The utility of age at puberty and anogenital distance as early-in-life predictors of an animal's genetic merit for fertility during lactation in New Zealand dairy cattle

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in

Animal Science

AL Rae Centre of Genetics and Breeding, Massey University,

Manawatū, New Zealand.

Melissa Anne Stephen

2023

ABSTRACT

Background. The New Zealand (NZ) dairy sector is predominantly pasture-based, with low-cost farm systems heavily dependent upon good alignment between the herd's feed demand and the seasonal supply of pasture. The feed demand of the herd varies across the season according to lactational performance and the physiological status of the cows. Therefore, management of the timing and spread of reproductive events are critical drivers of farm profit. Cows in NZ normally calve annually between July and September each year to ensure that the peak feed demand of the herd coincides with increased spring pasture growth. Adhering to a strictly annual calving interval is biologically challenging for dairy cows, and at least 10% of the national herd is culled each year due to reproductive failure. Selection for fertility provides a long-term tool that can genetically improve the reproductive performance of the national dairy herd. Gains in reproductive performance can contribute to improved pasture utilization, days in milk, cow longevity and, ultimately, farm profitability. Unfortunately, evaluating traits that represent reproductive success during lactation is challenging. The calving, breeding, and pregnancy date phenotypes required for evaluation typically have low heritabilities and are expressed relatively late in an animal's life, when cows are at least two years of age. Higher heritability traits that exhibit at least moderate genetic correlations with target fertility traits can have value as predictor traits, especially if they are expressed earlier in life than the target trait itself. Two candidate predictor traits for evaluating genetic merit for fertility during lactation are age at puberty (AGEP) and anogenital distance (AGD).

Objectives. There were four key objectives of this thesis. First, to investigate a cost-effective approach for measuring AGEP for the purpose of genetic evaluation. Second, to estimate the heritabilities of both AGEP and AGD in NZ Holstein-Friesian cattle, and third to estimate the genetic correlations between fertility during lactation and both AGEP and AGD. Finally, I aimed to undertake a Genome-Wide Association Study (GWAS) to identify genomic regions associated with variation in each of these candidate predictor traits.

Materials and Methods. I used data from a study population of 5,010 predominantly Holstein-Friesian and Holstein-Friesian cross Jersey cows, born in 2018 across 54 commercial pasture-based dairy herds. Elevated blood plasma progesterone (BP4) concentrations were used as an indicator of an animal's puberty status, with animals considered post-pubertal once their BP4 was >1 ng/mL. Each animal was blood tested on three occasions, when the average age of the animals in their herd cohort was around 10, 11 and 12 months of age. These age at first BP4 elevation (AGEP4) phenotypes (n = 4,688) are an example of a censored phenotype, as each animal's phenotype was only known to fall within a lower or upper bound, rather than being known precisely. Anogenital distance was measured at about 11 months of age (AGD1; n = 4,688) as the distance between the anus and the clitoris using digital calipers. These animals

were subsequently followed through first and second lactation, and binary calving (calved in the first 42 d of the seasonal calving period; first n = 4,327; second n = 3,575), breeding (bred within the first 21 d of the seasonal breeding period; first n = 4,111; second n = 3,507) and pregnancy (pregnant within the first 42 d of the seasonal breeding period; first n = 3,939; second n = 3,353) rate traits were recorded. A second measure of AGD was taken when the animals were around 29 months of age (AGD2) in a subset of herds (n=17; 1,956 animals).

Results. Overall, variance parameter and breeding value estimation for AGEP4 were remarkably robust to phenotype censoring, and reducing the blood testing regime down to a single BP4 test per animal may be sufficient for the purpose of genetic analysis. I used Markov chain Monte Carlo (MCMC) techniques applying a single site Gibbs sampler to obtain samples from the posterior distributions of (co)variance parameters between AGEP4, AGD and fertility during lactation. The AGEP4 trait had a moderate heritability with a posterior mean of 0.34 and 90% of estimated samples falling within a credibility interval (90% CRI) of 0.30 to 0.37. The heritabilities of AGD were slightly lower at 0.23 (90% CRI 0.20 to 0.26) and 0.29 (90% CRI 0.24 to 0.34) when measured at 11 months and 29 months of age, respectively. Calving, breeding, and pregnancy rate traits exhibited moderate genetic correlations with AGEP4 (0.11 to 0.60), AGD1 (0.19 to 0.52) and AGD2 (0.46 to 0.63). The GWAS analysis of AGEP identified 1 genomic window on chromosome 5 that was associated with variation in AGEP4. Another 4 regions of decreasing importance, located on chromosomes 14, 6, 1 and 11, were identified with suggestive associations with AGEP4. In addition, 2 regions on chromosome 20 and 13 were suggestively associated with variation in AGD1, but there were no associations with AGD2, possibly because this trait was measured in fewer animals.

Conclusion. I conclude that both AGEP and AGD are moderately heritable traits, which likely have value as early-in-life genetic predictors for reproductive success during lactation in NZ Holstein-Friesian and Holstein-Friesian cross Jersey cattle.

ACKNOWLEDGEMENTS

I am profoundly grateful for the support that I have received from my supervisors, co-authors, employer, colleagues, family, and friends during the course of my PhD.

To Dorian, Claire and Jennie, thank you for making time in your busy lives for me and my education, I could not have asked for a better team of supervisors. Dorian, it has been an absolute privilege to have you as my primary supervisor. Thank you for your teaching, advice and always being available to answer my questions. Claire, thank you for helping me keep my mind on the big picture, for guiding me through the internal reporting requirements that came with this PhD and for always looking out for me and my well-being. Jennie, thank you for your insights, you have a wealth of experience in cattle breeding across NZ and Australia and I greatly appreciate your contribution to my thesis.

I am thankful to NZAEL and DairyNZ, who have been my employers throughout my PhD journey. Thank you to the DairyNZ academic committee, and especially Susan Stokes for your steadfast support and encouragement. A special thanks to my managers during this time, Brian Wickham and then Andrew Fear at NZAEL, and David McCall and then Bridget Maclean at DairyNZ.

I wish to acknowledge that this work was a piece of a much larger research initiative 'Pillars of a Competitive and Responsible Dairy System: Improved Longevity and Reproductive Performance', funded by a partnership between NZ dairy farmers through DairyNZ Incorporated and the NZ Ministry of Business, Innovation and Employment (contract DRCX1302). Additional funding was provided under the DairyNZ-funded Resilient Dairy project – part of the LIC, DairyNZ, and the Ministry for Primary Industries "Innovative breeding for a sustainable future' programme.

My research would not have been possible without the generous support of the 54 herd owners and their graziers who provided access to their 2018-born dairy animals for sampling and measurements between 2019 and 2022 as part of the Puberty-at-Scale (PS) study. I would also like to thank the DairyNZ technical team and the participating veterinary clinics (VetEnt, Anexa FVC Morrinsville and Oamaru Vets) for sampling, lab analyses, and data collection. In particular, thank you to Nicole Steele, Chris Burke and Susanne Meier in the DairyNZ science team. Nicole, your attention to detail with data collection and storage made my job so much more enjoyable. Susanne and Chris, thank you for the provision of the Fertility Research Herd dataset for use in my thesis.

Finally, I would like to thank my family and friends, you have been everything I needed, and I am so fortunate to have you in my life.

PREFACE

I am providing this preface to explain the layout, formatting, and publishing of this thesis. I have written this thesis in the format of '*PhD by publication'*, and so my results chapters are presented in the format of a journal article. Each results chapter is written to be a stand-alone journal article, and so there is some repetition within the methods, discussion, and references sections. Hence, the formatting aligns with the requirements of the intended journal. For consistency I have used the *Journal of Dairy Science* referencing style for all chapters. Chapter 1 (literature review) and Chapter 7 (general discussion) have not been written as journal articles, but for these chapters, I have chosen to use the formatting required for publication in the *Journal of Dairy Science*. The figures and tables are numbers and labels as per the journal manuscript, but for the purpose of the Tables of Figures and Tables I have included the chapter number. For example, Table 1.1 refers to Table 1 in Chapter 1.

I was not directly involved with experimental design, enrolling herds, or measuring phenotypes. I collated and analysed phenotype data collected in the 'Puberty-at-Scale' trial, and the 'Fertility Research Herd' trial, both of which were part of the 'Pillars of a Competitive and Responsible Dairy System: Improved Longevity and Reproductive Performance' research programme. I selected the analytical approaches, completed all statistical analysis, interpreted the results, and wrote the manuscripts, under the guidance of my supervisors and co-authors.

The following section details the publication status of each chapter.

Chapter 1: **Literature Review.**
Unpublished June 2023.

Chapter 2: **Variance parameter estimation for age at puberty phenotypes under two levels of phenotype censorship.** M. Stephen, S. Meier, M. Price, J.E. Pryce, C. Burke, C. Phyn, D. Garrick.
Published in *JDS Communications*, 2022, 3(5), 339-342
https://doi.org/10.3168/jdsc.2022-0218

Chapter 3: **Comparison of methods for deriving phenotypes from incomplete observation data with an application to age at puberty.** Melissa A Stephen, Chris R Burke, Jennie E Pryce, Nicole M Steele, Peter R Amer, Susanne Meier, Claire VC Phyn, Dorian J Garrick.
Published in *Journal of Animal Science and Biotechnology*, 2023, 14(1), 119
https://doi.org/10.1186/s40104-023-00921-5

Chapter 4: **Estimating Heritabilities and Breeding Values from Censored Phenotypes Using a Data Augmentation Approach.** Melissa Stephen, Hao Cheng, Jennie Pryce, Chris Burke, Nicole Steele, Claire Phyn, Dorian Garrick.
Published in *Frontiers in Genetics,* 2022, 13
https://doi.org/10.3389/fgene.2022.867152

Chapter 5: **Genome-Wide Association Study of age at puberty and its (co)variances with fertility and stature in growing and lactating Holstein-Friesian dairy cattle.** M.A. Stephen, C.R. Burke, N. Steele, J.E. Pryce, S. Meier, P.R. Amer, C.V.C. Phyn, D.J. Garrick.
In review with the *Journal of Dairy Science, 2023*.

Chapter 6: **Genome-Wide Association Study of anogenital distance and its (co)variances with fertility in growing and lactating Holstein-Friesian dairy cattle.** M.A. Stephen, C.R. Burke, N. Steele, J.E. Pryce, S. Meier, P.R. Amer, C.V.C. Phyn, D.J. Garrick.
Published in *Journal of Dairy Science*, May 2023.
https://doi.org/10.3168/jds.2023-23427

Chapter 7: **General Discussion.**
Unpublished June 2023.

TABLE OF CONTENTS

TABLE OF FIGURES

TABLE OF TABLES

ABBREVIATIONS

AGD: Anogenital distance

AGD1: Anogenital distance measured when animals are approximately 12 mo

AGD2: Anogenital distance measured when animals are approximately 26 mo

AGEP: Age at puberty

AGEP4: Age at first elevation (> 1ng/mL) in blood plasma progesterone

AGEP4aug: A data augmentation method

AGEP4av: Age on visit method

AGEP4cat: Visit category method

AI: Artificial Insemination

BP4: Blood plasma progesterone

CR42: A binary trait denoting an animal's success (1) or failure (2) to calve within the first 42 days of the herds seasonal calving period.

CRI: Credibility interval

d: Day

E: Early

E/M/L: Early, Mid or Late

EBVs: Estimated Breeding Values

EL: Early and Late

EM: Early and Mid

FRH: Fertility Research Herd

GWAS: Genome-Wide Association Study

HF: Holstein-Friesian

J: Jersey

L: Late

M: Mid

MCMC: Markov Chain Monte Carlo

ML: Mid and Late

mo: Month

N: No

n: Number

PB21: A binary trait denoting an animal's success (1) or failure (2) to be presented for breeding within the first 21 days of the herds seasonal breeding period.

PR42: A binary trait denoting an animal's success (1) or failure (2) to become pregnant within the first 42 days of the herds seasonal breeding period.

SD: Standard deviation

SE: Standard Error

SNP: Single Nucleotide Polymorphism

XB: Cross-breed

Y: Yes

yr: Year

90CRI: The 5th and 95th percentile of a results vector

CHAPTER 1. Literature Review

Unpublished, June 2023

Introduction

The fertility of dairy cows is crucial for the economic success of dairy farm systems, particularly in countries where grazed pasture is the primary source of feed. Farming systems that are reliant on pasture experience seasonal fluctuations in feed supply, and strategic timing of the calving period enables farmers to match the feed demand of the herd with pasture growth (Macdonald and Roche, 2023). To achieve this, farmers aim for a condensed distribution of calving dates in late winter/early spring each year, which requires a strictly annual calving interval (Macdonald and Roche, 2023). Individual cows that fail to conceive in a timely manner and maintain a 365-d interval will calve later in the season. Late-calving cows that are retained will disrupt the balance between feed supply and demand and have limited days in milk (Garcia and Holmes, 1999); however, late-calving cows that are culled will increase the replacement rate, as will culling of cows that fail to become pregnant during the seasonally concentrated breeding period.

Genetic selection offers a means for farmers to improve the long-term fertility performance of their herd and increase the proportion of cows inherently capable of achieving an annual calving interval. That said, selection for improved reproduction and fertility traits is hampered by the low heritability and relatively late-in-life expression of targeted traits such as early calving date. Higher heritability traits that are expressed earlier-in-life and exhibit moderate to high genetic correlations with target fertility traits could provide value as predictor traits in the genetic evaluation of fertility.

In this review, I have two objectives: first, to provide an overview of the importance of fertility to seasonal, pasture-based dairy farming systems while highlighting current approaches to genetic evaluation of fertility; and second, to conduct a focused review of the utility of age at puberty (AGEP) and anogenital distance (AGD) as early-in-life predictor traits for reproductive success during lactation.

Importance of cow fertility and herd reproductive performance

It is essential for dairy cows to calve regularly to maintain milk production throughout their lifetime (Louca and Legates, 1968). Therefore, the ability of a cow to conceive is a vital aspect of its survival in a dairy herd. This is particularly true in New Zealand (NZ) where temperate climatic conditions mean that pasture-based diets are prevalent, and reliable reproductive performance is essential not only for sustaining milk production but also for synchronizing the herd's feed demand with seasonal fluctuations in pasture growth. Grazed pasture provides a cost-efficient source of feed for dairy cattle and so farmers who can

maximize the amount of pasture eaten per hectare tend to be more profitable (Neal and Roche, 2020; Macdonald and Roche, 2023). However, the seasonal nature of pasture growth means that farmers must control the feed demand of the herd. Nutritional requirements of cows vary according to their physiological state. For example, by stage of lactation, with a peak in feed intake occurring shortly after calving (Macdonald et al., 2008). Pasture-based farmers can manipulate the seasonal feed requirements of the herd by manipulating the timing and distribution of calving dates and the length of the lactation period (Macdonald and Roche, 2023). First, farmers ensure that the herd calves annually during late winter and early spring (July to September), targeting a condensed distribution of calving dates, which helps align the herd's peak in feed demand with abundant spring pasture growth. Second, in pasture-based farm systems, cows in the herd are generally dried-off by a fixed date, with the dry-off date being largely determined by pasture and supplementary feed availability, body condition score, and an allowance for a sufficient dry period (such as 60 d) before calving in the following season rather than the cow's production level *per se* (Macdonald et al., 2010). The profitable implementation of both management strategies depends on the reproductive performance of the herd.

Calving annually is somewhat biologically challenging for dairy cattle, because after a gestation of about 280 d (Norman et al., 2009), each animal has a relatively short window of just 85 d to resume normal estrous cycles and become pregnant again. Cows with extended intervals between calving and pregnancy will calve later the following season or are at increased risk of failing to become pregnant before the end of the restricted, seasonal breeding period. Non-pregnant cows are generally culled from the herd prior to the following season (Verkerk, 2003). However, late-calving cows compromise the alignment between feed supply and demand and, in the context of a fixed dry-off date, they have shorter, less profitable lactations. When a cow fails to maintain a 365-d calving interval, farmers are faced with a difficult decision. They can either use hormonal or management interventions to help ensure the cow is bred earlier in the seasonal breeding period and subsequently bring the cow's calving date back within the desired calving period, or alternatively farmers must prematurely cull what may be an otherwise healthy and productive animal. Neither of these options are desirable, as they incur economic, environmental, and social costs.

An example of a common hormonal intervention is the administration of progesterone using an intrauterine device, such as a CIDR® (Macmillan and Peterson, 1993). These devices can be effective at inducing ovulation and normal luteal function in anestrous (non-cycling) cows (Rhodes et al., 2003), but such interventions introduce costs into a farm system and may not be well accepted by health-conscious consumers. Management interventions generally involve reducing stress on cows' postpartum, to improve energy balance and support body condition gain (Rhodes et al., 2003). For example, farmers may choose to reduce the frequency of milking (e.g., from twice-a-day to once-a-day) or preferentially feed at-risk animals between

calving and breeding (Rhodes et al., 2003). Although these strategies can be effective, results can be unreliable and they may be associated with advantages and/or disadvantages to other aspects of the farming business; for example, once-a-day milking reduces time spent on milking-related tasks but also decreases milk production (Edwards, 2018).

Culling cows who are not pregnant, or are likely to calve late in the season, is a widely used strategy for mitigating the cost of reproductive failure in dairy cows (Kerslake et al., 2018; Macdonald and Roche, 2023). Fertility-related (e.g., late calving or non-pregnant) culls comprise over a third of the culling events recorded annually in NZ dairy cattle (Kerslake et al., 2018). This represents a loss of at least 300,000 cows each year, which is roughly 7% of the NZ national herd (DairyNZ and LIC, 2022). Although some annual culling is desired to facilitate genetic improvement in a dairy herd, it is preferable if farmers can select the cows that they wish to cull. Culling due to reproductive failure can be categorized as involuntary culling, as farmers have little choice over whether a cow can remain in the herd. Fertility success during lactation is a low heritability trait, meaning that selection based on phenotype (i.e., culling cows due to poor fertility performance) is unlikely to result in meaningful genetic improvement. Hence, fertility-related culling is an example of substantial wastage within the farm system that contributes to significant economic losses to the NZ dairy sector of over $7 million per annum (Kerslake et al., 2018). Moreover, consumers increasingly demand that agricultural production systems utilizing livestock ensure the animals have quality of life (Tonsor and Olynk, 2011), and premature culling does not align well with this philosophy.

The NZ dairy sector has had a long-standing target for 78% of the national herd to become pregnant within the first 42 d of the seasonal breeding period (known as the 6-week in-calf rate), with minimal hormonal interventions (Brownlie et al., 2014). This target is somewhat aspirational, given that the mean 6-week in-calf rate has remained around 67% between the 2011/12 and 2021/22 seasons (DairyNZ and LIC, 2022), but recognizes the enormous cost of reproductive failure to the NZ dairy industry. Addressing poor herd reproductive performance and reducing the proportion of cows that are non-pregnant or late-calving requires a multi-factorial approach to identify areas for improvement. These factors include: managing growth and health of young replacement animals; maintaining a condensed annual calving pattern; management of cow health, nutrition, and body condition score; estrus detection during the breeding period; mating practices, including bull management; and finally, the genetic merit of the herd (McDougall et al., 2014). In this thesis, I focus on the use of genetic selection to improve cow fertility.

Genetic selection for improved fertility

Genetic selection provides an important tool to improve the inherent fertility of dairy herds, strengthening resilience to suboptimal management, and reducing reliance on hormonal interventions. Unfortunately, fertility traits tend to have low heritabilities (<0.10; [Berry et al., 2002; Brotherstone et al., 2002; Bowley et al., 2015]), and this limits the rate of genetic progress that can be achieved in these traits. A low heritability indicates that a relatively small proportion of the variance observed in a trait is explained by the genetic merit of individuals and, in practice, this means that a larger quantity of phenotypes must be measured to obtain accurate estimated breeding values (EBVs; [Gonzalez-Recio et al., 2014]). Low heritability traits are challenging but not impossible to improve by selection and, in the case of fertility, the negative implications of a low heritability on genetic gain are partially offset by the scale of the genetic variance in fertility traits, as large differences in genetic merit exist within selection candidates (for example, Meier et al. [2021]), which provides good scope for genetic selection.

Despite the low heritability of commonly used breeding, calving and pregnancy-related traits, failing to consider fertility in a dairy breeding program can have dire consequences, as reproductive success becomes more challenging as milk production increases (Grosshans et al., 1997; Berry et al., 2014). Historically, these negative consequences have played out around the world, as selection for increased milk production is widespread, and many countries with established breeding programs did not initially include fertility traits in their selection indices (Pryce et al., 2014). Selection approaches that failed to consider fertility resulted in declining genetic trends. Those negative genetic trends and the associated decline in fertility phenotypes were arrested, and in many cases reversed, when fertility EBVs were directly and routinely incorporated in selection decisions (Pryce et al., 2014).

National selection index

In the NZ dairy sector, the national breeding objective (NBO) is to breed cattle whose progeny are efficient converters of feed into profit, within the context of a seasonal, pasture-based farm system (Harris, 2005). This objective is represented by the selection index ‘Breeding Worth’ (BWSI). The BWSI currently incorporates milk production (milk fat, milk protein, milk volume), somatic cell score (SCC), body weight (BW), body condition score, survival, udder overall, gestation length, and fertility (DairyNZ, 2023a). These traits have been selected as they have a measurable economic value to NZ dairy farmers, and their weighting within the BWSI is determined according to their economic value. The BWSI is revised on an annual basis, with the intention of being responsive to the changing economics and requirements of the dairy sector. The BWSI replaced an earlier selection index, called the breeding index (BI), in 1996 (Harris, 2005). The BI was initially focused exclusively on milk fat (Wickham et al., 1978), but that

selection approach later evolved to include milk protein and a penalty against milk volume (Harris, 2005). The introduction of the BWSI represented a move towards a more comprehensive selection approach, with non-production traits incorporated based on their economic value to farmers. Importantly, the first iteration of the BWSI included BW (Harris, 1998), recognizing that cows who weighed less have lower feed requirements for maintenance. Over time, more non-production traits have progressively been added, bringing us to the current 10-trait BWSI that NZ dairy farmers use today. The fertility trait was incorporated into the BWSI in the early 2000's (Harris, 2005; Pryce et al., 2014), and this was a particularly notable addition, as reproductive performance is a critical component of farm profitability in seasonal, pasture-based farm systems. The two major dairy cattle breeds in NZ are Holstein-Friesians and Jerseys. Jerseys tend to have higher fertility EBVs than their Holstein-Friesian counterparts; however, prior to the inclusion of fertility in the BWSI, the genetic trend for fertility in the national herd was negative for both breeds (Figure 1.1). This negative genetic trend was probably a result of the antagonistic relationship exhibited between fertility and milk production (Harris et al., 2005; Jayawardana et al., 2023), as the latter had been under direct selection for decades. The introduction of fertility into the BWSI lessened this negative genetic trend although the gains in fertility remain poor (Figure 1.1).

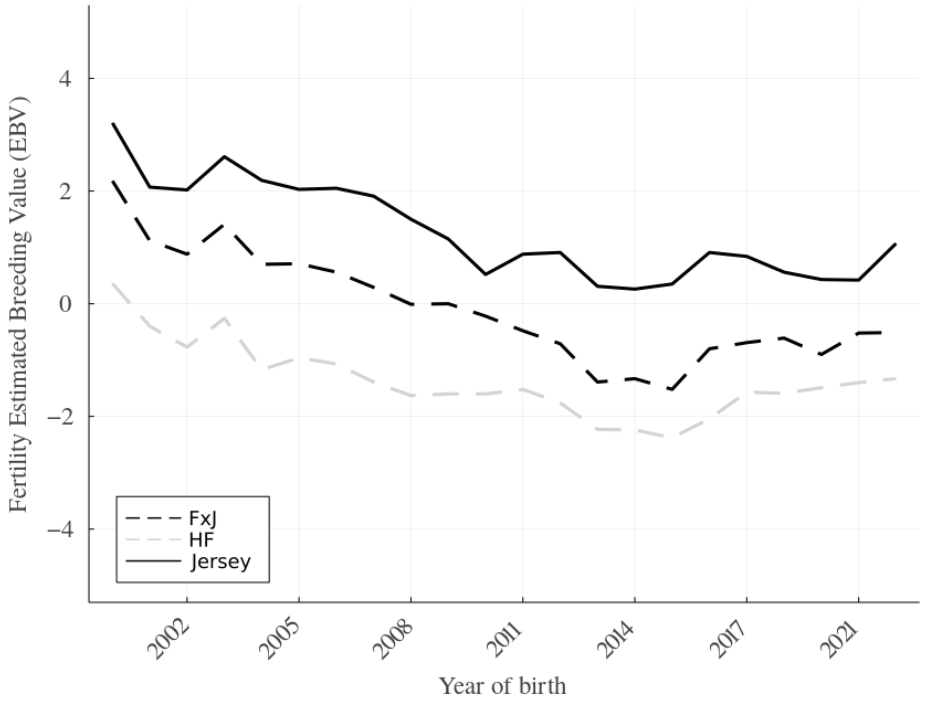


Figure 1.1. Genetic trend in fertility for Holstein-Friesian (dashed, grey), Jersey (solid) and Holstein-Friesian cross Jersey (dashed, black) bulls enrolled in the animal evaluation system in New Zealand (DairyNZ, 2023b).

