

Parameter	Pedigree estimate	Genomic estimate
Additive genetic variance (σ_a^2)	41.48 ± 4.85	38.94 ± 3.94
Residual variance (σ_a^2)	65.87 ± 3.76	67.53 ± 2.94
Heritability (h^2)	0.39 ± 0.04	0.37 ± 0.03

Table 4. Gain in reliability of breeding values with the incorporation of genomic information (ssGBLUP) for subgroups of genotyped animals compared with pedigree BLUP (pBLUP)

Genotyped group	pBLUP	ssGBLUP	Gain	Size
Young unphenotyped females	0.33 ± 0.06	0.45 ± 0.06	0.12	135
Phenotyped females	0.49 ± 0.03	0.55 ± 0.03	0.06	1,520
Unproven sires (no phenotyped daughters)	0.19 ± 0.11	0.33 ± 0.11	0.14	1,893
Unproven sires (1–5 phenotyped daughters)	0.40 ± 0.09	0.51 ± 0.07	0.11	655
Proven sires (≥10 phenotyped daughters)	0.70 ± 0.08	0.72 ± 0.06	0.02	129
Proven sires (≥30 phenotyped daughters)	0.81 ± 0.00	0.82 ± 0.04	0.01	22

better separation of genetic and environmental effects achieved with genomic information (Beaulieu et al., 2022). Additionally, we found a notable decrease in the standard error of the estimated variance components and heritability associated with the genomic evaluation, indicating increased accuracy of the estimates when genomics is included. Previous studies have also reported lower standard errors of variance components with the inclusion of genomic information (Bérénos et al., 2014; Forni et al., 2011). Genomic prediction is widely acknowledged to outperform pedigree methods due to its capacity to account for pedigree errors and Mendelian sampling effect (Daetwyler et al., 2012; VanRaden, 2008).

Incorporation of genomic information resulted in an overall increase in reliability of breeding values for genotyped individuals with higher frequency of reliability at or above 0.50 (Figure 2). Reliability of GEBVs were moderate to high across genotyped groups, with a gain of reliability ranging from 0.01 to 0.14 relative to pBLUP (Table 4). The highest gains were estimated for groups lacking individual or daughter phenotype information. Young, unphenotyped females showed substantial gain in estimated reliability of 0.12, whereas unproven sires without phenotyped daughters exhibited higher relative gain of 0.14 with genomic information. In contrast, the gain in reliability was less pronounced for proven sires (0.01 to 0.02) and phenotyped females (0.06). Most studies similarly report increased reliabilities with the incorporation of genomic information (Ashraf et al., 2016; Tsuruta et al., 2017; Lee et al., 2020). Forni et al. (2011) found no change in average reliability between pedigree and genomic prediction across their full dataset but observed some increases in reliability for genotyped animals, particularly for genotyped females. These findings underscore the potential for substantial improvements in reliability by genotyping young animals, which may reduce the generation interval for breeding and culling decisions.

Table 5. Spearman rank and Pearson correlation estimates for sires of phenotyped daughters from pedigree BLUP and single-step genomic BLUP separated by genotype status

Item	Spearman rank correlation	Pearson correlation
Genotyped Sires	0.87	0.84
Nongenotyped Sires	0.95	0.93
Overall	0.87	0.85

The comparison of breeding values showed sizable changes with the inclusion of genomic information. The Pearson correlation coefficient between EBVs and GEBVs was 0.85 for sires of phenotyped daughters, with slightly stronger correlation observed for nongenotyped sires (0.93) compared with genotyped sires (0.84; Table 5). Likewise, Spearman rank correlations for sires of phenotyped daughters ranged from 0.87 (genotyped sires) to 0.95 (nongenotyped sires), with an overall correlation of 0.87 for all sires. These results highlight that the higher reliability of GEBVs comes with some level of reranking of animals, which should be expected with the inclusion of genomic information in the evaluation.

Validation

The 5-fold cross-validation indicated a substantial gain in observed reliability for unphenotyped cows with the inclusion of genotype information (Table 6). The average theoretical reliability for masked cows with included phenotypes ranged from 0.49 ± 0.002 in the pBLUP analysis to 0.51 ± 0.002 in the ssGBLUP analysis. The average observed reliability in the masked analysis was estimated to be 0.42 ± 0.005 via pBLUP and 0.56 ± 0.002 for ssGBLUP.

Table 6. Average theoretical and observed reliabilities[1] across a 5-fold cross-validation and split forward validation for both pedigree BLUP (pBLUP) and single-step genomic BLUP (ssGBLUP) analyses

Validation type	pBLUP	ssGBLUP	Gain
5-Fold cross-validation			
Theoretical reliability	0.49 ± 0.002	0.51 ± 0.002	0.02
Observed reliability	0.42 ± 0.005	0.56 ± 0.005	0.14
Split forward validation			
Theoretical reliability	0.50 ± 0.002	0.56 ± 0.001	0.06
Observed reliability	0.35 ± 0.020	0.61 ± 0.030	0.26

[1] Theoretical reliabilities estimated using the method developed by VanVleck (1993), and observed reliabilities estimated using the square of Pearson correlation between breeding values for the full (available phenotype) and masked (missing phenotype) analyses.