Target fertility traits

The over-arching goal for fertility improvement within a seasonal farm system is to have cows that achieve long productive lives by repeatedly navigating the reproductive challenges associated with an annual calving regime. In a perfect world, we would gather information over a cow's lifetime, and selectively breed from sires whose daughters' productive lives were not truncated due to reproductive failure. Unfortunately, there are several negative implications that arise from traits expressed relatively late in life. First, establishing the lifetime fertility profile of a sire's daughters before widely using the sire would result in an extended generation interval for the fertility trait. This is particularly important in the context of a pedigree-based evaluation, where the accuracy of EBVs depends on the quantity of phenotypes measured on animals and their close relatives (i.e., their offspring). Second, it is difficult to make fair comparisons between animals across lifetime performance. For example, an animal that experiences reproductive failure in first lactation and is consequently culled, is not given the opportunity to express her fertility phenotype in subsequent lactations. This pre-selection increases with each lactation and can create bias in EBVs. Finally, the ongoing accuracy of genomic evaluations depends on the frequent 'refreshing' of the reference population, as recombination will erode the linkage between single nucleotide polymorphisms (SNP) markers and quantitative trait loci (QTL) across generations (Johnsson et al., 2022). A phenotype that is expressed late-in-life delays this refresh process and may limit the accuracy of genomic evaluations for younger generations of animals. Both the interval between generations and the accuracies of EBVs are key drivers of the rate of genetic gain in a trait (Rendel and Robertson, 1950), and so the value of a phenotype that is expressed late in life (i.e., lifetime fertility performance) must be weighed against the cost of compromising generation interval and EBV accuracy.

Furthermore, most countries implement a selection index approach (such as the BWSI) that combines several traits according to their economic (and sometimes perceived) value (Miglior et al., 2017), so selection for fertility does not occur in isolation. This approach becomes more problematic when traits within an index exhibit antagonistic genetic covariances, or there is a differential between heritabilities, or both, which is the case for fertility and milk production traits (Grosshans et al., 1997; Berry et al., 2014). In NZ, milk testing is widespread (DairyNZ and LIC, 2022), with milk production phenotypes readily available soon after a sires' daughters begin their first lactation. Accuracy of selection is a key driver to selection response and so to maintain balanced genetic progress it would be ideal to obtain fertility phenotypes during or before a cow's first lactation.

The phenotype that has been chosen to represent fertility within the national selection index in NZ is the day of calving, relative to the date that the seasonal calving period began (calving season day; CSD) in each herd (Bowley et al., 2015). In addition, a binary breeding

rate trait, denoting success or failure to be bred within the first 21 d of the herd's seasonal breeding period, is used as a predictor of calving date. Calving dates that initiate first to fourth lactation contribute to the fertility EBV, although the published EBV represents CSD in second to fourth lactation. First lactation CSD was incorporated into the EBV as a predictor trait relatively recently (Stachowicz et al., 2021), primarily due to the timing of phenotype expression. Prior to 2022, the NZ dairy industry used a binary calving date trait, denoting success or failure to (re)calve within the first 42 d of the herd's seasonal calving period (CR42) in second to fourth lactation. Although heritabilities of CSD and CR42 traits were similar (Harris et al., 2006; Stachowicz et al., 2021), EBVs produced using the CSD trait and incorporating CSD in first lactation appeared to improve the predictive ability of fertility EBVs (Stachowicz et al., 2021).

Other countries, such as the UK and Australia, use calving interval as their target trait, which is measured as the interval from first to second calving, second to third calving etc. (Brotherstone et al., 2002; Pryce et al., 2013). Calving interval traits have the benefit of capturing the animal's ability to re-calve within a 365-d interval, but they have the disadvantage of not being expressed until an animal's second calving. Furthermore, calving date traits better describe an animal's ability to adhere to a strictly seasonal calving pattern, which is necessary in the pasture-based systems that dominate the NZ dairy sector. If calving interval is the target trait, a multi-trait approach, which incorporates pregnancy or calving rate phenotypes can improve the timeliness of phenotype measurement. For example, the Australian dairy sector includes 6-week pregnancy rate as a predictor within their fertility evaluation (Pryce et al., 2013). At present, pregnancy rate or timing of conception traits are not included in the fertility EBVs in NZ.

There are several important limitations to using a breeding, calving or pregnancy date-based trait as the target for fertility selection. First, breeding, calving and pregnancy dates are each low heritability traits (Dearborn et al., 1973; Stachowicz et al., 2021). Second, although these fertility traits are often recorded routinely, there is a high incidence of missing data within contemporary groups, as animal's that fail to become pregnant, or become pregnant late in the breeding season, are generally removed from the herd before the subsequent calving period, and so their reproductive failure is not directly captured in a calving phenotype. Third, calving dates are expressed relatively late in a cow's life.

Calving date: A low heritability trait

Calving date, and related traits such as calving interval or calving rate (a binary trait indicating whether a cow calved within a certain timeframe or not), are low heritability traits (Pryce et al., 2000; Harris et al., 2006; Bowley et al., 2015), which means that there is only a weak relationship between phenotype and genotype and, therefore, reliable predictions of sire

merit need a large number of recorded daughters (Mrode, 2005). Calving date traits represent a composite phenotype that relates to performance across a wide range of fertility-related events. For example, animals must attain puberty, produce quality oocytes, exhibit estrus, be mated artificially or by a fertile bull in a timely manner, achieve conception and implantation, maintain a hospitable uterine environment for a developing fetus, calve safely, quickly resolve any postpartum uterine disorders, and then rapidly return to normal estrous cycles, ready for the next breeding season. At each of these stages, the underlying biological mechanisms will vary, and so broad selection for the end-stage phenotype of calving date is unlikely to capture the subtleties of each biological process. This lack of clarity of selection can contribute to a low heritability, as the genetic signal gets lost across so many competing influences. As a simplified example, a subset of cows may calve early because they have a high conception rate, whereas others calve early due to a high submission rate, but those with both poor conception and submission rates will calve late. Disentangling the effects of these two contributing factors alone is complex, and there are many more interactions in the underlying biology.

Calving date traits are also readily affected by management decisions, and management failings, and this further contributes to the low heritability of these traits. Some examples of management decisions that improve calving rate within a herd include use of estrus detection aids, preferential feeding of cows that are deemed to be at risk of conceiving late in the breeding period, reducing the milking frequency of at-risk cows, and the use of hormonal interventions such as CIDRs (Rhodes et al., 2003), which stimulate estrus in otherwise non-cycling cows. If the daughters of a bull with low genetic merit for fertility are systematically managed in a different way to their herd contemporaries, and these management differences are not captured in the data or model used for genetic analysis then the genetic inferiority of the bull, and the associated contribution to genetic variation in calving rate, will not be recognized. Conversely, some management interventions can limit the reproductive success of groups of animals. One example is the use of sexed semen, which is generally manufactured to permit a lower conception rate than unsexed semen (Norman et al., 2010; Butler et al., 2014; Oikawa et al., 2019). Sexed semen is a premium product sold by breeding companies that provides a near guarantee of female off-spring, providing the breeding results in a successful pregnancy. Farmers tend to preferentially breed their highest genetic merit cows to sexed semen; firstly, because the product is expensive, and they want to maximize the chance of a successful conception, and secondly, because the purpose of using sexed semen is generally to breed replacement heifers, and farmers generally want to keep replacements from their highest genetic merit cows. In practice, this means that the daughters of high fertility bulls may be systematically bred to sexed semen, while their lower genetic merit herd mates are bred to unsexed semen. The lowered conception rate among cows bred to sexed semen may make their sires appear less fertile, such that their true superiority for fertility will not be recognized.

Missing phenotypes

When an animal fails to become pregnant or becomes pregnant late in the breeding season, they are typically removed from the herd. These fertility-related culls occur toward the end of the seasonal milking period before the start of the next calving period. The timing of these culls means that animals who perform poorly during the breeding period have missing calving phenotypes for the following lactation season. The effect of these missing phenotypes can be mitigated by assigning culled animals a fertility phenotype based on a series of assumptions. For example, if an animal was mated as a two-year-old but did not calve as a three-year-old, it can be assumed that they were either a late calving or non-pregnant cow, and a calving phenotype can be developed accordingly. However, calculating phenotypes in this way will inevitably lead to some errors, which may compromise the accuracy of fertility EBVs. In addition to missing their calving phenotypes, which are relatively simple to predict, these animals will also not have the opportunity to express fertility phenotypes in subsequent milking seasons. For example, a cow that is culled due to reproductive failure in its first lactation will not have the opportunity to succeed or fail in second lactation. It is not simple to predict what its phenotype would have been, as fertility failure in first lactation does not necessarily predict fertility failure in future lactations. This is because the fertility failure in first lactation may have occurred for a wide range of reasons that are not relevant to second lactation; for example, an unusually difficult calving, a temporary injury, an undetected estrus and consequently missed submission for breeding, or illness during the breeding period that negativity impacted the animal's fertility. In some cases, a missing phenotype can be inferred; for example, if an animal was mated, and then subsequently culled in the previous lactation, then it is logical to assign them some sort of penalty phenotype, recognizing that if they had not been culled it is likely that they would have either calved late or failed to calve. However, if the animals were not mated in the previous season (i.e., they were culled prior to that breeding period), the calving phenotypes can only be set to missing, and analyses must somehow account for these missing phenotypes to avoid bias.

Phenotypes expressed later in life

The third key limitation to using calving date as a target fertility trait is the fact that these phenotypes are expressed relatively late in an animal's life. In NZ, dairy cows are around 2 yr old at their first calving. This means that bulls are at least 5 yr old before they have daughter fertility phenotypes. The dairy cattle genetic improvement industry in NZ still largely adheres to a 'progeny testing' phenotype collection design that was optimized for pedigree-based genetic evaluation, where daughters are specifically generated for sires of interest within designated progeny testing herds. These phenotype collection schemes tend to be very expensive, and so daughter numbers are tightly controlled. Relatively low daughter numbers,

coupled with the low heritability of calving date traits means that EBV accuracies remain low even when a bull is old enough to have daughter phenotypes. This delay in accuracy should result in a longer generation interval, but, in practice, farmers are often eager to use high genetic merit young bulls, and bulls tend to be used widely based on the accuracy of the EBVs for other traits, such as milk production, while their fertility EBVs are still relatively inaccurate. The selection differential realized for fertility from selection on an index would improve if steps were taken to increase the accuracy of EBVs earlier in an animal's life.

Strategies for improving the rate of genetic gain in fertility

There are two potential approaches that could be used to mitigate the limitations of fertility traits measured during lactation and improve the rate of improvement in fertility EBVs. First, genomic selection, and second, the use of genetically correlated predictor traits that have higher heritability and/or measured earlier-in-life than breeding, calving or pregnancy-based traits.

Genomic selection

Many countries have historically selected sires using EBVs that are based on parent and progeny information, but genomic evaluation systems have become increasingly commonplace around the world since 2009 (Weller et al., 2017). Genomic prediction involves the curation of a reference population, whereby animals have both phenotypes and genotypes measured. Associations between phenotypes and genotypes in this reference population are then used to predict the genetic merit of animals that have been genotyped but do not have phenotypes. The key benefit of genomic evaluation is the ability to utilize phenotype data obtained from older animals to estimate the genetic potential of younger animals, even if they are not closely related to those potential selection candidates. In practice, this extrapolation enables breeders to make selection decisions for young animals well before they or their daughters have a phenotype measured, shortening the generation interval for selection (Weller et al., 2017). Genomic selection is particularly useful when applied to traits like fertility, where genetic progress is limited by low accuracy of EBVs. That said, the size of the genomic reference population is a key driver of the accuracy of genomic predictions, particularly for low heritability target traits like calving date (Gonzalez-Recio et al., 2014). Although genomic technology is well established, implementation in NZ has been challenging. Early attempts were subject to an inflation bias (Winkelman et al., 2015), which was damaging for farmer confidence in the genomic technology. The source of this bias is not well articulated in the literature, but it is likely related to the highly ad-mixed nature of the NZ national herd, where around 50% of animals are Holstein-Friesian cross Jersey (DairyNZ and LIC, 2022). Implementation of a

national genomic evaluation system remains the primary focus for the genetic improvement sector in NZ.

Predictor traits

In some cases, it can be beneficial to apply selection to a trait that shares a genetic correlation with a target trait, rather than the target trait itself. For example, if the target trait is difficult or expensive to measure, is expressed late in an animal's life, or has a very low heritability. The trait somatic cell count (SCC) is incorporated into several national selection indices including in NZ (Harris, 2005; Byrne et al., 2016); it provides an example of a predictor trait that is under direct selection, in favor of the target trait, which is the incidence or susceptibility to mastitis. Directly measuring an animal's susceptibility to mastitis is expensive and invasive and, therefore, not feasible across large numbers of animals using current methodologies. In addition, mastitis has a low heritability of <5% (Lund et al., 1994; Green et al., 2004). Conversely, SCC phenotypes are routinely measured during normal milk testing, and the SCC trait is moderately heritable (Lund et al., 1994; Green et al., 2004). The genetic correlation between mastitis and SCC is estimated to be close to unity, and so SCC provides an ideal predictor of mastitis.

Selection for calving date traits may also benefit from the discovery of genetically correlated predictor traits, especially if the predictor trait was moderately heritable, and could be measured earlier in life than first lactation.

The value of predictor traits in the genomic selection era

Predictor traits have a somewhat different role within a genomic evaluation system, compared with a pedigree-based evaluation system. Genomic technology reduces the reliance on the selection candidates own phenotypes to obtain a reliable EBV. Accurate EBVs that do not depend on direct or daughter phenotypes mean that selection can take place at a younger age, reducing the generation interval, without foregoing reliable rankings on the candidates. However, phenotypes remain critically important within a genomic evaluation system, and as such predictor traits can still add value. First, traits with higher heritabilities require smaller reference populations (that is, animals with both phenotypes and genotypes) than their lower heritability counterparts to achieve comparable accuracies (Gonzalez-Recio et al., 2014), which means that the reliability of genomic EBVs for a higher heritability predictor trait may exceed those of the target trait, even if animals in the reference population have phenotypes for both. Depending on the strength of the genetic correlation between the predictor and the target trait, it may be beneficial to select directly for the predictor trait, as is the case for the SCC trait as a predictor of mastitis. Although the timing of phenotype expression and measurement is less

important within the context of genomic selection, earlier-in-life phenotypes do help maintain the relevance of associations between SNP markers and phenotypic variance across successive generations. For example, recombination events that occur regularly throughout the genome compromise the linkage between SNP markers and QTL (Johnsson et al., 2022). By including phenotypes measured on young animals, the changes in linkage between SNP and QTL can be captured and incorporated into genomic EBVs for the young animals, prior to selection. Similarly, a predictor trait can potentially accelerate the detection of relevant deleterious *de novo* mutations, providing early warning of selection candidates that should be disregarded. Furthermore, increased heritabilities can improve the statistical power of a GWAS analysis, leading to an improved ability to detect QTL. Enriching a SNP chip in the region surrounding a QTL might improve the accuracy of SNP predictions (Xiang et al., 2021).

Potential predictor traits for calving date

Timely calving as a 2 yr old and then maintaining a strict 365-d calving interval represents reproductive success across a wide range of fertility-related traits. However, the successful sequence of reproductive events starts well before an animal's first mating. Along this trajectory, there are measurable traits that have potential to add value as early predictors of an animal's genetic merit for reproductive success during lactation. Age at puberty and AGD are two examples of candidate predictor traits for fertility. The remainder of this review is divided into two parts, with the first part reviewing AGEP and the second part reviewing AGD as potential predictors of fertility EBVs.

Age at Puberty

Biological link between puberty and the estrous cycle

The onset of puberty encapsulates the period in a heifer's life when they become sexually mature, insofar as they can become pregnant. Age at puberty can be specifically defined as the age at which an animal experiences ovulation and behavioral estrus, followed by normal luteal function (Moran et al., 1989). The coordination of ovulation, estrus, and luteal function involves a series of complex endocrine interactions between the hypothalamus, pituitary, and the ovaries (Figure 1.2; [Day and Anderson, 1998]). Ovaries secrete estrogen from birth, and that estrogen initially enacts consistent negative feedback on the hypothalamus (Day and Anderson, 1998). When onset of puberty is imminent, heifers begin to lose sensitivity to this negative feedback (Day et al., 1984), and estrogen instead begins to stimulate the hypothalamus to produce gonadotrophin releasing hormone (GnRH) (Day and Anderson, 1998). The underling drivers of this switch from negative to positive feedback of estrogen on the hypothalamus are not well understood, although it is well established that nutrition plays a role,

as poor nutrition will delay the onset of puberty (Short and Bellows, 1971; Handcock et al., 2021).

The GnRH stimulates the pituitary gland to secrete both follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Each of these hormones has a role to play to support follicle maturation within the ovaries. Antral follicles are recruited in waves throughout life from shortly before puberty until menopause, and those follicles initially exhibit a growth response to FSH (Chun et al., 1996). As follicles mature, they increasingly develop sensitivity to LH, which manifests as a growth response (Mihm and Bleach, 2003). At the same time, a developing follicle will begin to secrete estrogen and inhibin, which act on the pituitary to inhibit FSH (Mihm and Bleach, 2003).

Cattle are generally mono-ovulatory, and in an environment of decreasing FSH, the follicle that exhibits the strongest growth response to LH will grow the fastest and become the dominant follicle (Mihm and Bleach, 2003). As the dominant follicle grows, it secretes increasing levels of estrogen. As discussed, prior to puberty, this additional estrogen will enact negative feedback on the hypothalamus, which suppresses pituitary release of LH. In the absence of LH, the dominant follicle will experience atresia, which lifts the inhibin and estrogen driven suppression of FSH and paves the way for recruitment of a subsequent follicular wave (Mihm and Bleach, 2003).

In post-pubertal animals, elevated estrogen can switch to enact positive feedback on the hypothalamus, which further stimulates the release of GnRH and downstream release of pituitary LH. A positive feedback loop is established whereby the increasing LH stimulates the dominant follicle to increase in size and produce more estrogen (Ginther et al., 2001), which, in turn, stimulates the release of more pituitary LH (Chenault et al., 1975). Increasing levels of circulating estrogen will usually manifest as behavioral estrus, whereby cows exhibit changes in behavior that provide visual signals of their imminent ovulation. These behavior changes include standing to be mounted, seeking out other cows in estrus, seeking out bulls, an increase in activity, loud vocalizations, and mounting of other cows (Gordon, 2011).

The feedback loop between estrogen and LH is self-perpetuating, and results in pulsatile releases of LH that are of increasing frequency and amplitude. This feedback loop eventually cumulates in a high amplitude pulse of LH that catalyzes ovulation of the dominant follicle (Forde et al., 2011). In conjunction with ovulation of the dominant follicle, the corpus luteum (CL) forms from the ovulatory cells remaining on the ovary at the site of ovulation. The CL is a temporary endocrine structure that has the primary function of secreting progesterone (P4) (Reynolds and Redmer, 1999; Forde et al., 2011). This P4, in conjunction with estrogen, enacts powerful negative feedback on the hypothalamus such that synthesis of LH and FSH within the pituitary are suppressed and subsequent ovulations are temporarily prevented (Forde et al., 2011). By preventing further ovulations, the CL contributes to stability within the uterine

environment, which facilitates the establishment and maintenance of a pregnancy. If a pregnancy is successfully established, the CL will continue to secrete P4 for the duration of the gestation period. If conception fails to occur, the uterine endometrium will begin to secrete pulsatile prostaglandin, which initiates the process of luteolysis, resulting in a decrease in circulating P4 concentrations. Once the P4 concentrations return to basal levels, the negative feedback of P4 and estrogen on the hypothalamus is lifted, and the pituitary gland resumes secretion of both LH and FSH.

A normal estrous cycle for a cow has a duration of about 21 d (Forde et al., 2011) and the length is largely dictated by the function of the CL. Short luteal phases resulting in truncated estrous cycles are relatively common during the onset of puberty (Berardinelli et al., 1979). It is unlikely that the heifer would conceive to a mating during one of these truncated cycles and so although the onset of puberty is imminent, the heifer does not meet the definition of being capable of reproduction until a normal 21-d cycle is achieved. There is some evidence that the endocrine control of the estrous cycles needs to be primed with P4 (Gonzalez-Padilla et al., 1975), and this may offer some explanation of the switch from negative to positive feedback of estrogen. In the prepubertal heifer, circulating P4 concentrations are negligible, and they do not increase until the first luteal tissue is established. This first P4 increase may serve to prime the complex feedback system, in that it increases the sensitivity of the hypothalamus to the positive feedback of estrogen that occurs in the absence of P4.

The interactions between estrogen, P4, GnRH, LH, FSH, inhibin etc. that are crucial to the onset of puberty continue to drive an animal's reproductive activity for the rest of her fertile life. It is, therefore, plausible that the onset of puberty has biological mechanisms in common with a range of fertility traits that influence lifetime reproductive success, including an animal's ability to conceive, maintain a pregnancy and then resume cycling post-calving. That said, of all of the reproduction-related processes, it seems likely that factors that influence AGEP would have the most in common with factors associated with resumption of cyclicity post-calving. This is because the period of anoestrus post-calving is similar to prepubertal anoestrus as it is the same mechanism of a switch from negative to positive feedback of estrogen that ultimately initiates both on-set of puberty and return to estrus post-calving (Berardinelli et al., 1979; Day and Anderson, 1998).

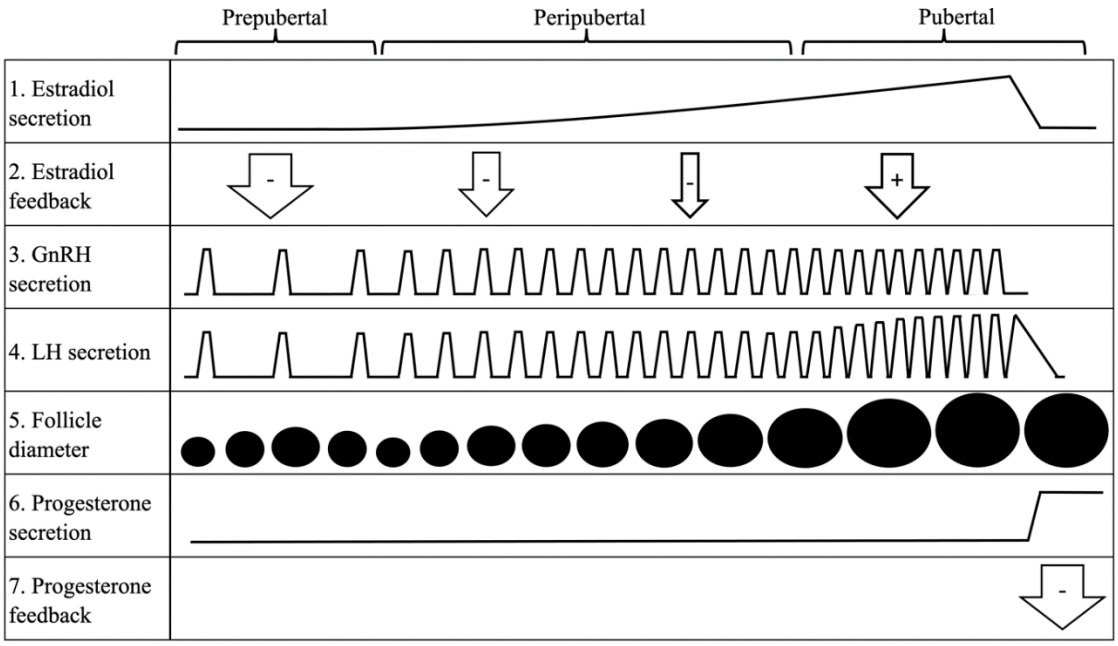


Figure 1.2. Model describing the endocrine changes that occur around the time of puberty as published by Day and Anderson, (1998), with a modification to include gonadotrophin-releasing hormone (GnRH) and progesterone. The peripubertal period is around 50 d prior to the on-set of puberty. 1. Estradiol secretion increases during the peripubertal period up to the point of ovulation. 2. Estradiol exerts gradually decreasing negative feedback on the hypothalamus until just prior to first ovulation, when feedback switches to positive. 3. Gonadotrophin-releasing hormone is secreted from the hypothalamus in a pulsatile pattern, and these pulses increase in frequency when the hypothalamus is positively stimulated. 4. Luteinizing hormone (LH) is secreted from the pituitary gland in response to GnRH in a pulsatile pattern that has increasing frequency and amplitude. 5. Circulating LH supports the development of a dominant follicle, which continues to increase in size and secrete an increasing amount of estrogen. The positive feedback loop between the ovaries and the hypothalamus cumulated in a high amplitude LH surge that initiates ovulation (Ginther et al., 2001). 6. A temporary endocrine structure called a CL is formed on the ovary in the place of the ovulated follicle, and this structure begins to secrete progesterone (Reynolds and Redmer, 1999; Forde et al., 2011). 7. Progesterone enacts negative feedback on the hypothalamus, inhibiting the secretion of GnRH, and so reducing the synthesis of pituitary hormones including LH (Forde et al., 2011). This negative feedback remains in place until the CL experiences atresia, maintaining a stable environment for conception and pregnancy to occur.

Measuring age at puberty

The precise definition of attainment of puberty is multi-faceted and mostly not externally visible, and so it can be an expensive trait to measure. Onset of puberty can be separated into

three components: behavioral estrus, ovulation, and normal luteal function. The following section describes methods that have been used to measure each of these components.

1. Behavioral estrus

Detecting behavioral estrus is common practice on dairy farms that use artificial insemination (AI), as identifying behavioral estrus enables farmers to correctly time the breeding of each cow (Gordon, 2011). Behavioral estrus can be detected by visual assessment without any specialist tools, but this involves a lot of time and a certain amount of skill, as each cow must be carefully observed on a daily basis (Gordon, 2011). As a cheap alternative to simple visual observation, farmers can apply paint to the sacrum of each cow and watch for signs of friction that indicate the cows was standing to be mounted. Tail paint can reduce the time and effort required to detect behavioral estrus, as farmers do not need to directly observe all estrus-related behaviors. That said, it is easier to see the evidence of mounting in fresh paint, so farmers who use this approach for estrus detection during mating tend to reapply the paint daily (Gordon, 2011). Several commercial devices have been developed to mimic the role of tail paint while reducing the labor associated with regularly repainting cows. For example, the 'Kamar' device (Kamar, Inc., Steamboat Springs, CO) involves a pressure-activated ink capsule that is glued to the animal's sacrum (Holman et al., 2011). A Kamar can remain in place for an extended period and the capsule is designed to burst if the animal is mounted by another animal. Once the ink capsule bursts, the color of the device changes, alerting farmers or researchers that the animal is experiencing behavioral estrus (Holman et al., 2011). Devices like Kamars are relatively inexpensive and are commonly used for the lactating herd on NZ dairy farms (Verkerk, 2003). Another estrus detection technology that is gaining popularity among farmers is the use of activity monitoring devices (Saint-Dizier and Chastant-Maillard, 2012; Dela Rue et al., 2019). If cows wear activity monitors for an extended period, a baseline activity level can be established, and an upward deviation in activity can provide a marker of an estrus event (Saint-Dizier and Chastant-Maillard, 2012). Activity monitors provide a low labor solution to estrus detection, but the hardware is still relatively expensive, and this is likely to be a barrier for uptake of the technology (Saint-Dizier and Chastant-Maillard, 2012). Additional expense for hardware is especially problematic in a seasonal farm system where costs are tightly controlled (Verkerk, 2003).

Most of the methods that have been developed to detect behavioral estrus are targeted for use during AI breeding; however, many can also be applied to detecting an animal's first behavioral estrus at puberty. There are several important differences to consider between detecting estrus events during a breeding period versus around the

onset of puberty. First, the breeding period on a seasonal dairy farm tends to be restricted to 8-12 weeks (Verkerk, 2003; Macdonald and Roche, 2023), as it is important that the calving period is condensed. Conversely, the AGEP of heifers can vary by over 200 d (Meier et al., 2021b) and so the required window of estrus detection can span many months. The longer timeframes involved with estrus detection during puberty mean that the labor requirements of an estrus detection method are a key consideration when measuring AGEP. Second, estrus detection within the lactating dairy herd occurs during a time of year when the animals are being milked (Verkerk, 2003). The regular handling of milking cows means that there is only minimal additional effort associated with activities such as daily checking and reapplying tail paint or 'Kamar' type devices. In contrast, peripubertal dairy heifers within seasonal farm systems are often farmed on extensive grazing properties, where they are only handled at infrequent intervals for activities such as drenching, vaccination, or weighing. Therefore, any method of detecting behavioral estrus that involves handling or observing heifers will require a significant additional labor commitment from farmers. Third, when a breeding cow is identified as being in estrus, urgent action is required to ensure she is mated at the optimal time to achieve conception (Gordon, 2011). Conversely, onset of puberty does not generally require any immediate action, rather it tends to be measured for longer-term objectives, such as research. Given the differences between estrus detection in lactating cows and heifers during the onset of puberty, researchers tend to modify how estrus detection technologies are used when the goal is to measure AGEP, to reduce effort. For example, instead of observing animals for signs of being mounted on a daily basis, this might be extended out to weekly intervals (Hickson et al., 2011).

2. Ovulation and length of luteal phase

Ovulation can be demonstrated as having occurred by determining the presence of a functional CL. The establishment of a CL is catalyzed by the ovulation of a dominant follicle (Reynolds and Redmer, 1999). Therefore, the presence of a CL indicates that an animal has ovulated. There are two main strategies for establishing the presence of a CL. First, ultrasound scanning the ovaries to visually observe a CL (Johnston et al., 2009), and second analysing blood plasma P4 (BP4) concentrations (Macdonald et al., 2007; Meier et al., 2021b). Of these two strategies, ultrasound scanning may be more reliable, as although the CL is the main source of BP4, there is evidence that prepubertal heifers can have elevated BP4 concentrations in the absence of a functioning CL (Berardinelli et al., 1979). Berardinelli et al. (1979) established that the source of this transient P4 in prepubertal heifers is the ovary. They identified a group of

heifers with prepubertal increases in BP4, and then demonstrated that the BP4 levels return to basal concentrations once the heifers were ovariectomized. In the same study, the authors concluded that it was likely P4 was secreted by compact luteal tissue that was not formed as a consequence of ovulation. These formations of luteal tissue were small, ranging from 1.5 to 6 mm, and were not palpable, or visible on an ultrasound scan. Transient BP4 in prepubertal heifers tends to be shorter in duration than the BP4 elevations that result from normal luteal function (Berardinelli et al., 1979; Dodson et al., 1988). Therefore, testing for BP4 elevation using an approach that also characterizes the length of the luteal phase (i.e., blood testing at intervals 1 week apart) can help confirm whether the elevation in BP4 was caused by a functional CL, or by some other source. The compact luteal tissue that is probably responsible for transient BP4 in prepubertal animals is too small to be seen using ultrasound scanning, and so phenotypes that relied on scanning to confirm ovulation would not be comprised by this presence of this tissue (Berardinelli et al., 1979); however, frequent scanning is still needed if a precise date of ovulation and the length of luteal phase are required.

Reducing the cost of measuring age at puberty

Those who measure AGEP in cattle tend to make a trade-off between the precision of their AGEP phenotypes and the number of phenotypes that they can feasibly measure. To measure a very specific and accurate phenotype, animals need to be subjected to daily measurements that span the > 200-d biological window for onset of puberty (Dennis et al., 2018), and these measurements need to be comprehensive enough to assess each of the three components of fertility (behavioral estrus, ovulation, and normal luteal activity). Although a comprehensive phenotyping approach that covers all these components of puberty may yield a best-practice AGEP phenotype, it is probably prohibitively expensive for most breeding programs. Moreover, such a phenotyping strategy could place stress on the animals involved that may not be justified for the marginal gain in phenotype precision. Researchers can reduce cost and effort associated with measuring AGEP by reducing the frequency of observations, decreasing the length of the observation window, and/or by simplifying the criteria that an animal must meet to be characterized as post-pubertal; for example, researchers may focus on only the occurrence of behavioral estrus, ovulation, or a normal luteal phase, rather than attempting to confirm all three.

Reducing the frequency of observations and the length of the observation window will introduce censoring into the AGEP phenotypes. For example, if researchers aimed to measure the age at which an animal experienced its first ovulation, an uncensored phenotype would require daily observations (either BP4 testing or ultrasound scanning for the presence of a CL). Reducing these observations to a frequency of weekly or monthly introduces interval censoring.

If the number of observations were further reduced by shortening the duration of the observation window, some phenotypes would be subjected to left or right censoring, as they would only be known with an upper or lower bound, respectively. Phenotype censoring reduces the precision of a phenotype, and this lack of precision may influence the utility of AGEP as a predictor of the target fertility traits. However, the potential gains from reducing observation are substantial, and so the implications of censored AGEP phenotypes should be investigated further.

Simplifying the definition of AGEP, alongside the strategic use of phenotype censoring, is commonly reported in the literature. For example, Mialon et al. (1999) observed some 350 Charolais heifers for estrus behavior twice daily for a period of 10 months, while also blood testing for BP4 bi-monthly. This phenotyping method yielded a very precise age of first behavioral estrus, while also providing a censored phenotype for age at first BP4 elevation (AGEP4). Together, these two phenotypes were combined to assess whether the animal met the criteria of exhibiting behavioral estrus and having a functional CL. The authors simplified the definition of AGEP insofar as the length of the luteal phase was not considered. In addition, the authors accepted a degree of phenotype censoring in their BP4 testing regime, as the AGEP4 phenotypes would only be known to fall within an upper and lower bound of the flanking blood test days. Johnston et al. (2009) further simplified the criteria for AGEP. They undertook serial rectal ultrasound scanning of heifers at intervals of around 6 weeks and recorded age at first CL (AGECL) as a proxy for AGEP. That phenotyping approach does not confirm that the animal expressed a behavioral estrus, nor does it confirm that the animal was exhibiting normal luteal function. In addition, their AGECL phenotypes were subject to left, interval and right censoring. Macdonald et al. (2007) and Meier et al. (2021) implemented a similar phenotyping strategy to Johnston et al. (2009), except that they used BP4 as an indicator of the presence of a functional CL, rather than ultrasound scanning, and the interval between observations was smaller. In both studies, animals were blood sampled on a weekly basis and categorized as post-pubertal once they had been observed with elevated BP4 concentrations in 2 of 3 consecutive weekly tests. The authors of these two papers reduced the risk of prepubertal BP4 fluctuations (Berardinelli et al., 1979) affecting their results by requiring animals to have a sustained elevation in BP4 (i.e., greater than 1 week). Blood testing for BP4 elevation can only indicate that ovulation has occurred, and a luteal phase was initiated, but the shorter interval of 1 week between observations means that the length of the luteal phase was able to be incorporated into the phenotype criteria.

Although the motivations for these phenotyping strategies were not directly discussed, it is likely that the authors of these studies accepted some loss in individual phenotype accuracy and precision in favor of limiting labor, costs, and animal stress.

Phenotypic distribution and factors affecting age at puberty

The average age of puberty in cattle varies depending on breed. In particular, breeds that fall within the classification of *Bos taurus* (European, temperate) tend to attain puberty earlier than their *Bos indicus* (tropical, zebu) counterparts (Abeygunawardena and Dematawewa, 2004). For example, the *Bos taurus* breeds Holstein-Friesian, Jersey, Angus and Charolais attain puberty at around 12 months old (Morris and Amyes, 2005; Macdonald et al., 2007; Hickson et al., 2011; Dennis et al., 2018; Meier et al., 2021b). Conversely, *Bos indicus* breeds such as Brahman tend to attain puberty later, at around 24 months old (Abeygunawardena and Dematawewa, 2004; Nogueira, 2004; Johnston et al., 2009).

The national dairy herd in NZ is predominantly of Holstein-Friesian and Jersey origin, as well as a significant proportion of ad-mixed Holstein-Friesian cross Jersey animals. There is evidence of breed differences within the *Bos taurus* classification. Morris et al. (1993) demonstrated the variance in AGEP phenotype among *Bos taurus* breeds by breeding sires from 11 breeds to Angus dams. The AGEP of the subsequent cross-bred calves depended on the sire breed, with a mean difference of 93 d between the youngest (Jersey) and oldest (Chianina) sire-breed groups. In addition, Hickson et al. (2011) reported that Jersey cattle attain puberty at ages up to 6 weeks younger than Holstein-Friesians. The AGEP trait appears to be normally distributed in NZ Holstein-Friesian (Hickson et al., 2011; Handcock et al., 2021; Meier et al., 2021b) and Jersey (Hickson et al., 2011) heifers.

Most farmers in NZ aim for heifers to calve for the first time at 2 yr of age, which means that breeding needs to commence when heifers are around 15 months old. This timeframe helps to ensure that heifers calve within the first 3 weeks of the season, which gives them a better chance of conceiving in a timely manner to maintain a 365-d calving interval and remain in the herd (Archbold et al., 2012). Hence, heifers need to reach puberty early enough to conceive during the first 3 weeks of the breeding season. Although, most dairy breeds attain puberty around 12 months old, the phenotypic distribution of AGEP spans a relatively wide age interval, with a standard deviation of 30 d (Dennis et al. 2018). A standard deviation of 30 d aligns well with the phenotypic data collected by Meier et al. (2021), where authors reported a difference of 200 d between the first and last animals to attain puberty, which would reflect about 6 standard deviations (that is, most of the observations fell within 3 standard deviations of the mean). Considering this phenotypic distribution, a proportion of animals may be prepubertal at the start of the seasonal breeding season. Indeed, McDougall et al. (2013) reported an observational study of 10 commercial herds in NZ, which indicated that only 60% of heifers had reached puberty by the start of the breeding season, with a huge range among farms of 9% to 93%.

The onset of puberty is closely related to body weight (BW) and BW gain (Little et al., 1981; Handcock et al., 2021), and it is well established that *Bos taurus* heifers reach puberty by

about 50% of their mature BW (Le Cozler et al., 2008). For example, puberty has been reported to occur at approximately 180 kg BW for Jerseys (Macdonald et al., 2005), and at approximately 250,and 275 kg BW for smaller NZ and larger North American Holstein-Friesian strains (Macdonald et al., 2005; Macdonald et al., 2007), respectively. It is recommended that heifers should be reared to achieve BW targets of 30%, 60% and 90% of estimated mature BW at 6 months, 15 months (breeding), and 22 months (pre-calving; (Roche et al., 2014) Poor growth rates relative to these targets will delay the onset of puberty and increase the risk of animals being prepubertal during the seasonal breeding period, which delays the timing of their first breeding, conception and subsequent calving, and reduces the likelihood of timely pregnancy during first lactation and, in turn, leads to increased culling risk (Archbold et al., 2012). In the study by McDougall et al. (2013), younger animals and those at a lower body condition score below 4.5 units were most at risk of not reaching puberty by the start of the seasonal breeding. Similarly, in an Irish study of seasonally managed dairy heifers, (Archbold et al., 2012) reported that heifers that were at least 14.5 months old and 4.5 body condition score units at the start of mating were more likely to have attained puberty. Unfortunately, the seasonal nature of feed supply and, therefore, heifer growth rates that are typical of NZ farm systems may delay the onset of puberty by up to 40 d (Handcock et al., 2021). Therefore, it is plausible that NZ farmers could almost eliminate the occurrence of profit-limiting late pubertal heifers by carefully managing feed allocations and growth rates in young stock to prevent subsequent issues with reproductive performance.

Heritability

The heritability of AGEP in dairy cattle has been reported by Lefebvre et al. (2021) and Price et al. (2017) to be around 0.40 (Table 1.1). Heritability estimates in beef cattle are more common in the literature, and tend to be around 0.30, with a range from 0.10 to 0.76 (Table 1.1; Smith et al., 1989; Morris and Amyes, 2005; Fortes et al., 2012). A heritability of 0.30 to 0.40 is relatively high in the context of reproductive traits, as traits relating to calving and mating performance have heritabilities of less than 0.10 (Pryce et al., 2000; Harris et al., 2005; Bowley et al., 2015). Furthermore, a heritability of 0.30 is comparable to those reported for highly selected milk production traits like milk fat and milk protein (Ahlborn and Dempfle, 1992). The approach for measuring AGEP (that is, categorizing animals as pre- or post-pubertal) varies among studies where authors have reported genetic parameters , but the estimated heritability for AGEP does not appear to depend on the specific definition of puberty (Table 1.1). The most common approach that authors have used when aiming to estimate genetic parameters is to categorize animals as pubertal based on observations of behavioral estrus (Smith et al., 1989; Gregory et al., 1995; Morris et al., 2000; Mialon et al., 2001). The heritability of AGEP using behavioral estrus to define puberty ranged from 0.10 to 0.38 (Smith et al., 1989; Gregory et al.,

1995; Morris et al., 2000; Mialon et al., 2001). Other common methods involve measuring the occurrence of the luteal phase of the estrous cycle, either by blood testing for BP4 (Mialon et al., 2001; Price et al., 2017; Lefebvre et al., 2021), or rectal ultrasound scanning for visual conformation of a CL (Johnston et al., 2009). The estimated heritabilities of AGEP when using the occurrence of the luteal phase to define puberty ranged from 0.28 to 0.76 (Mialon et al., 2001; Johnston et al., 2009; Price et al., 2017). Vargas et al. (1998) implemented a relatively thorough criteria for puberty, requiring animals to exhibit both behavioral estrus and evidence of a luteal phase before classifying them as pubertal. They reported a heritability for AGEP of 0.42, which falls within the range of estimated heritabilities from studies that used more simplified trait definitions. The heritability of AGEP has been reported in a range of difference breeds, including Hereford, Angus, Charolais, Holstein-Friesian and Brahman and those estimates do not appear to depend on breed (Table 1.1).

Table 1.1. Estimated heritabilities for age at puberty, or closely related phenotypes such as age at first blood plasma progesterone (BP4) elevation, age at first detected corpus luteum, or age at first observed behavioral estrus. * Standard error, ** Standard deviation.

Method	Number of animals	Breed	Heritability	Reference
Review – combined definitions.	.	Review – multiple breeds	0.40 (range of 0.07 to 0.67)	(Martin et al., 1992)
Observing behavioral estrus	779	Hereford and Angus	0.10 ± 0.17*	(Smith et al., 1989)
BP4 testing (7-d interval)	527	Holstein-Friesian	0.41 to 0.76	(Price et al., 2017)
Observing behavioral estrus	7,767	Beef	0.30 ± 0.04*	(Gregory et al., 1995)
Observing behavioral estrus	351	Charolais	0.38 ± 0.04*	(Mialon et al., 2001)
BP4 testing (10 to 20-d intervals)	351	Charolais	0.28 ± 0.05*	(Mialon et al., 2001)
Observing behavioral estrus	1,513	Angus	0.27 ± 0.04**	(Morris et al., 2000)
BP4 testing (30–42-d intervals)	2,115	Brahman (1,007) Tropical Composite (1,108)	Brahman: 0.57 ± 0.12* Tropical Composite: 0.52 ± 0.12*	(Johnston et al., 2009; Fortes et al., 2012)
Observing behavioral estrus, rectal palpation for a CL (28-d interval), BP4 testing (28-d interval)	292	Brahman	0.42	(Vargas et al., 1998)
BP4 testing (10-d intervals)	1,163	Holstein-Normande crossbred	0.38	(Lefebvre et al., 2021)

Associations between age at puberty and fertility

Several studies across various cattle breeds (Morris et al., 2000; Mialon et al., 2001; Lefebvre et al., 2021) indicate that the AGEP trait has a moderate genetic correlation with reproductive performance later in life. In the study reported by Morris et al. (2000), AGEP, calving date, and pregnancy rate (pregnant [1] or not [0] within the first six weeks of the seasonal breeding period) were measured in a multi-generation population of Angus cattle divergently selected for AGEP. The authors reported genetic correlations (± standard deviation) of 0.57 ± 0.17 between AGEP and calving date, and -0.29 ± 0.30 between AGEP and pregnancy rate. Mialon et al. (2001) reported similar results in a population of 351 Charolais cattle, with a genetic correlation (± standard error) of 0.43 ± 0.07 between AGEP and postpartum resumption of cyclicity (i.e., PPAI) when both traits were measured as first elevation in BP4, and 0.58 ± 0.08 when measured using first observed behavioral estrus. Further, Lefebvre et al. (2021) measured BP4 at 10-d intervals to determine AGEP and PPAI in a population of 1,163 Holstein-Normande cattle and reported a genetic correlation of 0.45 ± 0.23. However, a fourth study by Patterson et al. (1992) measured AGEP using different criteria (estrus, cohobated 6 -10 d later by a palpable CL and BP4 elevation), and the authors reported low genetic correlations of 0.12 ($P = 0.20$) and 0.05 ($P = 0.71$) in two groups of animals, Angus-cross Hereford and Brahman-cross Hereford cattle. Finally, Meier et al. (2021a;b) measured AGEP and subsequent breeding, calving and pregnancy rates during lactation in a population of 500 Holstein-Friesian heifers comprising 2 subgroups that were preselected to create divergence in NZ fertility EBVs but with minimal variance in other traits including live weight and milk production EBVs. Although the authors did not directly estimate the genetic correlation between AGEP and fertility later in life, they reported significant divergence in AGEP phenotypes between the two subgroups; heifers with a high fertility EBV (+5) reached puberty 27 d earlier than those with a low fertility BV (-5), which meant they were 20 kg BW lighter and at a lower percentage of mature BW (51 vs 55%; Figure 1.3). Consequently, 94% of high fertility EBV heifers had reached puberty by the start of the seasonal breeding period compared with 82% of low fertility EBV heifers. These high fertility EBV animals then demonstrated markedly superior reproductive performance during first and second lactations (Meier et al., 2021a) which suggests a genetic relationship between AGEP and subsequent fertility. Overall, these studies provide evidence of a non-zero genetic correlation between AGEP and fertility later in life, although there are some inconsistencies among studies that may be attributed to differences in study design and animal populations.

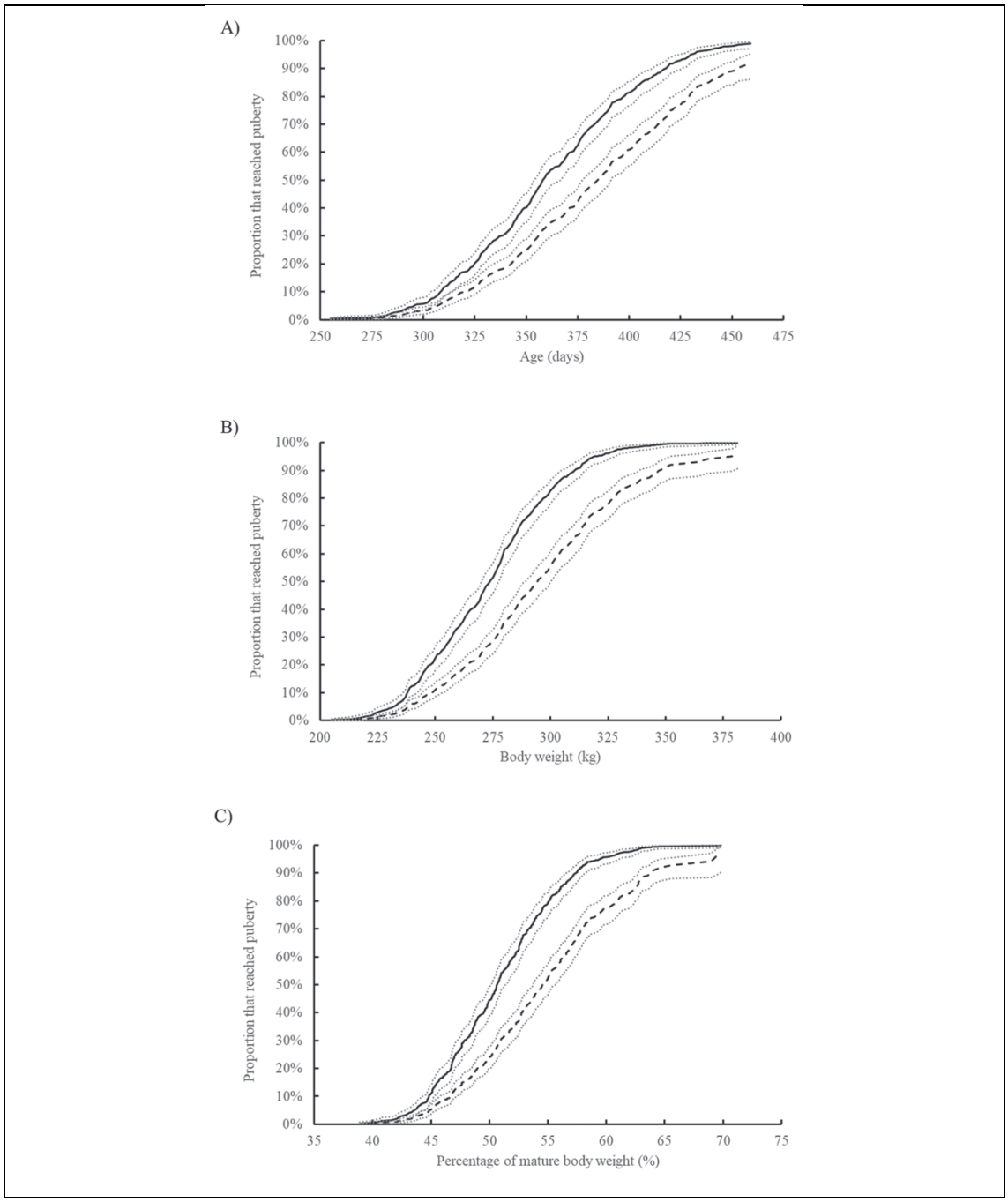


Figure 1.3 Survival estimations from the Cox proportional hazard model of (A) age (d, $P < 0.001$), (B) BW (kg, $P < 0.001$), and (C) percentage of estimated mature BW (%) at puberty ($P < 0.001$) of heifers with positive (solid line) and negative (dashed line) genetic merit for fertility traits. Dotted lines represent the 95% CI (Meier et al., 2021b).