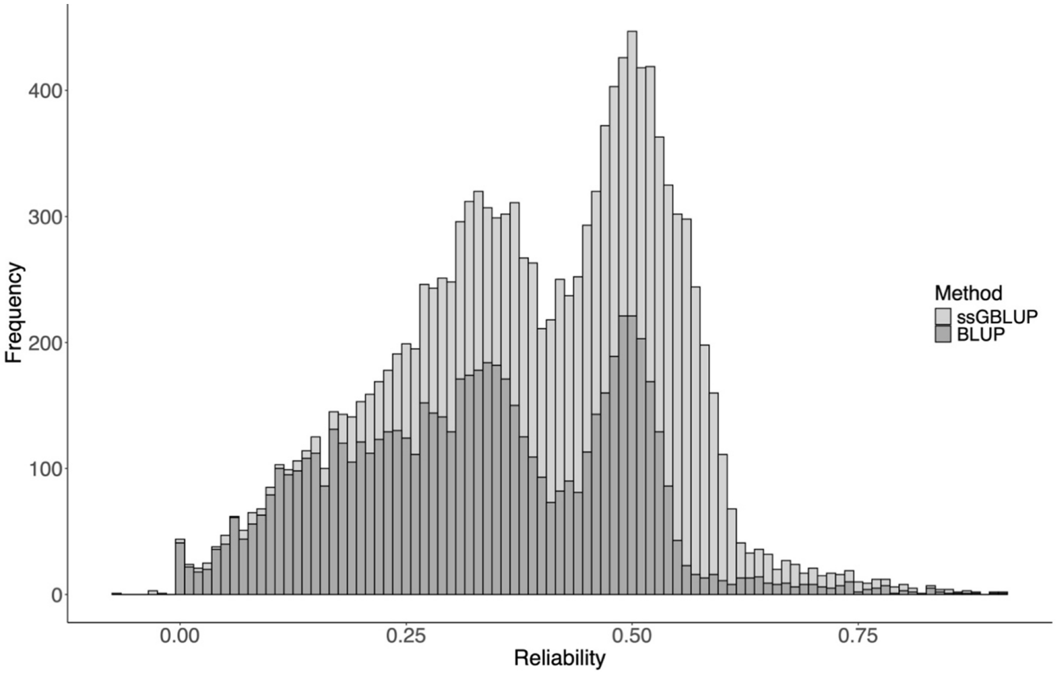

Figure 2. Comparing reliabilities of breeding values predicted using pedigree information (BLUP) and breeding values predicted incorporating genomic information (ssGBLUP) for genotyped animals (n = 6,631).

Similarly, the split forward validation demonstrated high theoretical and observed reliabilities for unphenotyped heifers, with a significant gain seen with the inclusion of genomics (Table 6). The average theoretical reliability for masked heifers when phenotypes were included was 0.50 ± 0.002 for pBLUP, increasing to 0.56 ± 0.005 with ssGBLUP. Observed reliability when phenotypes were masked increased from 0.35 ± 0.02 for pBLUP to 0.61 ± 0.03 for ssGBLUP.

The inclusion of genotype information increased both theoretical and observed reliabilities across validations. Higher observed reliability through ssGBLUP relative to pBLUP demonstrates the value of genomic data in improving prediction accuracy for unphenotyped cows and heifers. Additionally, observed reliabilities were higher than theoretical reliabilities for ssGBLUP in both the cross-validation and split forward validation. Although it is not typical to expect observed reliability to be higher than theoretical reliability, this can occur due to the high relatedness within the population. In the phenotyped population, ~2.8% had a full-sibling who was also phenotyped, and about 92.6% had a half-sibling who was phenotyped. Similarly, in the genotyped and phenotyped population, 4.3% had a full-sibling who was genotyped and phenotyped, and 88.6% had a half-sibling who was both genotyped and phenotyped. This high relatedness means that phenotypic information can be inferred from relatives, even if the individual's phenotype is not directly observed.

Breeding Value Correlations

Correlations between breeding values for AGD and breeding values for production and fertility traits were generally low (Table 7). Overall, correlations among breeding values for fertility traits of sires with phenotyped daughters were low to moderate. Among these fertility traits, AFS showed the highest unfavorable correlation with AGD at −0.29 ± 0.042. This result contrasts with earlier studies that reported phenotypic associations between shorter AGD and decreased age at puberty (Stephen et al., 2023; Vidal et al., 2024), as well as an in-

Table 7. Correlations of relative breeding values for AGD with production and fertility traits for sires of phenotyped daughters[1]

Trait	Sires of phenotyped daughters (n = 912)
Production	
305-d milk yield	−0.27
305-d fat yield	−0.33
305-d protein yield	−0.34
Fertility	
Age at first service	−0.29
First service to conception (heifers)	−0.11
First service to conception (cows)	0.02
56-d nonreturn rate (heifers)	−0.10
56-d nonreturn rate (cows)	−0.01
Calving to first service	0.09