Associations between age at puberty and stature

The onset of puberty in dairy cattle is intertwined with BW and growth rate, with AGEP largely determined by BW as a proportion of mature BW (Moran et al., 1989). Therefore, it may seem logical that the AGEP would exhibit strong associations with stature traits like height and

BW. However, the literature indicates that genetic associations are moderate at best. Gregory et al. (1995) measured AGEP in around 7,500 beef heifers using visual estrus detection. The heifers were weighed regularly throughout their lives, and genetic correlations between BW at different ages and AGEP were produced. The authors reported the strongest (although still relatively low) genetic correlation between AGEP and BW at 200 d of age ($r = 0.14 \pm 0.09$). The genetic correlations between AGEP and BW at other ages (birth, 1, 2 and 5 yr old) were not significantly different from 0. Phenotypic correlations reported by Gregory et al. (1995) were similar, ranging from 0 to -0.12. Smith et al. (1989) found similar results, reporting weak genetic correlations between AGEP and weaning weight (-0.04 ± 0.57), and AGEP and yearling weight (-0.14 ± 0.44) in some 700 predominantly Hereford heifers. Estimated phenotypic correlations were more consistent, but still low at -0.17 and -0.16 between AGEP and weaning and yearling weight, respectively. In slight contrast to these two papers, Wolcott et al. (2014) and Mialon et al. (2001) have reported moderate genetic associations between AGEP and BW. Wolcott et al. (2014) investigated AGEP in 1,027 Brahman and 1,132 Tropical composite females. They measured BW at 18 and 39 months of age and reported genetic correlations with AGEP ranging from 0.24 to 0.38, depending on breed and age of BW measurement. Mialon et al. (2001) measured BW for 351 yearling heifers and reported a correlation between these BW measures and AGEP of 0.32 ± 0.09 when AGEP was defined a first elevation in BP4, and -0.29 ± 0.04 when AGEP was defined as first observed behavioral estrus. In addition, Smith et al. (1989) reported moderate to high genetic correlations of 0.58 ± 0.55 between AGEP and birth weight, but the standard error for that estimate was notably high.

Genetic correlations reported between AGEP and stature are also weak to moderate. Gregory et al. (1995) reported genetic correlations of -0.11 ± 0.08, 0.17 ± 0.12 and 0.02 ± 0.11 with hip height measured at 12 months, 24 months and 5 yr of age, respectively. Wolcott et al. (2014) reported similar results in Braham ($n = 1027$) and Tropical composite cattle ($n = 1132$). The genetic correlation between AGEP and hip height in Brahman cattle was -0.03 ± 0.19 and -0.36 ± 0.18 at 18 months and 39 months of age, respectively. The genetic correlation between AGEP and hip height in Tropical composite cattle was -0.24 ± 0.18 and -0.20 ± 0.17 at 18 months and 39 months of age, respectively.

The general lack of a strong association between AGEP- and stature-related traits may be explained by slower maturation in heavier animals. For example, Macdonald et al. (2007) measured AGEP and BW in three strains of Holstein-Friesian cattle with differing mature BW and reported that the strains with lighter mature BW reached puberty earlier than their heavier counterparts. This study is consistent with other investigations that indicate that animals with a greater proportion of North American Holstein-Friesian ancestry, greater mature BW and/or a heavier live weight EBV take longer to reach puberty (Garcia-Muniz, 1998; McGrath et al.,

2001). Therefore, it is possible that the genetic correlation between AGEP and BW as a proportion of mature BW would better capture the interaction between puberty onset and BW.

GWAS of age at puberty

Several genomic association studies have been conducted to gain a better understanding of the genetic factors that influence AGEP and, perhaps, subsequent fertility performance in cattle. The results of those GWAS varied by study, which may be explained by the range of methodologies employed as well as the variety of breed populations (Table 1.2). That said, it is probable that AGEP is a trait that is influenced by large numbers of genes.

Although there is limited cross-over agreement in QTL detected between studies, there are several QTL that have been detected in multiple populations. For instance, a QTL on chromosome 14, located between 20 – 25 Mb has been reported in 3 populations of Nelore cattle (Irano et al., 2016; Mota et al., 2017, 2022) and Brahman cattle (Fortes et al., 2012; Hawken et al., 2012). The gene *PLAG1*, which is located at 25 Mb on chromosome 14 (Littlejohn et al., 2012), is a likely candidate for explaining a puberty QTL in this region. The *PLAG1* gene has an established effect on growth and stature traits (Littlejohn et al., 2012), which share a low to moderate covariance with AGEP. Two other QTLs located near the *PLAG1* gene on chromosome 14 have also been reported. The first QTL was found in Nelore cattle at 16.5 Mb (Mota et al., 2017), while the second QTL was identified in Holstein-Friesian cross Normande cattle between 36.6 and 37.6 Mb (Lefebvre et al., 2021). It is possible that these QTLs are in linkage with the *PLAG*1 gene in these populations, or there may be another gene in these regions that affects AGEP. According to Lefebvre et al. (2021), the QTL they identified on chromosome 14 is likely explained by the *NCOA2* gene, which is expressed in the hypothalamus and located at 36 Mb on chromosome 14 (Fortes et al., 2011). Given the proximity of this *NCOA2* to the QTL detected by Lefebvre et al. (2021), it is plausible that this gene is a more likely candidate for explaining variance in AGEP in their population than *PLAG1*. Another QTL on chromosome 13, likely between 59.3 and 67.3 Mb, has been reported in multiple populations, including Holstein-Normande (Lefebvre et al., 2021) and Nelore cattle (Mota et al., 2022). In that region, there is a candidate gene called *GHRH*, which codes for growth hormone releasing hormone and is known to influence growth and AGEP via modulation of the hypothalamic-pituitary-gonadal axis (Mota et al., 2022). A third QTL that has been detected in two different breeds is located on chromosome 8 between 68.42 and 72.2 Mb. Stegemiller et al. (2021) detected a QTL at this locus in a mixed-breed population of Angus, Hereford, Simmental, Simmental cross Angus and Shorthorn, while Mota et al. (2022) detected it in Nelore cattle. Within this region, there are two candidate genes associated with fertility: *LOXL2* and *STC1* (Mota et al., 2022). Regions on chromosome 2 (4.9 – 7.2 Mb) and chromosome 9 (41.0 – 49.5 Mb) may contain QTL that contribute to AGEP variation, although

these regions may only have relevance to Nelore cattle as reported by Mota et al. (2017, 2022). The genes *MAP3K2* (4.94–5.16 Mb on chromosome 2) and *GRIK2* (48.03–48.91 Mb on chromosome 9) have been linked to pituitary function and are potential candidate genes in these regions. Overall, genomic association studies have provided valuable insights into the genetic factors that influence AGEP and fertility performance in cattle. However, more research is needed to elucidate the genetic architecture of these traits, particularly in *Bos taurus* breeds, as most GWAS analyses have been undertaken in *Bos indicus* populations.

Table 1.2. Genomic regions associated with age at puberty (AGEP), or closely related phenotypes such as AGEP4 (age at first blood plasma progesterone elevation), AGECL (age at first detected corpus luteum), or age at first calving (AGECV).

Chromosome	Base pair location (Mb)	Trait	Breed (number of animals)	Citation
1	3.4 - 4.3	AGEP4	Holstein-Normande (1,163)	(Lefebvre et al., 2021)
1	128.2 – 129.5	Calved by 28 months of age	Nelore (8,545)	(Mota et al., 2022)
1	141.2 - 141.4	Calved by 26 months of age	Nelore (8,545)	(Mota et al., 2022)
2	4.9-5.1	Calved by 24 months of age	Nelore (8,545)	(Mota et al., 2022)
2	6.2 – 7.2	AGECV	Nelore (762)	(Mota et al., 2017)
2	97.3 - 97.3	Puberty status via reproductive tract scoring	Angus, Hereford, Simmental, Simmental-Angus (SimAngus) or Shorthorn (293)	(Stegemiller et al., 2021)
2	105.0-105.7	Calved by 28/30 months of age	Nelore (8,545)	(Mota et al., 2022)
3	29.8-29.9	Calved by 24 months of age	Nelore (8,545)	(Mota et al., 2022)
3	80.2 - 102.5	AGEP4	Holstein-Normande (1,163)	(Lefebvre et al., 2021)

4	92.6-93.9	Calved by 24/26/28 months of age	Nelore (8,545)	(Mota et al., 2022)
5	96	AGECL	Tropical Composite (866)	(Hawken et al., 2012)
5	8.8 – 10.1	Early pregnancy	Nelore (1,770)	(Irano et al., 2016)
5	16.0 – 17.1	Early pregnancy	Nelore (1,770)	(Irano et al., 2016)
5	65.7-67.3	Calved by 24/26/28/30 months of age	Nelore (8,545)	(Mota et al., 2022)
6	10.6 – 11.6	Early pregnancy	Nelore (1,770)	(Irano et al., 2016)
6	84.6-85.4	Calved by 24/26/28/30 months of age	Nelore (8,545)	(Mota et al., 2022)
7	Not specified	AGECL	Tropical Composite (866)	(Hawken et al., 2012)
7	3.1 – 3.8	Early pregnancy	Nelore (1,770)	(Irano et al., 2016)
7	17.2-17.6	Calved by 28/30 months of age	Nelore (8,545)	(Mota et al., 2022)
7	41.2 – 42.0	Early pregnancy	Nelore (1,770)	(Irano et al., 2016)
8	68.42 - 68.7	Calved by 30 months of age	Nelore (8,545)	(Mota et al., 2022)
8	72.3- 72.2	Puberty status via reproductive tract scoring	Angus, Hereford, Simmental, Simmental-Angus (SimAngus) or Shorthorn (293)	(Stegemiller et al., 2021)
8	106.3 – 107.3	AGECV	Nelore (762)	(Mota et al., 2017)

9	48.6 - 49.5	Calved by 24 months of age	Nelore (8,545)	(Mota et al., 2022)
9	41.0 – 46.6	AGECV	Nelore (762)	(Mota et al., 2017)
10	69.2	Puberty status via reproductive tract scoring	Angus, Hereford, Simmental, Simmental-Angus (SimAngus) or Shorthorn (293)	(Stegemiller et al., 2021)
11	13.4-16.1	Calved by 26/28/30 months of age	Nelore (8,545)	(Mota et al., 2022)
11	44.6 - 45.2	AGEP4	Holstein-Normande (1,163)	(Lefebvre et al., 2021)
11	90.0	Puberty status via reproductive tract scoring	Angus, Hereford, Simmental, Simmental-Angus (SimAngus) or Shorthorn (293)	(Stegemiller et al., 2021)
13	43.1 - 43.9	AGEP4	Holstein-Normande (1,163)	(Lefebvre et al., 2021)
13	59.3 - 62.2	AGEP4	Holstein-Normande (1,163)	(Lefebvre et al., 2021)
13	65.6-67.3	Calved by 28/30 months of age	Nelore (8,545)	(Mota et al., 2022)
14	16.5 – 16.5	AGECV	Nelore (762)	(Mota et al., 2017)
14	20 - 33	AGECL	Braham (843)	(Fortes et al., 2012; Hawken et al., 2012)
14	20.3 – 36.9	AGECV	Nelore (762)	(Mota et al., 2017)
14	22.6 – 23.3	Early pregnancy	Nelore (1,770)	(Irano et al., 2016)

14	23.8-25.6	Calved by 24/26/28/30 months of age	Nelore (8,545)	(Mota et al., 2022)
14	36.6 - 37.6	AGEP4	Holstein-Normande (1,163)	(Lefebvre et al., 2021)
15	31 – 38	AGECL	Brahman (843)	(Hawken et al., 2012)
16	0.8-2.1	Calved by 24/26/28/30 months of age	Nelore (8,545)	(Mota et al., 2022)
16	43.9 - 44.9	AGECV	Nelore (762)	(Mota et al., 2017)
16	68.2 – 69.2	AGECV	Nelore (762)	(Mota et al., 2017)
17	57.2 – 58.2	AGECV	Nelore (762)	(Mota et al., 2017)
18	4.2 – 4.9	Early pregnancy	Nelore (1,770)	(Irano et al., 2016)
18	33.9 - 34.2	Calved by 30 months of age	Nelore (8,545)	(Mota et al., 2022)
21	0.8 – 3.0	Early pregnancy	Nelore (1,770)	(Irano et al., 2016)
21	27.4 - 40.5	AGEP4	Holstein-Normande (1,163)	(Lefebvre et al., 2021)
21	61.9 – 62.5	Early pregnancy	Nelore (1,770)	(Irano et al., 2016)
22	54.1-55.1	Calved by 24/26/28 months of age	Nelore (8,545)	(Mota et al., 2022)
24	15.3 - 16.1	Calved by 30 months of age	Nelore (8,545)	(Mota et al., 2022)
27	9.9 – 10.5	Early pregnancy	Nelore (1,770)	(Irano et al., 2016)
28	10.1-10.7	Calved by 26/28 months of age	Nelore (8,545)	(Mota et al., 2022)

x	69 - 93	AGECL	Brahman (843)	(Fortes et al., 2012)

Anogenital Distance

Biological link between anogenital distance and fertility

AGD is a biomarker for testosterone exposure.

Anogenital distance describes the distance between an animals' anus and genitals (Figure 1.4). For females, this is commonly defined as the distance between the anus and the clitoris (Hotchkiss et al., 2007; Gobikrushanth et al., 2017), and for males, the distance between the anus and either the base of the scrotum or the base of the penis (Dean and Sharpe, 2013). The AGD phenotype is sexually dimorphic in mammals, with AGD in males tending to be at least 50% larger than the AGD of a female (Thankamony et al., 2016). It is likely that the divergence in AGD phenotypes between female and male mammals is driven by differences in pre-natal androgen exposure, as it is sustained exposure to androgens during key times within pregnancy that supports the development of the male reproductive tract (MacLeod et al., 2010). As such, male fetuses have higher fetal blood testosterone concentrations than their female counterparts (Challis et al., 1973; Mongkonpunya et al., 1975). In addition to AGD providing insight into the varying androgen concentrations associated with male verses female pregnancies, AGD phenotypes are also sensitive to within-sex variation in fetal androgen exposure.

The utility of AGD as a biomarker for within-sex variation in androgen exposure has been established in a range of mammalian species, including rats (Hotchkiss et al., 2007; MacLeod et al., 2010; van den Driesche et al., 2011), and sheep (Lamm et al., 2012). Hotchkiss et al. (2007) treated pregnant female rats with 1 of 3 concentrations of testosterone from d 14 to 18 of gestation, including a control treatment. The AGD of the female offspring responded to the level of testosterone administered to their mother, and fetuses with high testosterone exposure had larger AGD. The AGD of the male offspring was not significantly different across testosterone treatment groups. The lack of effect in the male offspring suggests that pre-natal exposure to abnormally high rates of testosterone may not affect the development of the male reproductive tract. However, van den Driesche et al. (2011) have shown that decreasing pre-natal exposure to testosterone (using either an androgen antagonist, or an androgen receptor antagonist) will result in shorter AGD phenotypes for male offspring. MacLeod et al. (2010) further demonstrated that reducing pre-natal testosterone exposure in male rat fetuses had a long-term effect on AGD, as well as other areas of the reproductive tract such as penis size. They demonstrated that these effects are not reversed by increasing concentrations of androgens after birth. Thankamony et al. (2016) reviewed several observational studies in humans, which

link AGD to other markers of androgen exposure, such as fertility outcomes, semen quality and behavior. They conclude that although data are limited due to difficulties associated with measuring fetal androgen concentrations in human studies, it is likely that AGD is a reliable marker of pre-natal androgen exposure in humans. Lamm et al. (2012) established the effects of increased androgen exposure in ovine fetuses by treating a group of 32 pregnant ewes with either testosterone (n=17) or dihydrotestosterone (a non-aromatizable metabolite of testosterone, n=15), which is another common androgen. They then compared a range of phenotypes associated with the reproductive tract across the two treatment groups and an untreated control group (n=16). They concluded that although the female progeny of treated ewes tended to have normal internal reproductive organs (ovaries, oviducts, and uteri), their external genitalia were masculinized. Notably, the AGD of females from treated ewes were significantly longer than those from the control group.

Reproductive failure related to pre-natal androgen exposure

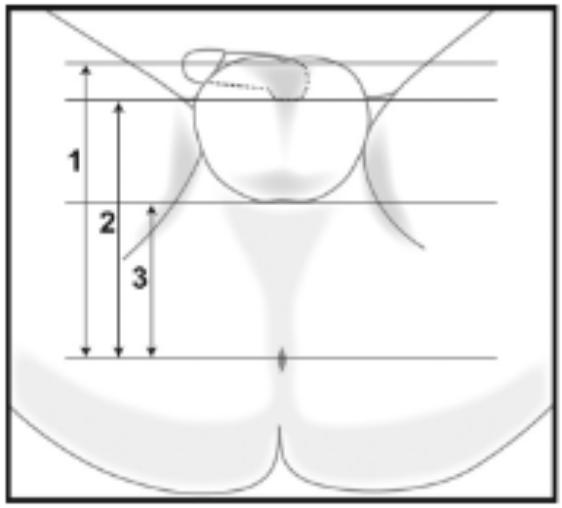
There is general agreement in the literature that pre-natal exposure to inappropriate androgen concentrations during important developmental phases can have lifelong reproductive and metabolic implications (Abbott et al., 1998; Hotchkiss et al., 2007; Welsh et al., 2008; Lamm et al., 2012). Welsh et al. (2008) demonstrated that male rats who were exposed to insufficient pre-natal androgen concentrations will have a smaller penis and are more likely to exhibit hypospadias and cryptorchidism. While over-androgenized females can exhibit permanent masculinization of their reproductive tract (Hotchkiss et al., 2007; Lamm et al., 2012). Bruns et al. (2004) showed that male Rhesus monkeys exposed to excess pre-natal testosterone have insulin resistance and impaired insulin secretion in response to a glucose challenge, putting them at greater risk of type 2 diabetes. This finding is consistent with the results of Abbott et al. (1998), who reported insulin resistance in over-androgenized female Rhesus monkeys. Further, Abbott et al. (1998) presented the female Rhesus monkey as an animal model for polycystic ovarian syndrome (PCOS) in female humans. In their study, the pre-natally androgenized female Rhesus monkeys presented with a wide range of conditions that are consistent with a PCOS diagnosis, including anovulation, enlarged ovaries, endocrine dysfunction, and pancreatic dysfunction (Abbott et al., 1998). These results suggest that PCOS may be a consequence of pre-natal exposure to excess androgens.

Measuring anogenital distance in cattle

Gobikrushanth et al. (2017) measured the AGD of 921 lactating cows using digital calipers; this method has been adopted by others and is now the standard approach in dairy cattle (Gobikrushanth et al., 2019; Carrelli et al., 2022; Rajesh et al., 2022). The AGD trait can be measured non-invasively from birth onwards, although the repeatability of the AGD

measurements may improve as animals mature (Rajesh et al., 2022). Rajesh et al. (2022) undertook serial measures of AGD in a relatively small cohort of 22 animals, from birth through until 16 months of age. Pearson's correlations between successive AGD phenotypes measured at 0, 2, 6, 9, 12 and 16 months of age indicated that AGD measured near maturity is more repeatable than AGD measured near birth. Rajesh et al. (2022) also took serial measurements of AGD in lactating cows, to determine the effect of stage of estrous cycle or stage of pregnancy on AGD. They reported that AGD was not affected by the estrous cycle and was highly repeatable throughout most of the gestation period, except for late gestation (270 d onwards) when AGD lengthened significantly. Rajesh et al. (2022) concluded that in lactating dairy cows, AGD could be measured anytime except late gestation. However, the results from Rajesh et al. (2022) indicate that the timing of the AGD measure could have implications on the association between AGD and other traits, for example fertility, particularly when AGD is measured in adolescent animals, and animals appear to re-rank substantially for the trait as they grow. Nevertheless, with just 22 animals this study did not have the statistical power to speculate on genetic correlations between measures taken at different ages, and it is possible that EBVs for AGD would be more robust to the timing of phenotype measurement.

Gobikrushanth et al. (2017)



Dean and Sharpe (2013)

Figure 1.4. Anogenital distance (AGD) in (a) cattle (Gobikrushanth et al., 2017) and (b) humans (Dean and Sharpe, 2013).

Phenotypic distribution

The AGD trait is highly variable and appears to follow a normal distribution in dairy cattle (Gobikrushanth et al., 2017, 2019; Carrelli et al., 2022), although the mean and variance of AGD may depend on selection history. Gobikrushanth et al. (2017) reported a mean and standard deviation of 131.0 ± 12.2 mm and in 921 North American Holstein-Friesians, with similar variances across parity. Gobikrushanth et al. (2019) measured AGD in a population of 1,180 Irish Holstein-Friesian cows reporting a smaller mean of 119.2 mm and a slightly smaller standard deviation of 11.6 mm. In a second cohort of North American Holstein-Friesians (n= 4,709), Carrelli et al. (2022) reported a mean of 132 mm and a standard deviation of 12 mm. The mean and variance in this larger population of North American Holsteins was consistent with those reported earlier by Gobikrushanth et al. (2017). The mean AGD has been shown to increase with age (Gobikrushanth et al., 2017; Rajesh et al., 2022), indicating that there is a relationship between BW or age and AGD. A relationship between AGD and BW is perhaps also evident in the smaller AGD measures observed in Irish Holstein-Friesians relative to North American Holstein-Friesians, as Irish Holstein-Friesians are selected for a pasture-based environment and tend to be smaller and lighter than their North American counterparts (Horan et al., 2005). Breed differences are an important consideration in the context of the NZ dairy industry, where the national herd is ad-mixed Jersey and Holstein-Friesian, two breeds which have a large difference in BW (Ahlborn and Dempfle, 1992). That said, the phenotypic correlations reported between AGD and BW and other stature traits is low (Gobikrushanth et al., 2017, 2019; Rajesh et al., 2022), which may suggest that the association exists between breed or maturity and AGD, rather than BW and AGD.

Heritability

Research into the genetic variance of AGD is very limited and, to my knowledge, the heritability of AGD has only been reported in one study to date. Gobikrushanth et al. (2019) reported a heritability of 0.37, with a standard error of 0.08, in their population of 1,130 mixed parity Irish Holstein-Friesian dairy cows. In the context of fertility traits, 0.37 is a relatively high heritability, and this is a promising indication that AGD could be a valuable predictor of correlated traits.

Association between AGD and Fertility

Non-zero phenotypic associations between AGD and reproductive development and performance have been reported for both males and females of several mammalian species, including rats (Zehr et al., 2001), mice (Hotchkiss et al., 2007), humans (Mendiola et al., 2012, 2016; Dean and Sharpe, 2013), and cattle (Gobikrushanth et al., 2017; Carrelli et al., 2022;

Madureira et al., 2022). The direction of these associations tends to be sexually dimorphic; in males, reproductive dysfunction has been associated with abnormally short AGD, whereas the opposite is true of females, wherein an abnormally long AGD is generally associated with reproductive dysfunction (Zehr et al., 2001; Hotchkiss et al., 2007; Madureira et al., 2022). Zehr et al. (2001) had a study population of 65 female rats, and they demonstrated that shorter AGD is associated with earlier puberty and shorter estrous cycles later in life. Similarly, Hotchkiss et al. (2007) measured a range of morphological traits in female mice that were exposed to varying pre-natal treatments of testosterone, which altered their AGD. Their study involved 24 pregnant female mice, that were evenly divided over a control and two testosterone treatment groups. They found that longer AGD was reliably associated with negative outcomes such as nipple retention and permanent masculinization of the reproductive tract (i.e., internal, and external genitalia was masculinized) in the female progeny of these trial mice. In humans, Mendiola et al. (2012) has associated longer AGD with increased follicle recruitment in a relatively small study population of 100 Spanish women, which could be a precursor to early depletion of follicular reserves. Interestingly, Mendiola et al. (2016) detected an association between shorter AGD and the presence of endometriomas in their case (n=114) and control (n=105) study, where cases were women who had been previously diagnosed with endometritis. The presence of endometriomas has negative implication for reproductive performance and so the results of this study were unusual, as most other studies involving female mammals have associated shorter AGD with positive fertility outcomes. Together, the results of Mendiola et al. (2012) and Mendiola et al. (2016) suggest that the association between AGD and fertility outcomes may not be linear in women. That is, both abnormally long and abnormally short AGD may be associated with poor fertility outcomes. Conversely, the association between AGD and reproductive development and performance in men seems to be more straight forward, with shorter AGD reliably associated with abnormalities of the reproductive tract, and poorer performance in traits such as sperm count (Dean and Sharpe, 2013). As far as I am aware there is no literature to support an association between abnormally long AGD in males, and negative reproductive outcomes.