[1] Correlations of genomic estimated breeding values or genomic relative breeding values were used if genomic information was available; estimated breeding values or relative breeding values were used otherwise (correlation SE ranged from 0.030 to 0.043).

creased rate of pregnancy by 450 d of age (Carrelli et al., 2021). These studies suggested that shorter AGD could be positively linked to earlier reproductive maturity and improved pregnancy outcomes. However, the moderate unfavorable correlation observed here between AGD and AFS hints at the possibility of a more complicated relationship. Current research suggests that AGD may be age-dependent in growing heifers, potentially increasing in length up to ~16 mo (Rajesh et al., 2022). If AGD is indeed highly variable in early stages of development, it could be less reliable as a fertility indicator in early life, which might contribute to the observed unfavorable relationship to heifer fertility. Additionally, AFS could be affected by various management practices, such as the requirement for heifers to achieve a specific percentage of their mature body weight before their first breeding (Duplessis et al., 2015), potentially obscuring any direct connection between AGD and age at puberty.

Low correlations were estimated between AGD and the remaining fertility traits, FSTC, NRR, and CTFS, for cows and heifers. For FSTCh, the correlation was −0.11 ± 0.037 for sires, indicating a low correlation. Similarly, the correlation for FSTCc was 0.02 ± 0.033 for sires, indicating no relationship with AGD. For NRRh, the correlation was −0.10 ± 0.036 for sires, suggesting a small unfavorable relationship between NRRh and AGD. However, the correlation for NRRc was close to zero at −0.01 ± 0.033 for sires. The correlation for CTFS was 0.09 ± 0.030 for sires, indicating a low favorable correlation. Low correlations suggest that AGD is not a strong predictor of FSTC, NRR, or CTFS, which might limit its utility as a selection criterion for improving postpartum cyclicity or conception. However, given the low heritability of these traits in both cows and heifers, more data may be necessary, and estimation of genetic correlations using multiple trait models should be carried out before definitive conclusions are drawn.

Correlations among breeding values for production traits were moderate for sires of phenotyped daughters (Table 7). The correlation for MY was moderate for sires (−0.27 ± 0.041). This indicates that a decrease in AGD could correlate with decreased milk production. Similarly, a previous study by Carrelli et al. (2022) reported a weak unfavorable phenotypic association between MY and AGD but concluded that the fertility gains would outweigh the reductions in milk production (5% vs. 0.17%). Similarly, correlations for FY (−0.33 ± 0.043) and PY (−0.34 ± 0.043) were moderate for sires. These findings indicate that although there are some unfavorable associations between AGD and milk components, the relationships are not strong enough to make AGD a difficult trait for selection. However, understanding these correlations can help in developing balanced breeding strategies that consider both fertility and production goals.

Although earlier studies suggested an antagonistic relationship between production and fertility in dairy cows (Lucy, 2001; Windig et al., 2006; Walsh et al., 2011), this view is increasingly seen as nuanced. The current understanding is that improvements in MY due to rapid genetic selection have led to increased metabolic demands and management challenges. If these demands are not adequately met, negative impacts on health and fertility may occur. However, in well-managed herds, there is no clear association between higher MY and reduced fertility (LeBlanc, 2013). This suggests that if AGD is confirmed as a reliable fertility indicator, it is likely that selecting for improved fertility traits such as AGD can be achieved without adversely affecting milk production.

It is important to note that these results are based on breeding value correlations, not estimated genetic correlations. Breeding value correlations reflect the shared variance due to selection rather than direct genetic relationships. Therefore, any conclusions drawn from these results should be considered preliminary. More definitive conclusions should await genetic correlations that can only be adequately estimated when more data becomes available.

CONCLUSIONS

In conclusion, anogenital distance shows notable genetic potential as a trait in dairy cattle. The study reports a moderate heritability estimate and moderate to high reliability of breeding values for anogenital distance relative to conventional fertility traits. Incorporation of genomic information resulted in substantial gains for young unproven animals with lower gain found for more proven animals. Cross-validation results indicated gain in reliability of breeding values for unphenotyped heifers when genomic information is included. Correlations of breeding values were weak to moderately unfavorable for fertility and production traits. Caution is warranted in immediate application of anogenital distance, as further research is needed to explore the genetic potential of anogenital distance across diverse management systems and validate its long-term effectiveness in improving reproductive efficiency. Future studies should focus on expanding phenotype data, particularly across lifetimes and across breeds, and on estimating genetic correlations with other traditional reproduction and production traits using multiple trait models.

NOTES

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Nonstandard abbreviations used: AFS = age at first service; AGD = anogenital distance; CTFS = calving to first service; FSTC = first service to conception; FSTCc = FSTC in cows; FSTCh = FSTC in heifers; FY = fat yield; GPBV = genomic proof breeding value; GRBV = genomic relative breeding value; HYS = herd, year, and season of birth; MY = milk yield; NRR = nonreturn rate; NRRc = NRR in cows; NRRh = NRR in heifers; pBLUP = pedigree BLUP; PBV = proof breeding value; PY = protein yield; RBV = relative breeding value; ssGBLUP = single-step GBLUP.

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