In dairy cattle, the focus of research to date has been in females. The interest in AGD has primarily centered on evaluating the utility of AGD to predict fertility later in life. Gobikrushanth et al. (2017) investigated the relationship between AGD and two fertility phenotypes expressed during lactation (pregnancy rate to first AI and cumulative pregnancy rate by 250 d in milk). Their analysis included a total of 921 North American Holstein-Friesian cows, grouped according to parity (first-, second- or third and greater parities). They characterized cows as either short or long (≤ or >127.1 mm) AGD, and then compared performance across the two AGD groups. They reported that for the first and second parity cohorts, the cows in the longer AGD group required more inseminations to attain pregnancy and

had a reduced likelihood of being in calf by 250 d after calving. They did not detect any association between AGD and fertility outcomes in the oldest group of cows in their third or later parity, although poor fertility animals are likely to have been culled prior to attaining third and later parities. Gobikrushanth et al. (2019) then undertook a similar analysis in a population of 1,180 pasture-based Irish Holstein-Friesian cattle, where they categorized cows into quartiles, based on their AGD. They then compared the performance of the cows within each group across 6 fertility phenotypes. These phenotypes included the proportion of cows that presented for mating in the first 21 d of the seasonal mating period, proportion of the cows that were pregnant to their first AI, the proportion of cows that were pregnant within the first 21, 42 and 84 d of the seasonal mating period, and the number of times each cow was bred. They did not detect an association between any of these fertility phenotypes and the AGD quartiles. These results differed from those reported by Gobikrushanth et al. (2017) but there were several key differences between the two studies.

First, Gobikrushanth et al. (2017) separated their analysis by parity, while Gobikrushanth et al. (2019) included all parities in a single analysis. Later parity animals have generally been subjected to performance-based selection, where cows that fail to become pregnant again after each calving are removed from the herd (Macdonald and Roche, 2023). This pre-selection could affect the association between AGD and fertility outcomes and could explain the interaction of parity on the association that Gobikrushanth et al. (2017) reported between AGD and fertility traits, where they did not detect an association between AGD and fertility in cows that were in their third parity or later. It is possible that if Gobikrushanth et al. (2019) had separated their analysis into parity groups, they would have detected an association between AGD and fertility in lower parity cows. That said, Carrelli et al. (2022) have subsequently measured AGD in a much larger population of 4,790 North American Holstein-Friesians, with the primary purpose of validating the phenotypic analysis of Gobikrushanth et al. (2017). They reported that longer AGD was associated with poorer fertility outcomes across a range of fertility measures, including proportion of cows pregnant to first and second AI, total number of inseminations, number of inseminations to pregnancy, days open and proportion pregnant by 150 d and 250 d post-calving. This outcome occurred despite having analysed animals of all parities together (first: n = 1,719, second: n = 1,228 and third or greater: n = 1,762). Akbarinejad et al. (2019) also reported an association between AGD and fertility outcomes in a population of 86 mixed-parity Iranian Holstein-Friesians. In their study, they specifically tested the effect of parity within their analysis, and reported it as insignificant, although with just 86 animals they may have lacked the statistical power to detect an interaction.

The second difference between the Gobikrushanth et al. (2017) and Gobikrushanth et al. (2019) studies was the management systems that the animals were farmed within. The former

study population studied by Gobikrushanth et al. (2017) were housed in a barn and fed a total mixed ration diet with a year-round calving system, whereas the latter study population studied by Gobikrushanth et al. (2019) were managed in a pasture-based farm system with seasonal calving. It is possible that there is an interaction of environment on the association between AGD and fertility, and this interaction may lead to the association between AGD and fertility being non-significant in some populations.

The third difference between the two studies is the history of genetic selection for fertility, as selection for fertility in the last two decades has been more aggressive in Irish herds relative to North American herds. It is possible that this selection has modified the association between AGD and fertility. That said, NZ and Irish Holstein-Friesians have similar management conditions and a comparable history of genetic selection for fertility, and Grala et al. (2021) have reported results that align with Gobikrushanth et al. (2017). Grala et al. (2021) measured AGD in a relatively small population of 500 NZ born Holstein-Friesians that had either extremely high (+5) or extremely low (-5) parent average fertility EBVs. They reported an association between longer AGD and poorer outcomes for several fertility phenotypes in first parity cows. These phenotypes included proportion pregnant by week 3 and week 6 of the seasonal mating period, proportion pregnant following the completion of the mating period, and interval between calving and conception. Given the similarities between Irish and NZ Holstein-Friesians, it seems unlikely that the null associations reported by Gobikrushanth et al. (2019) are a result of grazing conditions or selection history.

The value of AGD as a predictor trait for fertility will be greater if AGD can be measured early in an animal's life. Most of the studies that have investigated the association between AGD and fertility in dairy cattle have involved cows that are first parity or later. Carrelli et al. (2021) addressed the question of whether AGD measured in nulliparous heifers was associated with fertility outcomes later in life. Their study included 1,692 North American Holstein-Friesian heifers from 16 commercial dairy herds in Canada. They reported that longer AGD was associated with an increased number of inseminations per conception (1.7 vs. 1.5), increased interval between calving and conception date (454.3 vs. 448.4 d), a lower proportion pregnant to first insemination (49.6 vs. 58.3%), and a greater risk of cows failing to become pregnant by the time they were 450 d old. The results of Carrelli et al. (2021) demonstrate that AGD could provide a predictor for fertility that can be measured months earlier than fertility traits expressed during lactation.

It is important to understand the association between AGD and other potential predictors of fertility in dairy cattle. A non-zero association between AGD and an existing predictor of reproductive success can improve our confidence in AGD as a predictor trait itself. Conversely, a null association between AGD and an alternative predictor trait may mean that they each add independent value to the prediction of fertility trait. Two traits that has been

previously investigated as predictors of fertility are antral follicle count (AFC) and concentrations of anti-Mullerian hormone (AMH). The AMH trait is a hormonal marker for AFC, and both traits have been established as predictors of reproductive competence in dairy cattle (Alward and Bohlen, 2020). Akbarinejad et al. (2019) measured both AGD and AMH in their population of 86 Iranian Holstein-Friesian cows and reported an association between longer AGD and higher AMH. This was an unexpected finding, as AMH is generally an indication of ovarian reserves, and therefore a higher AMH value is considered a desirable phenotype. Akbarinejad et al. (2019) discussed the possibility that high AMH in cattle may also be indicative of an analogous condition to polycystic ovaries (PCOS) in humans. Elevated AMH can be indicative of PCOS in women, which manifests as poor fertility outcomes. If the high AMH cattle in the Akbarinejad et al. (2019) study were suffering from PCOS-like symptoms, then the association between long AGD and high AMH would indicate a poorer fertility outcome for long AGD cows. It is worth noting that in the same population of cows, Akbarinejad et al. (2019) reported an association between long AGD and poorer fertility outcomes. Grala et al. (2021) measured AGD and AFC in their population of some 500 NZ Holstein-Friesian cows. They did not find any association between these two traits. In summary, it is possible that AGD shares an association with AMH; however, the complexities of the AMH trait make it difficult to interpret, as both high and low AMH have been established as predictors of poor fertility outcomes in mammals.

Madureira et al. (2022) investigated the associations between AGD and several potential predictors of fertility success in a population of 178 North American Holstein-Friesian heifers. The traits they analyzed included length and strength of estrus measured as the percent increase in activity, diameter of the CL, and PPAI measured as proportion of cows returned to estrus within 50-d postpartum. They reported that across all parity cohorts, longer AGD was associated with poorer performance, although they did note that there was an interaction of parity on the association between AGD and strength of estrus, whereby cows in second parity and later who were classified with long AGD had stronger estrus events than their short AGD counterparts. Another potential predictor of fertility success is the age that a heifer attains puberty. I am not aware of any studies that report the association between AGD and AGEP in dairy cattle; however, Zehr et al. (2001) reported that longer AGD was associated with a higher age at first estrus in a population of 65 female rats. With this in mind, it possible that AGD and AGEP are associated traits in dairy cattle, although further work is required to confirm this.

There is general agreement in the literature that that shorter AGD in cows is phenotypically associated with favorable fertility outcomes in dairy cattle. This association seems to be consistent across parities, although some researchers did detect an interaction of parity on the association between AGD and fertility. I note that the genetic association between AGD and fertility outcomes has not yet been established. While evidence of a phenotypic

association positions AGD as a promising candidate predictor of fertility EBVs, it is important that the genetic associations are directly estimated.

Association between AGD and non-fertility traits

The AGD trait may also exhibit non-zero genetic associations with traits other than fertility. It is important that these associations are understood prior to widespread use of AGD as a selection criterion, as selection could result in unexpected and detrimental genetic changes within a population. To our knowledge, the genetic associations between AGD and other traits in dairy cattle have not been estimated. However, as with the associations between AGD and fertility traits, several papers have included phenotypic associations between AGD and important non-fertility traits. Milk yield is a high priority trait for most dairy industries. Carrelli et al. (2022) reported a low positive association (r = 0.0017; $P < 0.01$) between 305 d milk yield and AGD in their population of 4,709 North American Holstein-Friesians. They concluded that selection favoring shorter AGD animals would likely compromise milk yield, but that this decrease in milk yield would be minor. Body size traits are sometimes included within a selection index as they provide information on the maintenance feed requirements of an animal. Gobikrushanth et al. (2017) reported a low positive association between AGD and hip height ($R^2 = 0.04$; $P < 0.01$) in a population of 921 lactating North American Holstein-Friesian cows. Gobikrushanth et al. (2019) subsequently confirmed this finding in a population of 1,190 lactating Irish Holstein-Friesians, where they reported a small but significant correlation ($R^2 = 0.06$; $P < 0.01$) between AGD and hip height. Gobikrushanth et al. (2019) also reported low but significant phenotypic association between AGD and both BW ($R^2 = 0.10$; $P < 0.01$) and body condition score ($R^2 = 0.02$; $P < 0.01$). In a third study, Rajesh et al. (2022) reported low positive associations between AGD and both body weight ($R^2 = 0.08$; $P = 0.04$) and hip height ($R^2 = 0.07$; $P = 0.06$). Although that study differed slightly as AGD, BW and hip height were measured at birth, but in a much smaller population of 48 heifer calves. The results of these three studies suggest that selection favoring shorter AGD might result in a slight size decrease in cattle. Further work is required to estimate genetic associations between AGD and important traits under selection.

GWAS of anogenital distance

Genome-wide association studies can help improve our understanding of the genetic architecture of not only the target trait, but also genetically correlated traits. For example, if AGD and fertility traits are genetically correlated, then a GWAS of AGD may help us understand the genetic architecture of fertility traits as well. Although a GWAS on the fertility trait of interest is a more direct approach, the low heritability of fertility traits compromises the

statistical power of a GWAS, meaning that where animal numbers are constant, a GWAS of a higher heritability predictor trait may yield more useful results. Most genomic evaluation systems make use of genotypes from commonly available versions of 50K SNP chips, where SNPs were initially selected to achieve even coverage across the genome. However, enriching a future SNP chip in a genomic region that is associated with a trait of interest can improve the accuracy of SNP predictions (Xiang et al., 2021). If AGD and target fertility traits are positively or negatively genetically correlated, the regions that are highlighted in the GWAS of AGD may also be relevant to target fertility traits. Therefore, enrichment of SNP chips in these areas could help improve genomic predictions of target fertility traits.

To my knowledge, there have only been two studies published that included GWAS analysis of AGD, and both of those studies involved Holstein-Friesian dairy cattle. Gobikrushanth et al. (2019) reported 6 SNP that they highlighted from their GWAS. The SNP were located on chromosomes 6 (1 SNP, 32.427–32.427 Mb), 15 (3 SNP, 40.947–41.007 Mb), 20 (1 SNP, 25.028–25.028 Mb) and 26 (1 SNP, 2.383–2.383 Mb). None of those SNP remained significantly associated with AGD once the authors had adjusted for multiple testing. A second GWAS analysis was published by Grala et al. (2021). They reported a single SNP that was significantly associated with AGD following application of a Benjamini-Hochberg–corrected 5% chromosome-wise significance threshold. No SNPs were associated with variation in AGD in common across both of these studies. This lack of concordance may be due to the genetic differences between the two study populations, as the cattle were Irish- (Gobikrushanth et al., 2019) versus NZ-born (Grala et al., 2021) and so may segregate differently at some loci. In addition, the population size in both studies were somewhat small and, as a result, they both will have been underpowered for GWAS analysis. Poor statistical power may explain both the lack of associated SNP, as well as the lack of SNP in common.

Conclusion

Both AGEP and AGD are moderately heritable and can be measured earlier in life than economically important fertility traits that are expressed during lactation, such as PPAI, mating events, successful or failed conceptions, and calving date traits. These characteristics position both traits as appealing predictors of fertility EBVs, but their utility to predict fertility EBVs depends on the strength of the genetic association between each candidate predictor and target fertility traits measured during lactation.

Current literature supports a genetic association between AGEP and fertility traits measured during lactation, although genetic correlations between AGEP and breeding, calving and pregnancy phenotypes in dairy cattle have not been directly published. Genetic analysis of the AGD trait has not been widely reported and, to date, the genetic correlations between AGD

and fertility phenotypes in dairy cattle are unknown. Furthermore, literature that includes parametrization of genetic (co)variance of AGD is limited, as most publications involving this trait have focused on phenotypic analysis.

Directly estimating of the genetic correlations between both AGEP and AGD and target fertility traits is a critical step towards establishing their utility as predictors of fertility. A robust genetic analysis requires large numbers of phenotyped individuals (that is, some 2,000 individuals or more). Therefore, a large-scale phenotyping initiative is required. Existing literature can provide insights into designing this phenotyping initiative, particularly for the AGEP trait where cost-savings can be made by simplifying the criteria an animal must meet to be classified as post-pubertal, and also accepting a degree of phenotyping censoring.

Knowledge of the genetic correlations between AGEP, AGD, and target fertility traits will provide the final piece of information that is required to quantify the utility of these two traits as predictors of fertility EBVs.

Thesis objectives

In this thesis, my key aim was to investigate the utility of AGEP and AGD as predictors of an animal's genetic merit for key fertility traits; breeding, calving and pregnancy in first and second lactation. The value of a predictor trait depends on the cost of measuring the trait, its heritability, and the genetic correlation that it exhibits with a trait under selection. In chapters 2 to 4, my objective was to providence evidence towards developing a cost-effective approach for measuring and evaluating AGEP across thousands of animals. In chapters 5 and 6, my objectives were three-fold. My first and main objective was to characterize the (co)variance parameters of AGEP, AGD and breeding, calving and pregnancy traits in first and second lactation. Secondly, I aimed to estimate the (co)variance parameters of AGEP, AGD and body stature traits measured as yearling heifers (height, length, body weight). Finally, I aimed to undertake a GWAS of both candidate predictor traits. I hypothesized that both AGD and AGEP would be moderately heritable in a study population of predominantly Holstein-Friesian NZ dairy cattle, and exhibit a moderate genetic correlation with breeding, calving and pregnancy phenotypes. Further, I hypothesized that my GWAS analysis would identify genomic regions associated with variance in each of these candidate predictor traits.

References

Abbott, D.H., D.A. Dumesic, J.R. Eisner, R.J. Colman, and J.W. Kemnitz. 1998. Insights into the development of Polycystic Ovary Syndrome (PCOS) from studies of prenatally androgenized female Rhesus monkeys. Trends in Endocrinology & Metabolism 9:1043–2760. doi:10.1016/S1043-2760(98)00019-8.

Abeygunawardena, H., and C.M.B. Dematawewa. 2004. Pre-pubertal and postpartum anestrus in tropical Zebu cattle. Anim Reprod Sci 82–83:373–387. doi:10.1016/j.anireprosci.2004.05.006.

Ahlborn, G., and L. Dempfle. 1992. Genetic parameters for milk production and body size in New Zealand Holstein-Friesian and Jersey. Livest Prod Sci 31:205–219. doi:10.1016/0301-6226(92)90018-Y.

Akbarinejad, V., F. Gharagozlou, M. Vojgani, E. Shourabi, and M.J.M. Makiabadi. 2019. Inferior fertility and higher concentrations of anti-Müllerian hormone in dairy cows with longer anogenital distance. Domest Anim Endocrinol 68:47–53. doi:10.1016/j.domaniend.2019.01.011.

Alward, K.J., and J.F. Bohlen. 2020. Overview of Anti-Müllerian hormone (AMH) and association with fertility in female cattle. Reproduction in Domestic Animals 55:3–10. doi:10.1111/rda.13583.

Archbold, H., L. Shalloo, E. Kennedy, K.M. Pierce, and F. Buckley. 2012. Influence of age, body weight and body condition score before mating start date on the pubertal rate of maiden Holstein-Friesian heifers and implications for subsequent cow performance and profitability. Animal 6:1143–1151. doi:10.1017/S1751731111002692.

Berardinelli, J.G., R.A. Dailey, R.L. Butcher, and E.K. Inskeep. 1979. Source of progesterone prior to puberty in beef heifers. J Anim Sci 49:1276–1280. doi:10.2527/jas1979.4951276x.

Berry, D.P., F. Buckley, P. Dillon, R.D. Evans, M. Rath, and R.F. Veerkamp. 2002. Genetic parameters for level and change of body condition score and body weight in dairy cows. J Dairy Sci 85:2030–2039. doi:10.3168/jds.S0022-0302(02)74280-X.

Berry, D.P., E. Wall, and J.E. Pryce. 2014. Genetics and genomics of reproductive performance in dairy and beef cattle. Animal 8:105–121. doi:10.1017/S1751731114000743.

Bowley, F.E., R.E. Green, P.R. Amer, and S. Meier. 2015. Novel approaches to genetic analysis of fertility traits in New Zealand dairy cattle. J Dairy Sci 98:2005–2012. doi:10.3168/jds.2014-8266.

Brotherstone, S., G. Banos, and M.P. Coffey. 2002. Evaluation of yield traits for the development of a UK fertility index for dairy cattle. Pages 1–28 in World Congress on Genetics Applied to Livestock Production.

Brownlie, T.S., J.M. Morton, C. Heuer, J. Hunnam, and S. McDougall. 2014. Reproductive performance of seasonal-calving, pasture-based dairy herds in four regions of New Zealand. N Z Vet J 62:77–86. doi:10.1080/00480169.2013.848499.

Bruns, C.M., S.T. Baum, R.J. Colman, J.R. Eisner, J.W. Kemnitz, R. Weindruch, and D.H. Abbott. 2004. Insulin resistance and impaired insulin secretion in prenatally androgenized male rhesus monkeys. Journal of Clinical Endocrinology and Metabolism 89:6218–6223. doi:10.1210/jc.2004-0918.

Butler, S.T., I.A. Hutchinson, A.R. Cromie, and L. Shalloo. 2014. Applications and cost benefits of sexed semen in pasture-based dairy production systems. Animal 8:165–172. doi:10.1017/S1751731114000664.

Byrne, T.J., B.F.S. Santos, P.R. Amer, D. Martin-Collado, J.E. Pryce, and M. Axford. 2016. New breeding objectives and selection indices for the Australian dairy industry. J Dairy Sci 99:8146–8167. doi:10.3168/jds.2015-10747.

Carrelli, J.E., M. Gobikrushanth, M. Corpron, I. Rajesh, W. Sandberg, M.G. Colazo, A. Ahmadzadeh, M. Oba, and D.J. Ambrose. 2021. Relationship of anogenital distance with fertility in nulliparous Holstein heifers. J Dairy Sci 104:8256–8264. doi:10.3168/jds.2020-19940.

Carrelli, J.E., M. Gobikrushanth, M. Corpron, W. Sandberg, I. Rajesh, A. Ahmadzadeh, M. Oba, and D.J. Ambrose. 2022. Associations between anogenital distance and measures of fertility in lactating North American Holstein cows: A validation study. J Dairy Sci 105:6339–6352. doi:10.3168/jds.2021-20827.

Challis, J.R.G., C.K. Kim, F. Naftolin, H.L. Judd, S.S.C. Yen, and K. Benirschke. 1973. The concentrations of androgens, oestrogens, progesterone and luteinizing hormone in the serum of foetal calves throughout the course of gestation. Journal of Endocrinology 60:107–115. doi:10.1677/joe.0.0600107.

Chenault, J.R., W.W. Thatcher, P.S. Kalra, R.M. Abrams, and C.J. Wilcox. 1975. Transitory changes in plasma progestins, estradiol, and luteinizing hormone approaching ovulation in the bovine. J Dairy Sci 58:709–717. doi:10.3168/jds.S0022-0302(75)84632-7.

Chun, S.-Y., K.M. Eisenhauer, S. Minami, H. Billig, E. Perlas, and A.J.W. Hsueh. 1996. Hormonal regulation of apoptosis in early antral follicles: follicle-stimulating hormone as a major survival factor. Endocrinology 137:1447–1456. doi:10.1210/endo.137.4.8625923.

Le Cozler, Y., V. Lollivier, P. Lacasse, and C. Disenhaus. 2008. Rearing strategy and optimizing first-calving targets in dairy heifers: A review. Animal 2:1393–1404. doi:10.1017/S1751731108002498.

DairyNZ. 2023a. All about BW. Accessed. https://www.dairynz.co.nz/animal/animal-evaluation/interpreting-the-info/breeding-worth/.

DairyNZ. 2023b. DairyNZ Bull Search. Accessed June 28, 2023. https://www.dairynz.co.nz/animal/animal-evaluation/bull-search/.

DairyNZ, and LIC. 2022. New Zealand Dairy Statistics.

Day, M.L., and L.H. Anderson. 1998. Current concepts on the control of puberty in cattle. J Anim Sci 76:1–15. doi:10.2527/1998.76suppl_31x.

Day, M.L., K. Imakawa, M. Garcia-Winder, D.D. Zalesky, B.D. Schanbacher, R.J. Kittok, J.E. Kinder, N. Lincoln, and R.L. Hruska. 1984. Endocrine mechanisms of puberty in heifers:

estradiol negative feedback regulation of luteinizing hormone secretion. Biol Reprod 31:332–241. doi:10.1095/biolreprod31.2.332.

Dean, A., and R.M. Sharpe. 2013. Anogenital distance or digit length ratio as measures of fetal androgen exposure: Relationship to male reproductive development and its disorders. Journal of Clinical Endocrinology and Metabolism 98:2230–2238. doi:10.1210/jc.2012-4057.

Dearborn, D.D., R.M. Koch, L. V Cundiff, K.E. Gregory, and G.E. Dickerson. 1973. An analysis of reproductive traits in beef cattle. J Anim Sci 36:1032–1040. doi:10.2527/jas1973.3661032x.

Dennis, N.A., K. Stachowicz, B. Visser, F.S. Hely, D.K. Berg, N.C. Friggens, P.R. Amer, S. Meier, and C.R. Burke. 2018. Combining genetic and physiological data to identify predictors of lifetime reproductive success and the effect of selection on these predictors on underlying fertility traits. J Dairy Sci 101:3176–3192. doi:10.3168/jds.2017-13355.

Dodson, S.E., B.J. McLeod, W. Haresign, A.R. Peters, and G.E. Lamming. 1988. Endocrine changes from birth to puberty in the heifer. J Reprod Fertil 82:527–538. doi:10.1530/jrf.0.0820527.

van den Driesche, S., H.M. Scott, D.J. MacLeod, M. Fisken, M. Walker, and R.M. Sharpe. 2011. Relative importance of prenatal and postnatal androgen action in determining growth of the penis and anogenital distance in the rat before, during and after puberty. Int J Androl 34:e578–e586. doi:10.1111/j.1365-2605.2011.01175.x.

Edwards, J.P. 2018. Comparison of milk production and herd characteristics in New Zealand herds milked once or twice a day. Anim Prod Sci 59:570–580. doi:10.1071/AN17484.

Forde, N., M.E. Beltman, P. Lonergan, M. Diskin, J.F. Roche, and M.A. Crowe. 2011. Oestrous cycles in Bos taurus cattle. Anim Reprod Sci 124:163–169. doi:10.1016/j.anireprosci.2010.08.025.

Fortes, M.R.S., S.A. Lehnert, S. Bolormaa, C. Reich, G. Fordyce, N.J. Corbet, V. Whan, R.J. Hawken, and A. Reverter. 2012. Finding genes for economically important traits: Brahman cattle puberty. Anim Prod Sci 52:143–150. doi:10.1071/AN11165.

Fortes, M.R.S., A. Reverter, S.H. Nagaraj, Y. Zhang, N.N. Jonsson, W. Barris, S. Lehnert, G.B. Boe-Hansen, and R.J. Hawken. 2011. A single nucleotide polymorphism-derived regulatory gene network underlying puberty in 2 tropical breeds of beef cattle. J Anim Sci 89:1669–1683. doi:10.2527/jas.2010-3681.

Garcia, S.C., and C.W. Holmes. 1999. Effects of time of calving on the productivity of pasture-based dairy systems: A review. New Zealand Journal of Agricultural Research 42:347–362. doi:10.1080/00288233.1999.9513384.

Garcia-Muniz, J.G. 1998. Studies of Holstein-Friesian cattle bred for heavy or light mature live weight. PhD Thesis. Massey University, Palmerston North.

Ginther, O.J., D.R. Bergfelt, M.A. Beg, and K. Kot. 2001. Follicle selection in cattle: role of luteinizing hormone. Biol Reprod 64:197–205. doi:10.1095/biolreprod64.1.197.

Gobikrushanth, M., T.C. Bruinjé, M.G. Colazo, S.T. Butler, and D.J. Ambrose. 2017. Characterization of anogenital distance and its relationship to fertility in lactating Holstein cows. J Dairy Sci 100:9815–9823. doi:https://doi.org/10.3168/jds.2017-13033.

Gobikrushanth, M., D.C. Purfield, J. Kenneally, R.C. Doyle, S.A. Holden, P.M. Martinez, E.R. Canadas, T.C. Bruinjé, M.G. Colazo, D.J. Ambrose, and S.T. Butler. 2019. The relationship between anogenital distance and fertility, and genome-wide associations for anogenital distance in Irish Holstein-Friesian cows. J Dairy Sci 102:1702–1711. doi:https://doi.org/10.3168/jds.2018-15552.

Gonzalez-Padilla, E., G.D. Niswender, and J.N. Wiltbank. 1975. Puberty in beef heifers. Effect of injections of progesterone and estradiol-17β on serum LH, FSH and ovarian activity. J Anim Sci 40:1105–1109. doi:10.2527/jas1975.4061105x.

Gonzalez-Recio, O., M.P. Coffey, and J.E. Pryce. 2014. On the value of the phenotypes in the genomic era. J Dairy Sci 97:7905–7915. doi:10.3168/jds.2014-8125.

Gordon, P. 2011. Farm animal practice: Oestrus detection in dairy cattle. In Pract 33:542–546. doi:10.1136/inp.d7479.

Grala, T.M., M.D. Price, B. Kuhn-Sherlock, C.R. Burke, and S. Meier. 2021. Investigating anogenital distance and antral follicle count as novel markers of fertility within a herd of cows with positive or negative genetic merit for fertility traits. J Dairy Sci 104:12939–12952. doi:10.3168/jds.2020-19948.

Green, M.J., L.E. Green, Y.H. Schukken, A.J. Bradley, E.J. Peeler, H.W. Barkema, Y. De Haas, V.J. Collis, and G.F. Medley. 2004. Somatic cell count distributions during lactation predict clinical mastitis. J Dairy Sci 87:1256–1264. doi:10.3168/jds.S0022-0302(04)73276-2.

Gregory, K.E., L. V Cundiff, R.M. Koch4, and R.L. Hruska. 1995. Genetic and phenotypic (co)variances for production traits of female populations of purebred and composite beef cattle. J Anim Sci 73:2235–2242. doi:10.2527/1995.7382235x.

Grosshans, T., Z.Z. Xu, L.J. Burton, D.L. Johnson, and K.L. Macmillan. 1997. Performance and genetic parameters for fertility of seasonal dairy cows in New Zealand. Livest Prod Sci 5:41–46. doi:10.1016/S0301-6226(97)00104-8.

Handcock, R.C., C.M.C. Jenkinson, R. Laven, L.R. McNaughton, N. Lopez-Villalobos, P.J. Back, and R.E. Hickson. 2021. Linear versus seasonal growth of dairy heifers decreased age at puberty but did not affect first lactation milk production. New Zealand Journal of Agricultural Research 64:83–100. doi:10.1080/00288233.2019.1607404.

Harris, B.L. 1998. Breeding dairy cattle for economic efficiency: A New Zealand pasture based system. Page in Proceedings of the 6th World Congress on Genetics Applied to Livestock Production.

Harris, B.L. 2005. Breeding dairy cows for the future in New Zealand. N Z Vet J 53:384–389. doi:10.1080/00480169.2005.36582.

Harris, B.L., J.E. Pryce, Z.Z. Xu, and W.A. Montgomerie. 2005. Fertility Breeding Values in New Zealand, the Next Generation. Interbull Bulletin 33:47–50.

Harris, B.L., J.E. Pryce, Z.Z. Xu, and W.A. Montgomerie. 2006. Development of new fertility breeding values in the dairy industry. Pages 107–112 in Proceedings of the New Zealand Society of Animal Production. New Zealand Society of Animal Production, Napier.

Hawken, R.J., Y.D. Zhang, M.R.S. Fortes, E. Collis, W.C. Barris, N.J. Corbet, P.J. Williams, G. Fordyce, R.G. Holroyd, J.R.W. Walkley, W. Barendse, D.J. Johnston, K.C. Prayaga, B. Tier, A. Reverter, and S.A. Lehnert. 2012. Genome-wide association studies of female reproduction in tropically adapted beef cattle. J. Anim. Sci 90:1398–1410. doi:10.2527/jas2011-4410.

Hickson, R.E., C.C. Balcomb, K.R. Fraser, N. Lopez-Villalobos, P.R. Kenyon, and S.T. Morris. 2011. The effect of breed on the onset of puberty in heifers. Pages 51–54 in Proceedings of the Association for the Advancement of Animal Breeding and Genetics, Perth, Australia.

Holman, A., J. Thompson, J.E. Routly, J. Cameron, D.N. Jones, D. Grove-White, R.F. Smith, and H. Dobson. 2011. Comparison of oestrus detection methods in dairy cattle. Veterinary Record 169:47–47. doi:10.1136/vr.d2344.

Horan, B., P. Dillon, P. Faverdin, L. Delaby, F. Buckley, and M. Rath. 2005. The interaction of strain of Holstein-Friesian cows and pasture-based feed systems on milk yield, body weight, and body condition score. J Dairy Sci 88:1231–1243. doi:10.3168/jds.S0022-0302(05)72790-9.

Hotchkiss, A.K., C.S. Lambright, J.S. Ostby, L. Parks-Saldutti, J.G. Vandenbergh, and L.E. Gray. 2007. Prenatal testosterone exposure permanently masculinizes anogenital distance, nipple development, and reproductive tract morphology in female sprague-dawley rats. Toxicological Sciences 96:335–345. doi:10.1093/toxsci/kfm002.

Irano, N., G.M.F. de Camargo, R.B. Costa, A.P.N. Terakado, A.F.B. Magalhães, R.M.D.O. Silva, M.M. Dias, A.B. Bignardi, F. Baldi, R. Carvalheiro, H.N. de Oliveira, and L.G. de Albuquerque. 2016. Genome-wide association study for indicator traits of sexual precocity in Nellore cattle. PLoS One 11. doi:10.1371/journal.pone.0159502.

Jayawardana, J.M.D.R., N. Lopez-Villalobos, L.R. McNaughton, and R.E. Hickson. 2023. Heritabilities and genetic and phenotypic correlations for milk production and fertility traits of spring-calved once-daily or twice-daily milking cows in New Zealand. J Dairy Sci 106:1910–1924. doi:10.3168/jds.2022-22431.

Johnsson, M., A.M. Johansson, T. Klingström, and E. Rius-Vilarrasa. 2022. The effect of recombination rate on genomic selection in simulation. Pages 853–856 in Proceedings of 12th World Congress on Genetics Applied to Livestock Production (WCGALP) . Wageningen Academic Publishers.

Johnston, D.J., S.A. Barwick, N.J. Corbet, G. Fordyce, R.G. Holroyd, P.J. Williams, and H.M. Burrow. 2009. Genetics of heifer puberty in two tropical beef genotypes in northern Australia and associations with heifer- and steer-production traits. Anim Prod Sci 49:399–412. doi:10.1071/EA08276.

Kerslake, J.I., P.R. Amer, P.L. O'Neill, S.L. Wong, J.R. Roche, and C.V.C. Phyn. 2018. Economic costs of recorded reasons for cow mortality and culling in a pasture-based dairy industry. J Dairy Sci 101:1795–1803. doi:10.3168/jds.2017-13124.

Lamm, C.G., P.M. Hastie, N.P. Evans, and J.E. Robinson. 2012. Masculinization of the distal tubular and external genitalia in female sheep with prenatal androgen exposure. Vet Pathol 49:546–551. doi:10.1177/0300985811419533.

Lefebvre, R., H. Larroque, S. Barbey, Y. Gallard, J.J. Colleau, A.L. Laine, D. Boichard, and P. Martin. 2021. Genome-wide association study for age at puberty and resumption of cyclicity in a crossbred dairy cattle population. J Dairy Sci 104:5794–5804. doi:10.3168/jds.2020-18228.

Little, W., C.B. Mallinson, D.N. Gibbons, and G.J. Rowlands. 1981. Effects of plane of nutrition and season of birth on the age and body weight at puberty of British Friesian heifers. Anim Prod 33:273–279. doi:10.1017/S0003356100031652.

Littlejohn, M., T. Grala, K. Sanders, C. Walker, G. Waghorn, K. MacDonald, W. Coppieters, M. Georges, R. Spelman, E. Hillerton, S. Davis, and R. Snell. 2012. Genetic variation in PLAG1 associates with early life body weight and peripubertal weight and growth in Bos taurus. Anim Genet 43:591–594. doi:10.1111/j.1365-2052.2011.02293.x.

Louca, A., and J.E. Legates. 1968. Production losses in dairy cattle due to days open. J Dairy Sci 51:573–583. doi:10.3168/jds.S0022-0302(68)87031-6.

Lund, T., F. Miglior, J.C.M. Dekkers, and E.B. Burnside. 1994. Genetic relationships between clinical mastitis, somatic cell count, and udder conformation in Danish Holsteins. Livest Prod Sci 39:243–268. doi:10.1016/0301-6226(94)90203-8.

Macdonald, K.A., C.B. Glassey, and R.P. Rawnsley. 2010. The emergence, development and effectiveness of decision rules for pasture based dairy systems. Proceedings of the 4th Australasian Dairy Science symposium 199–209.

Macdonald, K.A., L.R. McNaughton, G.A. Verkerk, J.W. Penno, L.J. Burton, D.P. Berry, P.J.S. Gore, J.A.S. Lancaster, and C.W. Holmes. 2007. A comparison of three strains of Holstein-Friesian cows grazed on pasture: Growth, development, and puberty. J Dairy Sci 90:3993–4003. doi:10.3168/jds.2007-0119.

Macdonald, K.A., J.W. Penno, A.M. Bryant, and J.R. Roche. 2005. Effect of feeding level pre- and post-puberty and body weight at first calving on growth, milk production, and fertility in grazing dairy cows. J Dairy Sci 88:3363–3375. doi:10.3168/jds.S0022-0302(05)73020-4.

Macdonald, K.A., J.W. Penno, J.A.S. Lancaster, and J.R. Roche. 2008. Effect of stocking rate on pasture production, milk production, and reproduction of dairy cows in pasture-based systems. J Dairy Sci 91:2151–2163. doi:10.3168/jds.2007-0630.

Macdonald, K.A., and J.R. Roche. 2023. Farm systems research at Ruakura–a 60-year legacy underpinning profitable and sustainable pasture-based dairy systems. New Zealand Journal of Agricultural Research 1–118. doi:10.1080/00288233.2023.2168707.

MacLeod, D.J., R.M. Sharpe, M. Welsh, M. Fisken, H.M. Scott, G.R. Hutchison, A.J. Drake, and S. Van Den Driesche. 2010. Androgen action in the masculinization programming window and development of male reproductive organs. Int J Androl 33:279–287. doi:10.1111/j.1365-2605.2009.01005.x.

Macmillan, K.L., and A.J. Peterson. 1993. A new intravaginal progesterone releasing device for cattle (CIDR-B) for oestrous synchronisation, increasing pregnancy rates and the treatment of post-partum anoestrus. Anim Reprod Sci 33:1–25. doi:10.1016/0378-4320(93)90104-Y.

Madureira, A.M.L., T.A. Burnett, J.E. Carrelli, M. Gobikrushanth, R.L.A. Cerri, and D.J. Ambrose. 2022. Anogenital distance is associated with postpartum estrous activity, intensity of estrous expression, ovulation, and progesterone concentrations in lactating Holstein cows. J Dairy Sci. doi:10.3168/jds.2022-21897.

Martin, L.C., J.S. Brinks, R.M. Bourdon, and L. V. Cundiff. 1992. Genetic effects on beef heifer puberty and subsequent reproduction. J Anim Sci 70:4006–4017. doi:10.2527/1992.70124006x.

McDougall, S., C. Heuer, J. Morton, and T. Brownlie. 2014. Use of herd management programmes to improve the reproductive performance of dairy cattle. Animal 8:199–210. doi:10.1017/S1751731114000457.

McDougall, S., F.M. Rhodes, and C.W.R. Compton. 2013. Evaluation of three synchrony programs for pasture-based dairy heifers. Theriogenology 79:882–889. doi:10.1016/j.theriogenology.2012.12.013.

McGrath ME, Mee JF, Snijders SEM, and O'Callaghan D. 2001. The effect of genotype on the onset of puberty and subsequent fertility in dairy heifers. Page 46 in Proceedings of the Agricultural Research Forum, Tullamore, Ireland.

Meier, S., B. Kuhn-Sherlock, P.A. Amer, J.R. Roche, and C.R. Burke. 2021a. Positive genetic merit for fertility traits is associated with superior reproductive performance in pasture-based dairy cows with seasonal calving. J Dairy Sci 104:10382–10398. doi:10.3168/jds.2021-20195.

Meier, S., L.R. McNaughton, R.C. Handcock, P.R. Amer, P.R. Beatson, J.R. Bryant, K.G. Dodds, R. Spelman, J.R. Roche, and C.R. Burke. 2021b. Heifers with positive genetic merit for fertility traits reach puberty earlier and have a greater pregnancy rate than heifers with negative genetic merit for fertility traits.. J Dairy Sci 104:3707–3721. doi:10.3168/jds.2020-19155.

Mendiola, J., M. Roca, L. Mínguez-Alarcón, M.P. Mira-Escolano, J.J. López-Espín, E.S. Barrett, S.H. Swan, and A.M. Torres-Cantero. 2012. Anogenital distance is related to ovarian follicular number in young Spanish women: a cross-sectional study. Environ Health 11:1–8. doi:10.1186/1476-069X-11-90.

Mendiola, J., M.L. Sánchez-Ferrer, R. JimCrossed Dénez-Velázquez, L. Cánovas-López, A.I. Hernández-Peñalver, S. Corbalán-Biyang, A. Carmona-Barnosi, M.T. Prieto-Sánchez, A. Nieto, and A.M. Torres-Cantero. 2016. Endometriomas and deep infiltrating endometriosis in adulthood are strongly associated with anogenital distance, a biomarker for prenatal hormonal environment. Human Reproduction 31:2377–2383. doi:10.1093/humrep/dew163.

Mialon, M.M., G. Renand, D. Krauss, and F. Ménissier. 1999. Puberty of Charolais heifers in relation to growth rate. 1. Phenotypic variability. Animal Research 48:413–426. doi:10.1051/animres:19990601.

Mialon, M.M., G. Renand, D. Krauss, and F. Ménissier. 2001. Genetic relationship between cyclic ovarian activity in heifers and cows and beef traits in males. Genetics Selection Evolution 33:273–287. doi:10.1186/1297-9686-33-3-273.

Miglior, F., A. Fleming, F. Malchiodi, L.F. Brito, P. Martin, and C.F. Baes. 2017. A 100-Year Review: Identification and genetic selection of economically important traits in dairy cattle. J Dairy Sci 100:10251–10271. doi:10.3168/jds.2017-12968.

Mihm, M., and E.C.L. Bleach. 2003. Endocrine regulation of ovarian antral follicle development in cattle. Anim Reprod Sci 78:217–237. doi:10.1016/S0378-4320(03)00092-7.

Mongkonpunya, K., Y.C. Lin, P.A. Noden, W.D. Oxender, and H.D. Hafs. 1975. Androgens in the bovine fetus and dam. Pages 489–493 in Proceedings of the Society for Experimental Biology and Medicine.

Moran, C., J.F. Quirke, and J.F. Roche. 1989. Puberty in heifers: a review. Anim Reprod Sci 18:167–182. doi:10.1016/0378-4320(89)90019-5.

Morris, C.A., and N.C. Amyes. 2005. Response to selection for age at puberty in an angus herd. Pages 157–160 in Proceedings of the Association for Advancement of Animal Breeding and Genetics.

Morris, C.A., R.L. Baker, S.M. Hickey, D.L. Johnson, N.G. Cullen, and J.A. Wilson. 1993. Evidence of genotype by environment interaction for reproductive and maternal traits in beef cattle. Anim Prod 56:69–83. doi:10.1017/S0003356100006176.

Morris, C.A., J.A. Wilson, G.L. Bennett, N.G. Cullen, S.M. Hickey, and J.C. Hunter. 2000. Genetic parameters for growth, puberty, and beef cow reproductive traits in a puberty selection experiment. New Zealand Journal of Agricultural Research 43:83–91. doi:10.1080/00288233.2000.9513411.

Mota, L.F.M., A.B. Carvajal, P.A. Bernardes, M.E. Buzanskas, F. Baldi, R.B. Lôbo, and D.P. Munari. 2022. Integrating genome-wide association study and pathway analysis reveals physiological aspects affecting heifer early calving defined at different ages in Nelore cattle. Genomics 114. doi:10.1016/j.ygeno.2022.110395.

Mota, R.R., S.E.F. Guimarães, M.R.S. Fortes, B. Hayes, F.F. Silva, L.L. Verardo, M.J. Kelly, C.F. de Campos, J.D. Guimarães, R.R. Wenceslau, J.M. Penitente-Filho, J.F. Garcia, and S. Moore. 2017. Genome-wide association study and annotating candidate gene networks affecting age at first calving in Nellore cattle. Journal of Animal Breeding and Genetics 134:484–492. doi:10.1111/jbg.12299.

Mrode, R.A. 2005. Linear Models for the Prediction of Animal Breeding Values.

Neal, M., and J.R. Roche. 2020. Profitable and resilient pasture-based dairy farm businesses in New Zealand. Anim Prod Sci 60:169–174. doi:10.1071/AN18572.

Nogueira, G.P. 2004. Puberty in South American Bos indicus (Zebu) cattle. Anim Reprod Sci 82–83:361–372. doi:10.1016/j.anireprosci.2004.04.007.

Norman, H.D., J.L. Hutchison, and R.H. Miller. 2010. Use of sexed semen and its effect on conception rate, calf sex, dystocia, and stillbirth of Holsteins in the United States. J Dairy Sci 93:3880–3890. doi:10.3168/jds.2009-2781.

Norman, H.D., J.R. Wright, M.T. Kuhn, S.M. Hubbard, J.B. Cole, and P.M. VanRaden. 2009. Genetic and environmental factors that affect gestation length in dairy cattle. J Dairy Sci 92:2259–2269. doi:10.3168/jds.2007-0982.

Oikawa, K., T. Yamazaki, S. Yamaguchi, H. Abe, H. Bai, M. Takahashi, and M. Kawahara. 2019. Effects of use of conventional and sexed semen on the conception rate in heifers: A comparison study. Theriogenology 135:33–37. doi:10.1016/j.theriogenology.2019.06.012.

Price, M.D., M.D. Camara, J.R. Bryant, S. Meier, and C.R. Burke. 2017. Genetic parameters of puberty estimated using two genetically divergent groups of Holstein-Friesian dairy heifers. Pages 529–532 in Proceedings of the Association for the Advancement of Animal Breeding and Genetics.

Pryce, J.E., M.P. Coffey, and S. Brotherstone. 2000. The genetic relationship between calving interval, body condition score and linear type and management traits in registered Holsteins. J Dairy Sci 83:2664–2671. doi:10.3168/jds.S0022-0302(00)75160-5.

Pryce, J.E., M. Haile-Mariam, P. Bowman, T. Nguyen, K. Konstantinov, G.J. Nieuwhof, and B.J. Hayes. 2013. Improving the reliability of fertility breeding values in Australian dairy cattle. Pages 33–36 in In Proceedings of the Association for the Advancement of Animal Breeding and Genetics. Association for the Advancement of Animal Breeding and Genetics.

Pryce, J.E., R. Woolaston, D.P. Berry, E. Wall, M. Winters, R. Butler, and M. Shaffer. 2014. World trends in dairy cow fertility. Pages 1–10 in 10th World Congress of Genetics Applied to Livestock Production.

Rajesh, I., M. Gobikrushanth, J.E. Carrelli, M. Oba, and D.J. Ambrose. 2022. Repeatability of anogenital distance measurements from birth to maturity and at different physiological states in female Holstein cattle. J Dairy Sci 86. doi:10.3168/jds.2021-21419.

Rendel, J.M., and A. Robertson. 1950. Estimation of genetic gain in milk yield by selection in a closed herd of dairy cattle. J Genet 50:1–8. doi:10.1007/BF02986789.

Reynolds, L.P., and D.A. Redmer. 1999. Growth and development of the corpus luteum. J Reprod Fertil Suppl 54:181–191.

Rhodes, F.M., S. McDougall, C.R. Burke, G.A. Verkerk, and K.L. Macmillan. 2003. Invited review: Treatment of cows with an extended postpartum anestrous interval. J Dairy Sci 86:1876–1894. doi:10.3168/jds.S0022-0302(03)73775-8.

Roche, J.R., N.A. Dennis, K.A. Macdonald, C.V.C. Phyn, P.R. Amer, R.R. White, and J.K. Drackley. 2014. Heifer rearing to optimise farm profitability. Pages 281–297 in Proceedings of the Australasian Dairy Science Symposium.

Dela Rue, B.T., C.R. Eastwood, J.P. Edwards, and S. Cuthbert. 2019. New Zealand dairy farmers preference investments in automation technology over decision-support technology. Anim Prod Sci 60:133–137. doi:10.1071/AN18566.

Saint-Dizier, M., and S. Chastant-Maillard. 2012. Towards an automated detection of oestrus in dairy cattle. Reproduction in Domestic Animals 47:1056–1061. doi:10.1111/j.1439-0531.2011.01971.x.

Short, R.E., and R.A. Bellows. 1971. Relationships among weight gains, age at puberty and reproductive performance in heifers. J Anim Sci 32:127–131. doi:10.2527/jas1971.321127x.

Smith, B.A., J.S. Brinks, and G.V. Richardson. 1989. Estimation of genetic parameters among reproductive and growth traits in yearling heifers. J Anim Sci 67:2886–2891. doi:10.2527/jas1989.00218812006700110007x.

Stachowicz, K., P.R. Amer, B. Falconer, and D.P. Garrick. 2021. New model for genetic evaluation of fertility in New Zealand dairy cattle. Pages 373–376 in Association for the Advancement of Animal Breeding and Genetics.

Stegemiller, M.R., G.K. Murdoch, T.N. Rowan, K.M. Davenport, G.M. Becker, J.B. Hall, and B.M. Murdoch. 2021. Genome-wide association analyses of fertility traits in beef heifers. Genes (Basel) 12:1–15. doi:10.3390/genes12020217.

Thankamony, A., V. Pasterski, K.K. Ong, C.L. Acerini, and I.A. Hughes. 2016. Anogenital distance as a marker of androgen exposure in humans. Andrology 4:616–625. doi:10.1111/andr.12156.

Tonsor, G.T., and N.J. Olynk. 2011. Impacts of Animal Well-Being and Welfare Media on Meat Demand. J Agric Econ 62:59–72. doi:10.1111/j.1477-9552.2010.00266.x.

Vargas, C.A., M.A. Elzo, C.C. Chase, P.J. Chenoweth, and T.A. Olson. 1998. Estimation of genetic parameters for scrotal circumference, age at puberty in heifers, and hip height in brahman cattle. J. Anim. Sci 76:2536–2541. doi:10.2527/1998.76102536x.

Verkerk, G. 2003. Pasture-based dairying: Challenges and rewards for New Zealand producers. Theriogenology 59:553–561. doi:10.1016/S0093-691X(02)01239-6.

Weller, J.I., E. Ezra, and M. Ron. 2017. Invited review: A perspective on the future of genomic selection in dairy cattle. J Dairy Sci 100:8633–8644. doi:10.3168/jds.2017-12879.

Welsh, M., P.T.K. Saunders, M. Fisken, H.M. Scott, G.R. Hutchison, L.B. Smith, and R.M. Sharpe. 2008. Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. Journal of Clinical Investigation 118:1479–1490. doi:10.1172/JCI34241.

Wickham, B.W., M.A. Belsey, R.G. Jackson, and W. Rumball. 1978. Evidence of genetic improvement of New Zealand dairy cattle. New Zealand Journal of Experimental Agriculture 6:101–113. doi:10.1080/03015521.1978.10426024.

Winkelman, A.M., D.L. Johnson, and B.L. Harris. 2015. Application of genomic evaluation to dairy cattle in New Zealand. J Dairy Sci 98:659–675. doi:10.3168/jds.2014-8560.

Wolcott, M.L., D.J. Johnston, and S.A. Barwick. 2014. Genetic relationships of female reproduction with growth, body composition, maternal weaning weight and tropical adaptation in two tropical beef genotypes. Anim Prod Sci 54:60–73. doi:10.1071/AN13012.

Xiang, R., I.M. MacLeod, H.D. Daetwyler, G. de Jong, E. O'Connor, C. Schrooten, A.J. Chamberlain, and M.E. Goddard. 2021. Genome-wide fine-mapping identifies pleiotropic and functional variants that predict many traits across global cattle populations. Nat Commun 12:1–13. doi:10.1038/s41467-021-21001-0.

Zehr, J.L., S.E. Gans, and M.K. McClintock. 2001. Variation in reproductive traits is associated with short anogenital distance in female rats. Dev Psychobiol 38:229–238. doi:10.1002/dev.1017.

CHAPTER 2. Variance parameter estimation for age at puberty phenotypes under two levels of phenotype censorship

Published in JDS Communications, 2022
https://doi.org/10.3168/jdsc.2022-0218

Variance parameter estimation for age at puberty phenotypes under two levels of phenotype censorship

M. Stephen*[1,4], S. Meier[1], M. Price[1], J.E. Pryce[2,3], C. Burke[1], C. Phyn[1], D.Garrick[4]

[1] DairyNZ Ltd, 605 Ruakura Road, Hamilton 3240, New Zealand

[2] Agriculture Victoria Research, AgriBio, Centre for AgriBioscience, Bundoora, Victoria 3083, Australia

[3] School of Applied Systems Biology, La Trobe University, Bundoora, Victoria 3083, Australia

[4] AL Rae Centre for Genetics and Breeding - Massey University, Ruakura, Hamilton 3214, New Zealand

* Corresponding Author

Melissa Stephen

DairyNZ Ltd, 605 Ruakura Road, Hamilton 3240, New Zealand

+6427 836 6294

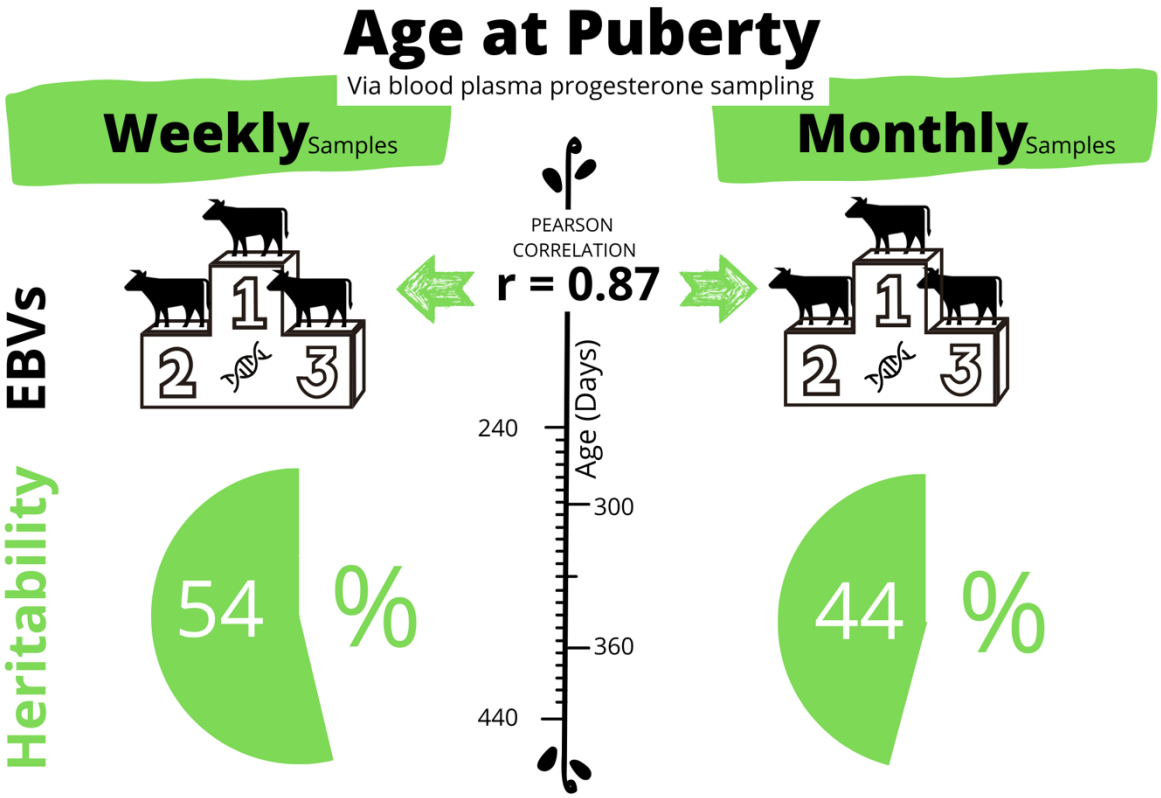
melissa.stephen@dairynz.co.nz

ORCID: https://orcid.org/0000-0002-1210-5139

Highlights

- Age at puberty (AGEP) is moderately heritable in NZ Holstein-Friesian cattle
- The estimated heritability of AGEP is robust to phenotype censorship.
- AGEP EBVs for animals in this population are robust to phenotype censorship.

Graphical Abstract



Summary: We measured AGEP in a closely monitored population of around 500 Holstein-Friesian cows, born in 2015 and managed under a seasonal, pasture-based dairy system. Animals were blood tested weekly from approximately 240 to 440 days old and were deemed to have reached puberty once blood plasma progesterone (BP4) elevation (>1 ng/mL) was detected in two of three consecutive blood tests (AGEP_Weekly). To simulate a simplified phenotyping strategy based upon monthly herd visits (AGEP_Monthly), we selectively disregarded data from all but three blood test events, when animals were around 300, 330 and 360 days old (SD = 14.5 days). The correlation between EBVs for AGEP_Weekly and AGEP_Monthly was 0.87 with a 90% credibility interval (CRI) of 0.84 to 0.89. The posterior mean of estimated heritabilities for AGEP_Weekly was 0.54 (90% CRI 0.41 to 0.66). Our results support the strategic use of censoring to reduce costs and animal ethics considerations associated with collection of puberty phenotypes.

Abstract

Age at puberty (AGEP) is a moderately heritable trait in cattle that may be predictive of an animal's genetic merit for reproductive success later in life. In addition, under some mating strategies (for example, where mating begins before all animals have attained puberty) animals that attain puberty at a relatively young age will also likely conceive earlier than their herd mates, and thus begin their productive life earlier. Unfortunately, AGEP is challenging to measure as animals must be observed over a period of several months. Our objectives for this study were two-fold. First, to produce variance components for AGEP. Second, to investigate the implications of a simplified phenotyping strategy for AGEP, when the interval between repeated blood plasma progesterone (BP4) measures was extended from weekly to monthly, increasing the extent of left-, interval- and right- censoring. We measured AGEP in a closely monitored population of around 500 Holstein-Friesian heifers, born in 2015 and managed under a seasonal, pasture-based dairy system. Animals were blood tested weekly from approximately 240 to 440 days of age and were deemed to have reached puberty when BP4 elevation (>1 ng/mL) was detected in two of three consecutive blood tests (AGEP_Weekly). To simulate a simplified phenotyping strategy based upon monthly herd visits (AGEP_Monthly), we selectively disregarded data from all but three blood test events, when animals were around 300, 330 and 360 days of age (SD = 14.5 d). The posterior mean of estimated heritabilities for AGEP_Weekly was 0.54, with a 90% credibility interval (90% CI) of 0.41 to 0.66, whereas it was 0.44 (90% CI 0.32 to 0.57) for AGEP_Monthly. The correlation between EBVs for AGEP_Weekly and AGEP_Monthly was 0.87 (90% CI, 0.84 to 0.89). We conclude that in this population, AGEP is a moderately heritable trait. Further, increasing phenotype censorship from weekly to monthly observations would not have altered the main conclusions of this analysis. Our results support the strategic use of censoring to reduce costs and animal ethics considerations associated with collection of puberty phenotypes.

Key words: Puberty, Gibbs Sampling, MCMC, Heritability, Censored

Body of Paper

Reproductive success is a key driver of a dairy cow's lifetime profitability. This is especially true under seasonal, pasture-based grazing systems, where a strictly annual calving pattern dictates that cows must normally resume estrus activity and become pregnant within an 85-d window post-calving. As such, fertility is an important component of the national breeding objective for dairy cattle both in New Zealand (NZ), and around the world (Pryce et al., 2014). Unfortunately, many fertility phenotypes that are of direct economic importance have low

heritability, and those that are easy to measure are not expressed before the cow is well into its first lactation. The current breeding objective trait for fertility in NZ is derived from the timing of a cow's calving in second lactation relative to the herd's seasonal calving start date. That trait has a heritability of less than 10% (Harris et al., 2006; Bowley et al., 2015), and the phenotype is not expressed until a cow is a 3-yr old. Hence, phenotypes that can be measured earlier in an animal's life and can provide a good prediction of an animal's genetic merit for reproductive success would be of high value, particularly if the phenotype has a moderate to high heritability.

Age at puberty (AGEP) is a possible candidate trait that meets these criteria. The reported heritabilities of AGEP in cattle range from 0.10 to 0.56 (Smith et al., 1989; Fortes et al., 2012), indicating that it is a moderately heritable trait. Dairy heifers typically reach puberty when they are around 12-mo old, which means AGEP phenotypes are measured substantially earlier than mating- and calving-related phenotypes measured during lactation. There is also a growing body of evidence supporting a genetic relationship between AGEP and subsequent fertility success. For example, Meier et al. (2021) reported that two lines of NZ Holstein-Friesian heifers with a divergence of around 1.3 genetic standard deviations in parent average cow fertility had a 28-d phenotypic difference in AGEP. Furthermore, Lefebvre et al. (2021) estimated a genetic correlation of 0.45 (SE ± 0.23) between AGEP and post-calving interval to resumption of cyclicity in a population of French Holstein-Normandy cross cattle.

Unfortunately, AGEP is challenging to measure precisely. Animals in a contemporary group can attain puberty over a window of time that spans several months. Numerous and frequent-repeated observations are required to ascertain an animal's precise AGEP. Two common methods for determining an animal's pubertal status involve detecting the presence or absence of an active corpus luteum. The first using ultrasound scanning to visualize the animals' ovaries (Fortes et al., 2012), and the second using blood testing for elevated blood plasma progesterone (BP4) concentrations, for example, (Lefebvre et al., 2021). A third common method involves visually monitoring the animals for signs of estrus, such as mounting behavior (Morris and Amyes, 2005). These indicator phenotypes require a substantial amount of effort and resources to obtain, and daily observations across large cohorts of animals are simply not feasible; however, costs can be constrained by reducing the frequency of observations, and that strategy is common in the literature. The phenotypes analyzed by Fortes et al. (2012) were derived from ultrasonography conducted at approximately monthly intervals, whereas the phenotypes analyzed by Lefebvre et al. (2021) were derived from blood samples taken at 10-d intervals. Although phenotype censoring is a useful strategy for reducing effort and resource requirements, there is an unavoidable trade-off against phenotype accuracy. That said, Donoghue et al. (2004) investigated this trade-off in the context of right-censored fertility phenotypes, where they simulated uncensored, 12% and 20% right-censored phenotypes. They did not report significant differences in variance parameters and determined that sire EBV

rankings were largely consistent across censorship scenarios. Here, we aimed to characterize the heritability of AGEP in a research population of approximately 500 Holstein-Friesian dairy cows (Meier et al., 2021). A second key aim was to determine the implications of left-, interval- and right-censoring of AGEP on the outcomes of subsequent analysis. Our hypothesis was that phenotype censorship would not meaningfully alter the estimated heritability of AGEP in our population, nor the EBV rankings of animals.

We used data collected from a purpose-bred research herd of approximately 500 NZ Holstein-Friesian cows, born in 2015. The population and sampling procedure were described comprehensively by Meier et al. (2021). Briefly, these cattle resulted from planned seasonal matings (where inbreeding coefficients between mating pairs were <12%) designed to generate a herd with extreme divergence in parent average genetic merit for the NZ Fertility Breeding Value (positive line; POS +5% EBV, negative line; NEG -5% EBV). Milk volume, fat, protein and liveweight parent average EBVs and proportion of North American Holstein ancestry were constrained to be similar (within 1 SD) in the two lines. A total of 67 sires were represented in this population, with 24 and 43 in the POS and NEG fertility groups, respectively. Following rearing, animals were managed on the same farm location in one of four different herd grazing groups, which were based upon date of birth, while balanced for fertility group.

Animals were blood sampled weekly from approximately 190 kg BW (~240-d of age) through until they either met the criterion for having attained puberty, or 3-wk after the start of the seasonal breeding period (~440-d of age). Concentrations of BP4 were measured in all these samples, as previously described by Meier et al. (2021).

We used two methods to derive an AGEP phenotype using BP4 for every animal. The first method included all available BP4 values, resulting in a weekly testing interval for most animals. Under this method, an animal's AGEP was defined as their age on the day when BP4 concentrations were first observed >1 ng/mL, provided BP4 was also elevated on either of the next two blood test days (AGEP_Weekly). If an animal had no measured elevation in BP4 during the study (n=36), their AGEP was set as their age on the last blood test day plus a 7-d penalty. The mean difference in AGEP_Weekly between the POS and NEG fertility groups was approximately 28-d. That said, there was substantial crossover in the distributions of phenotypes from the two groups. The min and max phenotypes in the POS and NEG groups were 255 to 457-d and 285 to 472-d, respectively. The second, more censored version of this phenotype was derived from the same data, but we selectively disregarded most weekly blood test days to simulate a herd visit testing regime with only 3 visits at ~30-d intervals (AGEP_Monthly). We chose which 3 blood test days we would include based on the average age of the animals at each visit. We aimed to have roughly even numbers of animals with left- and right- censored phenotypes, and so we selected the second blood test day for when the average age of the animals in each grazing group was ~330-d of age. At this age we would expect roughly 45% of

the animals to have attained puberty (Dennis et al., 2018). To produce our AGEP_Monthly phenotype, we defined the AGEP for each animal as their age on the first of the three visits that their BP4 concentration was observed >1 ng/mL. If an animal had no measured elevation in BP4 during these visits (n=299), their AGEP was set as their age on the last of the three blood test visits plus a 31-d penalty. This penalty was chosen based on the assumption that most animals would have attained puberty prior to a fourth herd visit. AGEP_Monthly represents a phenotype with left-, interval- and right censorship.

The blood sampling regime for this trial meant that once an animal had been confirmed as pubertal (that is, the criteria for AGEP_Weekly had been met), future samples from the animal were not quantified for BP4. This meant that due to missing data, some animals that attained puberty during the trial were not observed to have BP4 elevation on any test days that were included to produce the AGEP_Monthly phenotype. In this situation, the animal's AGEP_Monthly phenotype was set as their age on the first (monthly) test that was included after they met the AGEP_Weekly criteria, as if the sampling approach had actually been monthly, these animals would not have been missing and would have been confirmed as post-pubertal on this visit. For example, under the AGEP_Monthly scenario, between 13% and 20% of animals were missing on the visit 3 blood test day from each grazing group because they had previously been confirmed as pubertal.

On average, around 10% of animals were also missing from each of the AGEP_Monthly test days for other reasons (that is, they were not sampled on the test day and had not previously met the AGEP_Weekly criteria). These animals did not have the opportunity to be observed as pubertal until at least the following visit, and as such their AGEP_Monthly phenotype may have been inflated by 30-d.

All the animals were genotyped by GenomNZ (AgResearch, NZ) using a GeneSeek GGP Bovine 150K SNP array (Illumina, USA). Most other relatives in our genotype database were tested on the Weatherbys Versa 50k SNP array (Weatherbys, Ireland), so we imputed the genotypes to that content using FImpute software (Sargolzaei et al., 2014). The GeneSeek GGP Bovine 150K SNP array has around 40K SNP in common with the Weatherbys Versa 50k SNP array. For the analyses, we used only the SNP content from the Weatherbys Versa 50k SNP array, disregarding a further 2,120 SNP with a minor allele frequency < 1%, leaving some 46,577 SNP.

We fitted a linear SNP effects model to AGEP phenotypes to estimate fixed herd grazing group effects, random SNP effects, and variance parameters. Matrix representation of the linear mixed model equation is:

$$\mathbf{y=Xb+Ma+e} \qquad \text{Equation 1}$$

where **y** is a vector of phenotypes (one phenotype per study animal, either AGEP_Weekly or AGEP_Monthly), **b** is a vector of herd grazing group effects, and **a** is a vector of SNP effects. The vector **e** is a vector of residuals corresponding to each of the phenotypes. The incidence matrix **X** relates each phenotype record to relevant fixed effects. The covariate matrix **M** relates each phenotype record to the alleles present at each SNP locus. **M** has a column for each SNP locus, and a row for each phenotype. We ran two versions of this model, the first (model 1) included only herd grazing group (4 levels) as a fixed effect. The second (model 2) included herd grazing group and fertility group (2 levels) as fixed effects. Model 2 was included to provide a lower bound for variance parameter estimation, recognizing that the divergent herd structure could be manifesting as inflated heritabilities in our analysis using model 1 (Price et al., 2017), see the following section for further details.

A Markov Chain Monte Carlo (MCMC) technique was applied using a single site Gibbs sampler to obtain samples from the posterior distributions of variance parameters as well as the fixed and SNP effects. BayesC methodology was used, where Pi (the proportion of SNP loci with 0 effect) was assumed to be 0.99, which meant about 460 SNPs were fit in each sample to explain differences between 525 animals. The MCMC comprised 50,000 samples of every unknown parameter, with the first 10,000 samples disregarded as a burn-in. Prior values for genetic and residual variances were 297 d^2 and 603 d^2, respectively for all analyses based on existing NZ data (Dennis et al., 2018). We produced 90% CI based on thresholds for the 5% (lower bound) and 95% (upper bound) percentiles. We tested our analyses for evidence of non-convergence using the method described by (Geweke, 1992). In addition to this diagnostic, we observed trace plots to visually assess the convergence of each parameter.

The extent of re-ranking between EBVs produced using the AGEP_Weekly and the AGEP_Monthly phenotypes was quantified using the Pearson's correlation coefficient. Correlations included all animals with phenotypes (n = 525). We used a bootstrap method with replacement (Zhu, 1997) to generate the mean and 90% CI for the correlations between EBVs. These statistics represent a total of 1000 bootstrap samples.

The heritability and variance components for AGEP_Weekly and AGEP_Monthly phenotypes using both model 1 and model 2 are presented in Table 2.1. Using either phenotype there was a difference of around 0.10 in the heritabilities estimated using model 1 and model 2. The heritabilities estimated using AGEP_Weekly tended to be about 0.10 higher than those estimated using AGEP_Monthly, but there was a large overlap in 90% CI for both model 1 and 2. Conversely, the variances themselves were quite different, with higher genetic and residual variances observed when AGEP_Weekly phenotypes were used, compared with AGEP_Monthly phenotypes. This heterogeneous variance across the two phenotypes was observed using both model 1 and 2.

Table 2.1: Variance parameters for 'age of puberty' (AGEP) produced using weekly (AGEP_Weekly) or monthly (AGEP_Monthly) sampling. This table includes results (with 90% CI in parentheses) from two model equations. Model 1 included only herd grazing group as a fixed effect. Model 2 included both herd grazing group and fertility group as fixed effects.

	Model 1		Model 2	
Parameter	AGEP_Weekly	AGEP_Monthly	AGEP_Weekly	AGEP_Monthly
Genetic Variance	1000 (743,1262)	344 (239,449)	757 (508,1015)	250 (153,355)
Residual Variance	866 (633,1109)	431 (331,530)	967 (733,1213)	473 (371,578)
Phenotypic Variance	1866 (1710,2034)	775 (706,850)	1724 (1571,1890)	724 (656,796)
Heritability	0.54 (0.41,0.66)	0.44 (0.32,0.57)	0.44 (0.30,0.57)	0.35 (0.22,0.48)

The correlation between EBVs produced from model 1 and model 2 using the AGEP_Weekly phenotypes was 0.90 (90% CI: 0.90, 0.92). Similarly, the correlation between EBVs produced from model 1 and model 2 using the AGEP_Monthly phenotypes was 0.91 (90% CI 0.90, 0.92). Within fertility group these across-model correlations were >0.99. The correlation between EBVs produced using either AGEP_Weekly or AGEP_Monthly phenotypes from model 1 was 0.87 (90% CI 0.84, 0.89). The correlation between EBVs produced using either AGEP_Weekly or AGEP_Monthly phenotypes from model 2 was 0.84 (90% CI 0.81, 0.86). Within each fertility group these across-phenotype correlations ranged from 0.77 to 0.87. Using either model 1 and model 2 the correlations within the NEG fertility group tended to be lower than those within the POS fertility group.

Our AGEP phenotypes were measured in a research herd that consisted of two sub-populations with extremely divergent fertility EBVs. This pre-selection on fertility contributed to a divergence of 28-d in the AGEP_Weekly phenotype between the two divergent populations (Meier et al., 2021), and a slightly non-normal distribution in AGEP phenotypes in this research herd. We have analyzed AGEP in a univariate context, and as such, pre-selection on fertility EBVs is not implicitly taken into account. To address this pre-selection, we included a second analysis, using an alternative model equation (model 2) where fertility group is included as a fixed effect (Price et al., 2017). In model 1, herd grazing group was the only fixed effect, while in model 2, both herd grazing group and fertility group were fitted as fixed effects. Both models included SNP effects. Under model 1, pre-selection on fertility is ignored, and it is possible that this gives rise to an upward bias in our estimates of genetic variance and heritability of AGEP in this population. Under model 2, pre-selection on fertility is accounted for by omitting comparisons between animals across the two fertility groups; however, analysis using model 2

is likely to produce a downward bias in our estimates of the genetic variance and heritability. In this way, the results produced by model 1 and model 2 provide somewhat of an upper and lower bound for heritability in this pre-selected population. Hence, we estimate that the heritability of AGEP in this population falls between 0.30 (lower 90% CI under model 2) and 0.66 (upper 90% CI under model 1) when using weekly BP4 testing. Under increased censorship using AGEP_Monthly phenotypes, these lower and upper bounds are 0.22 and 0.57, respectively. Therefore, in this population, AGEP was a moderately heritable trait, similar to the range of heritabilities reported in other populations (Smith et al., 1989; Fortes et al., 2012). Moreover, we did not observe meaningfully different heritability estimates under increased phenotype censorship by reducing the frequency of BP4 testing or the length of the observation period.

The high correlation between EBVs estimated using AGEP_Weekly phenotypes and those estimated using AGEP_Monthly phenotypes indicated that increasing phenotype censorship did not substantially affect animal EBV rankings. This finding is relevant for animal breeders aiming to apply selection to the AGEP trait in large populations. It is likely that a simplified phenotyping strategy of monthly observations can be implemented, without substantial implications on animal selection decisions.

The variances of the AGEP_Weekly and AGEP_Monthly phenotypes were heterogeneous. This would make it difficult to combine phenotypes that were collected under different phenotyping regimes (for example, where AGEP was measured monthly in one population, and weekly in another). This should not be a problem in a research context, where the same phenotyping strategy is applied to all animals; however, implementation in large-scale animal evaluation schemes may require further work to establish a method for combining censored and uncensored phenotypes in a single analysis. For example, a multi-trait approach could be explored.

The main benefits of the AGEP_Monthly phenotyping strategy are the reduced costs, increased practicality and improved animal ethics considerations associated with measuring the trait. Reducing these barriers to data collection would likely result in an increased number of cows with phenotypes, relative to the number cows that could feasibly be measured for AGEP_Weekly. To capture the value of these additional phenotypes, we would need to manipulate the number of animals contributing phenotypes to each of the AGEP_weekly and AGEP_Monthly analyses. That is, reduce the number of animals included in the AGEP_Weekly analysis. Our current dataset is too small to test the implications of a relative difference in population size between censorship levels. Our results could be extended either using simulated AGEP phenotypes, or those collected from a larger scale phenotyping initiative. Moreover, collecting AGEP phenotypes for a larger number of animals would be beneficial to investigate the suitability of AGEP as an early predictor trait of subsequent reproductive success. Using a

more censored approach to collect AGEP phenotypes less frequently and over a shorter period will enable this research to progress.

We conclude that AGEP is a moderately to highly heritable trait in dairy cattle. If AGEP were to be measured at a large scale, phenotype censorship could provide a useful strategy for reducing costs and logistical challenges associated with phenotype collection, without compromising the integrity of the subsequent analysis.

Notes

This study was funded by NZ dairy farmers through DairyNZ Inc. and by the NZ Ministry of Business, Innovation and Employment (DRCX1302). Additional in-kind support and animal data were gratefully received from Livestock Improvement Corporation (LIC) and CRV (Hamilton, NZ).

Authors have not stated any conflicts of interest.

References

Bowley, F.E., R.E. Green, P.R. Amer, and S. Meier. 2015. Novel approaches to genetic analysis of fertility traits in New Zealand dairy cattle. J Dairy Sci 98:2005–2012. doi:10.3168/jds.2014-8266.

Dennis, N.A., K. Stachowicz, B. Visser, F.S. Hely, D.K. Berg, N.C. Friggens, P.R. Amer, S. Meier, and C.R. Burke. 2018. Combining genetic and physiological data to identify predictors of lifetime reproductive success and the effect of selection on these predictors on underlying fertility traits. J Dairy Sci 101:3176–3192. doi:10.3168/jds.2017-13355.

Donoghue, K.A., R. Rekaya, and J.K. Bertrand. 2004. Comparison of methods for handling censored records in beef fertility data: Simulation study. J Anim Sci 82:351–356. doi:https://doi.org/10.2527/2004.822351x.

Fortes, M.R.S., S.A. Lehnert, S. Bolormaa, C. Reich, G. Fordyce, N.J. Corbet, V. Whan, R.J. Hawken, and A. Reverter. 2012. Finding genes for economically important traits: Brahman cattle puberty. Anim Prod Sci 52:143–150. doi:10.1071/AN11165.

Geweke, J. 1992. Evaluating the accuracy of sampling-based approaches to the calculation of posterior moments. Bayesian Statistics 169–193.

Harris, B.L., J.E. Pryce, Z.Z. Xu, and W.A. Montgomerie. 2006. Development of new fertility breeding values in the dairy industry. Pages 107–112 in Proceedings of the New Zealand Society of Animal Production. New Zealand Society of Animal Production, Napier.

Lefebvre, R., H. Larroque, S. Barbey, Y. Gallard, J.J. Colleau, A.L. Laine, D. Boichard, and P. Martin. 2021. Genome-wide association study for age at puberty and resumption of

cyclicity in a crossbred dairy cattle population. J Dairy Sci 104:5794–5804. doi:10.3168/jds.2020-18228.

Meier, S., L.R. McNaughton, R.C. Handcock, P.R. Amer, P.R. Beatson, J.R. Bryant, K.G. Dodds, R. Spelman, J.R. Roche, and C.R. Burke. 2021. Heifers with positive genetic merit for fertility traits reach puberty earlier and have a greater pregnancy rate than heifers with negative genetic merit for fertility traits.. J Dairy Sci 104:3707–3721. doi:10.3168/jds.2020-19155.

Morris, C.A., and N.C. Amyes. 2005. Response to selection for age at puberty in an angus herd. Pages 157–160 in Proceedings of the Association for Advancement of Animal Breeding and Genetics.

Price, M.D., M.D. Camara, J.R. Bryant, S. Meier, and C.R. Burke. 2017. Genetic parameters of puberty estimated using two genetically divergent groups of Holstein-Friesian dairy heifers. Pages 529–532 in Proceedings of the Association for the Advancement of Animal Breeding and Genetics.

Pryce, J.E., R. Woolaston, D.P. Berry, E. Wall, M. Winters, R. Butler, and M. Shaffer. 2014. World trends in dairy cow fertility. Pages 1–10 in 10th World Congress of Genetics Applied to Livestock Production.

Sargolzaei, M., J.P. Chesnais, and F.S. Schenkel. 2014. A new approach for efficient genotype imputation using information from relatives. BMC Genomics 15. doi:10.1186/1471-2164-15-478.

Smith, B.A., J.S. Brinks, and G.V. Richardson. 1989. Estimation of genetic parameters among reproductive and growth traits in yearling heifers. J Anim Sci 67:2886–2891. doi:10.2527/jas1989.00218812006700110007x.

Zhu, W. 1997. Making bootstrap statistical inferences. Res Q Exerc Sport 68:44–55. doi:10.1080/02701367.1997.10608865.

CHAPTER 3. Comparison of methods for deriving phenotypes from incomplete observation data with an application to age at puberty.

Published in the Journal of Animal Science and Biotechnology
https://doi.org/10.1186/s40104-023-00921-5

Comparison of methods for deriving phenotypes from incomplete observation data with an application to age at puberty.

Melissa A Stephen*[1,4], Chris R Burke[1], Jennie E Pryce[2,3], Nicole M Steele[1], Peter R Amer[5], Susanne Meier[1], Claire VC Phyn[1], Dorian J Garrick[4]

[1] DairyNZ Ltd, 605 Ruakura Road, Hamilton 3240, New Zealand

[2] Agriculture Victoria Research, AgriBio, Centre for AgriBioscience, Bundoora, Victoria 3083, Australia

[3] School of Applied Systems Biology, La Trobe University, Bundoora, Victoria 3083, Australia

[4] AL Rae Centre for Genetics and Breeding - Massey University, Ruakura, Hamilton 3214, New Zealand

[5]AbacusBio, Dunedin, New Zealand

* Corresponding Author
Melissa Stephen
DairyNZ Ltd, 605 Ruakura Road, Hamilton 3240, New Zealand
+6427 836 6294
melissa.stephen@dairynz.co.nz
ORCID: https://orcid.org/0000-0002-1210-5139

Highlights

- The estimated heritability of age at first blood plasma progesterone elevation (> 1ng/mL) is robust to phenotype censorship.
- Using a data augmentation approach, a single observation per offspring yields comparable sire EBV rankings to three observations per offspring.

Abstract

Background: Many phenotypes in animal breeding are derived from incomplete measures, especially if they are challenging or expensive to measure precisely. Examples include time-dependent traits such as reproductive status, or lifespan. Incomplete measures for these traits result in phenotypes that are subject to left-, interval- and right-censoring, where phenotypes are only known to fall below an upper bound, between a lower and upper bound, or above a lower bound respectively. Here we compare three methods for deriving phenotypes from incomplete data using age at first elevation (> 1ng/mL) in blood plasma progesterone (AGEP4), which generally coincides with onset of puberty, as an example trait.

Methods: We produced AGEP4 phenotypes from three blood samples collected at about 30-day intervals from approximately 5,000 Holstein-Friesian or Holstein-Friesian x Jersey cross-bred dairy heifers managed in 54 seasonal-calving, pasture-based herds in New Zealand. We used these actual data to simulate seven different visit scenarios, increasing the extent of censoring by disregarding data from one or two of the three visits. Three methods for deriving phenotypes from these data were explored: 1) ordinal categorical variables which were analysed using categorical threshold analysis; 2) continuous variables, with a penalty of 31 days assigned to right-censored phenotypes; and 3) continuous variables, sampled from within a lower and upper bound using a data augmentation approach.

Results: Credibility intervals for heritability estimations overlapped across all methods and visit scenarios, but estimated heritabilities tended to be higher when left censoring was reduced. For sires with at least five daughters, the correlations between estimated breeding values (EBVs) from our three-visit scenario and each reduced data scenario varied by method, ranging from 0.65 to 0.95. The estimated breed effects also varied by method, but breed differences were smaller as phenotype censoring increased.

Conclusion: Our results indicate that using some methods, phenotypes derived from one observation per offspring for a time-dependent trait such as AGEP4 may provide comparable sire rankings to three observations per offspring. This has implications for the design of large-scale phenotyping initiatives where animal breeders aim to estimate variance parameters and estimated breeding values (EBVs) for phenotypes that are challenging to measure or prohibitively expensive.

Additional keywords: Gibbs sampler, Markov-chain Monte Carlo (MCMC), Cattle, Puberty

Background

Time-dependent traits can be logistically challenging and expensive to measure precisely, as animals need to be observed regularly over a long period of time. Some examples

include age at puberty (AGEP), mating and calving dates (particularly in the context of beef cattle) and lifespan (which requires culling or mortality dates). In the case of AGEP, indicator traits may be measured repeatedly over a period, with some pre-determined criteria to define an animal as either pubertal or non-pubertal at any given time. Possible measures include behavior monitoring to identify an estrus event, ultrasonography of ovaries to detect the presence or absence of a corpus luteum or testing for elevated blood plasma progesterone (BP4) concentrations that indicate the presence of a functioning corpus luteum (Morris et al., 2000; Macdonald et al., 2007; Hickson et al., 2011). Measuring these indicator traits often requires skilled professionals, specialized equipment, facilities, or laboratory resources, as well as a significant commitment from the herd owners who must make their animals available on multiple occasions while somewhat invasive measurements are obtained. These logistical and economic challenges mean that if the AGEP trait is to be measured at sufficient scale for genetic evaluations, it is preferable to establish a phenotype for each animal using as few observations as possible. Therefore, the AGEP trait provides a useful case study of a trait that is rarely measured precisely, and so phenotypes are often subject to censoring.

Past experiments which define the AGEP of individual heifers have measured animals at a range of intervals, including monthly (Johnston et al., 2009), weekly (Hickson et al., 2011) or daily (Grass et al., 1982; Morris et al., 2000). Researchers must optimize both the length of the observation window and the frequency of measures, according to the cost and effort associated with each additional measure. Optimizing measurement regimes requires assessing the added value of reducing left censoring (animals that are pubertal prior to the start of the observations), interval censoring (animals that become pubertal between two observations), and right censoring (animals that are not pubertal before the end of the observation window).
It is difficult to directly assess the implications of censoring on the genetic analysis of a trait like AGEP, as precise phenotypes are prohibitively difficult to measure at a large-scale. That said, high, positive correlations have been reported between EBVs produced using simulated phenotypes that were either uncensored, or subject to various left-, interval- or right-censoring combinations (Stephen et al., 2022a). Those findings indicate that heritability estimates and EBVs can be robust to phenotype censoring, although this may depend on the methods used to derive AGEP phenotypes from censored observations.

There are several methods that can be used for deriving phenotypes from incomplete observation data which involve converting the incomplete observations into categorical or continuous variables. Researchers often convert censored AGEP observation data into a continuous variable by defining AGEP as the age of the animal when it was first observed to meet the puberty criteria within a set observation period (Macdonald et al., 2007; Hickson et al., 2011; Fortes et al., 2012). According to that definition, left and interval censoring are usually ignored, but a penalty approach is often used to handle right censoring. Alternatively, a data

augmentation method can be used, where a lower and upper bound are established using incomplete observation data, and an animal's plausible phenotypes are sampled from a truncated normal distribution using a Gibbs sampling technique (Tanner and Wong, 1987). Data augmentation has been used previously to analyze simulated phenotypes subject to right-censoring (Donoghue et al., 2004a) and left-, interval- and right-censoring (Stephen et al., 2022a) with authors reporting only minimal differences between the analysis of censored and uncensored phenotypes. The method used to derive a phenotype from incomplete observations may be important, and a data augmentation approach has been shown to have a slight advantage over other common approaches, particularly for variance parameter estimation (Donoghue et al., 2004b).

Our primary objective was to investigate the sensitivity of estimated variance parameters and breeding values (EBVs) to varying degrees of observation censoring for a time-dependent trait, using real-life phenotype data. Our second objective was to compare three methods for deriving phenotypes from censored observations. We hypothesized that EBVs and variance parameters would be robust to phenotype censoring, regardless of statistical method. We used AGEP4 as an example trait, but the results of this study may be applicable to many other phenotypes that are derived from incomplete observation data.

Methods

Animals

The Ruakura Animal Ethics Committee (Hamilton, New Zealand) approved this study and all manipulations (AE application: 14448). Data were collected from 5,010 dairy heifers, born between July and September 2018 and reared in 54 seasonal calving; pasture-based herds located across three regions (Waikato, Taranaki, Otago) of New Zealand. The average number of heifers from each herd was 88 animals ± 45 (± standard deviation; SD). These 54 herds were selected based on the quality of the existing animal records and predominant breed, with a preference towards herds with mostly Holstein-Friesian animals. The breed proportions for each animal were provided by DairyNZ (Hamilton, New Zealand), and were derived using pedigree records. The resultant study animals were predominantly Holstein-Friesian (i.e., >90% Holstein-Friesian, n=2,307) or admixed Holstein-Friesian x Jersey crossbred (Holstein-Friesian and Jersey proportions sum to >90%, but neither Holstein-Friesian or Jersey are >90% independently, n=2,364), and a small number were predominantly Jersey (>90% Jersey, n=24). Our analysis also included 50 animals who could not be assigned to the Holstein-Friesian, Jersey or Holstein-Friesian x Jersey breed categories (Other, n=50). Animals with incomplete parentage (n = 132), incomplete observations data (n = 129), or issues with identification (n = 4)

were excluded from analysis, leaving 4,745 animals remaining (Table 3.1). A total of 103 sires were represented by at least 5 daughters.

Table 3.1 Population descriptive statistics of dairy heifers enrolled in age at first blood plasma progesterone elevation phenotype analysis.

Breed[1]	No. of Animals	No. of Herds	Average no. of Heifers	No. of Sires	No. Sires with > 5 Daughters
All	4,745	54	88	260	103
HF	2,307	54	43	166	66
J	24	1	24	7	2
XB	2,364	53	45	220	66
OTHER	50	19	3	35	0

[1]Breeds are defined as: > 90% Holstein-Friesian (HF); > 90% Jersey (J); HF + J > 90% but HF < 90% and J < 90% (XB); and HF + J < 90% (OTHER).

Sampling and measurements

Three sampling visits at approximately 30-day intervals were conducted for each of the 54 herds between May and August 2019. The timing was chosen to meet a target of the animals in each herd being, on average, 327 days old at the second visit, when 45% were predicted to be post-pubertal based up on the stochastic model of (Dennis et al., 2018). Accordingly, animals were, on average, 299, 327, and 354 days old (± 14.5) on the first, second and third visits, respectively. At each visit, blood was collected from a coccygeal vessel of animals using blood tubes containing lithium heparin (BD Vacutainers, BD New Zealand, Auckland, New Zealand). Blood samples were immediately placed on ice and were centrifuged (at 4°C, 1,900 × g for 12 min) on the same day as collection. Plasma was separated and stored at -20 °C until BP4 concentration was analysed using a commercial radioimmune assay kit, as previously described (Meier et al., 2021). An animal was classified as have elevated BP4 once it had one blood test result indicating a BP4 concentration >1 ng/mL. This aligns with the criteria previously implemented to characterize onset of puberty in a population of around 500 Holstein-Friesian cows (Meier et al., 2021).

Age at first blood plasma progesterone elevation phenotype analyses

We investigated three methods for deriving an AGEP4 phenotype from incomplete observation data. In the analyses presented here, every phenotype was derived from incomplete observations.

Firstly, for the visit category method (AGEP4cat), we defined the phenotype for each animal as the consecutive number of the first visit it was observed with BP4 >1 ng/mL (Table 3.2). Animals that were observed to have elevated BP4 on the first visit were assigned a score of one (left-censored phenotypes), whereas those first observed with BP4 elevation on the second or third visit were assigned scores of two or three, respectively. Animals with BP4 <1 ng/mL for the entire trial were assigned a score of four (right-censored phenotypes). We fitted a threshold model to these ordered categorical scores that assumed an underlying normally distributed liability variable, with fixed thresholds that mapped the unobserved liability to the visit score (Gianola and Foulley, 1983). Sire, rather than animal, was fitted as a random effect, herd was fitted as a fixed effect and breed was fitted as a fixed covariate to estimate variance components. Animal models were not used to analyze phenotypes from this method as convergence failure of the Gibbs sampler is not uncommon for this kind of categorical data ((Luo et al., 2001).

Secondly, we used an age on visit method (AGEP4av), with the phenotype for each animal defined as its age at the first visit that it was observed with BP4 >1 ng/mL (Table 3.2). This phenotype was treated as a continuous variable. Animals with BP4 <1 ng/mL for the entire trial (right-censored records) were assigned a penalized phenotype of 31 days older than their age on the last visit. A model fitting herd and breed as fixed effects and animal as a random effect was used to analyze this continuous trait (Henderson, 1988).

Thirdly, we used a data augmentation method (AGEP4aug), whereby the unobserved continuous variable representing actual age at first BP4 elevation was treated as an unknown variable whose value must fall between known upper and lower bounds (Table 3.2), and plausible values within these bounds were sampled using data augmentation (Tanner and Wong, 1987). The upper bound was the age of the animal at the visit it was first observed with BP4 >1 ng/mL (that is, we knew that they had experienced BP4 elevation on or before this age). The lower bound was the age of the animal at the previous visit (when it had BP4 <1 ng/mL). For example, the lower and upper bounds of an animal with BP4 >1 ng/mL on the second visit, would be its age on the first and second visits, respectively. The lower bounds for animals with BP4 >1 ng/mL on the first visit (left-censored phenotypes) were set to the very young value of 200 days of age, as we would expect all of the animals to be prepubertal, with basal BP4 levels at 200-d old (Dennis et al., 2018). The upper bounds for animals with BP4 <1 ng/mL throughout the three visits (right-censored phenotypes) were set to the very old value of 500 days, as we would expect all animals to be post-pubertal, with BP4 elevation by 500-d old (Dennis et al., 2018). Plausible AGEP4 phenotypes for each animal were sampled to produce a Markov-chain Monte Carlo (MCMC) posterior distribution for each animal's AGEP4 based on simultaneous sampling of fixed herd, fixed breed, and random animal effects and variance parameters using single site Gibbs sampling (Sorensen et al., 1998)

Table 3.2 Example phenotypes (age in days) for animals that have elevated blood plasma progesterone (BP4) or not (Y/N) at each herd visit (one, two or three) for: A) an animal with elevated blood plasma progesterone at visit one; B) an animal whose BP4 became elevated between visit one and visit two; C) an animal whose BP4 became elevated between visit two and visit three; and D) an animal whose BP4 became elevated after the third visit.

Example Animal	A	B	C	D
Actual age at BP4 elevation (AGEP4)	270	380	340	400
Age at visit one (BP4 status visit one)	280 (Y)	350 (N)	300 (N)	330 (N)
Age at visit two (BP4 status visit two)	310 (Y)	380 (Y)	330 (N)	360 (N)
Age on visit three (BP4 status visit three)	340 (Y)	410 (Y)	360 (Y)	390 (N)
AGEP4cat[1]	1	2	3	4
AGEP4av[2]	280	380	360	421
AGEP4aug[3]	200-280	350-380	330-360	390-500

[1] The number of the visit where the animal was first observed with elevated BP4.

[2]Age (in days) at the visit the animal was first observed with elevated BP4.

[3]Lower and upper bounds of age (in days) when the animal first had elevated BP4.

Model equation

We fitted a linear model to these data to estimate variance parameters, fixed herd and breed effects, and to obtain EBVs. Matrix representation of the linear mixed model equation is:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{e} \qquad \text{Equation 1}$$

where **y** is a vector of unobserved liabilities or phenotypes (as defined for each method), **b** is a vector of fixed effects, **u** is a vector of breeding values (random effects). The vector **e** is a vector of residuals corresponding to each of the phenotypes. **X** is an incidence matrix relating each phenotype record to relevant fixed effects. All analyses included herd as a fixed effect and proportion Jersey as a fixed covariate. The incidence matrix **Z** relates phenotypes to their corresponding EBVs, with a row for each phenotype and a column for each animal represented in **u**.

Visit scenarios

We produced a 'control' analysis for each of the three methods tested, where observations from all three visits were used. The results of these control analyses were then

compared (within method) with results of seven alternate test visit scenarios (Figure 3.1). Test visit scenarios varied in timing or frequency of the observations that were retained. Each test scenario had a proportion of data selectively excluded to alter left, right, or interval censoring. In total, we defined eight scenarios (Fig. 1). The first scenario (early, mid, and late; EML) represented the actual experiment comprising of three visit observations. The second scenario (early, mid, or late; E/M/L) simulated only one randomly assigned visit observation for each herd. The third, fourth and fifth scenarios simulated that all herds were only visited once and that visit is either E, M or L, respectively. In these visit scenarios (2 to 5) all phenotypes were subject to either left or right censoring, and the ratio was varied depending to which visit was included. The sixth, seventh and eighth scenarios simulated two observations per herd: early and mid (EM); mid and late (ML); or early and late (EL), respectively. In these visit scenarios (6 to 8) all phenotypes were subject to either left-, interval- or right- censoring. The ratio of phenotypes that were subject to left-, interval- or right-censoring varied depending on which visits were included.

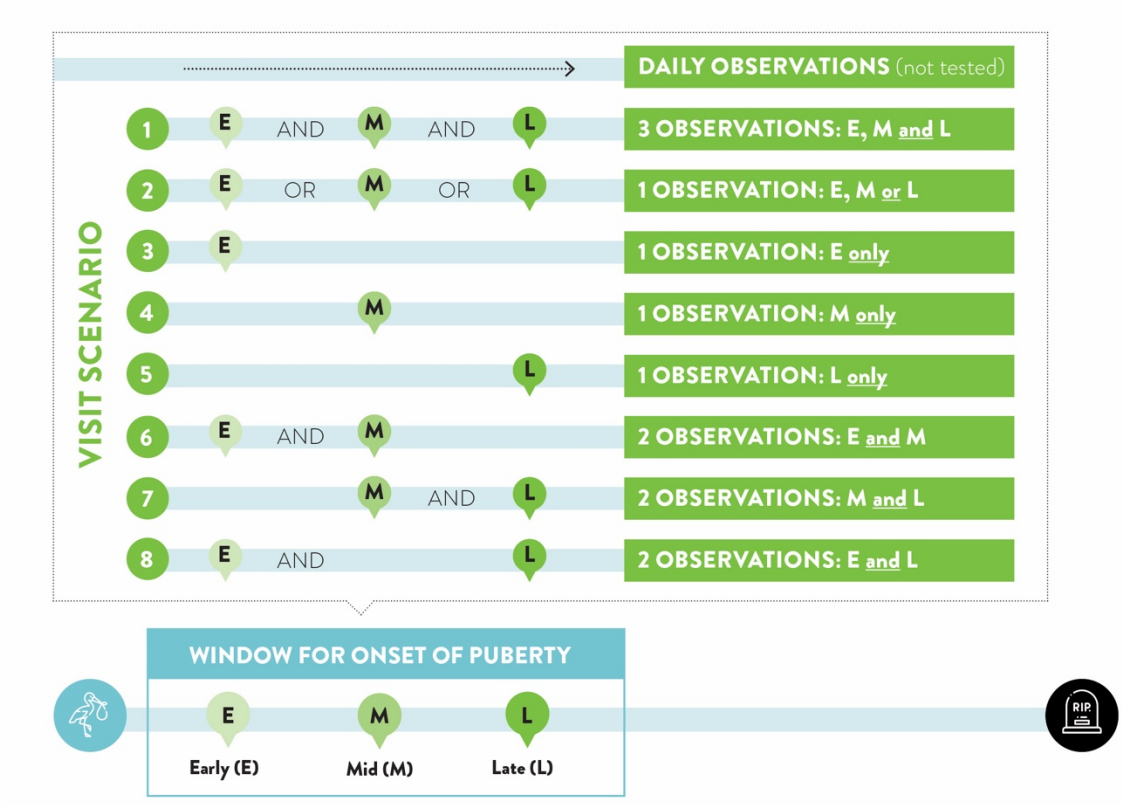


Figure 3.1 Eight scenarios varying the timing and number of herd visit observations to collect blood progesterone concentrations for measuring age at puberty (AGEP). The first scenario (early, mid, and late; EML) represented the actual experiment comprising of three visit observations. The second scenario (early, mid or late; E/M/L) simulated only one randomly assigned visit observation for each herd. The third, fourth and fifth scenarios simulate that all herds are only visited once E, M or L, respectively. The sixth, seventh and eighth scenarios

simulate two observations per herd: early and late (EL); mid and late (ML); or early and late (EL), respectively.

Software and solver

We used command line bash scripts to pre-process observation data and produce files containing the phenotypes for each method and visit scenario combination. We performed the genetic analysis and post-processing using the JWAS package (Cheng et al., 2018) implemented in Julia (Bezanson et al., 2017). A MCMC technique was applied using a single site Gibbs sampler to obtain samples from the posterior distributions for fixed and random effects, variance parameters, and in the case of the AGEP4aug method, plausible phenotypes within the known lower and upper bounds for each animal. The MCMC comprised 100,000 samples of every unknown, with the first 50,000 samples disregarded as a burn-in. The Julia packages CSV, StatsPlots, DataFrames were used to post-process the results. We assessed MCMC convergence by grouping post burn-in samples consecutively in lots of 10,000 (group one = sample 50,000 to 60,000, group two = sample 60,000 to 70,000 etc.) and comparing the mean and distribution of sample groups. The models were considered to have converged when the 95% credibility intervals consistently overlapped across groups.

We produced 95% credibility intervals that were thresholds for the 2.5% (lower bound) and 97.5% (upper bound) percentile of all values samples within the MCMC. That is, 95% of the plausible values fell within the credibility intervals presented.

Criteria for comparison

We used the stability of estimated variance parameters, breeding values (EBVs) and fixed effect solutions across varying degrees of phenotype censoring to assess the sensitivity of these parameters to increased phenotype censoring. That is, if a given parameter was impervious to increased phenotype censoring, we would expect no change in estimated variance parameters, breeding values (EBVs) and fixed effect solutions as phenotype censoring was increased. To assess stability of estimated variance parameters we compared the posterior mean and 90CRI of heritability estimates. Similarly, we also compared the posterior mean and 90CRI of fixed breed covariate solutions. To assess the stability of EBVs and fixed herd effect solutions we calculated the Pearson correlation coefficient between EBVs produced using our control scenario (early, mid and late visits included) and the EBVs produced using each test scenario. A higher correlation demonstrated greater stability.

Results

EBV correlations between visit scenarios

Correlations between sire EBVs (sires with >5 daughters, n=103) from the control scenario (EML) and scenarios E/M/L, E, M, L, EM, ML, and EL were positive and mostly high (AGEP4cat r=0.67 to 0.93, AGEP4av r=0.65 to 0.95, AGEP4aug r=0.75 to 0.95) (Table 3.3). Correlations decreased as phenotype censoring increased. Correlations with the control scenario, which had the most complete dataset, were lowest with scenario E/M/L, which had just one randomly timed visit per herd.

Among the methods, the AGEP4aug method generally had the highest correlations between the control scenario and the other scenarios. For scenarios E, M and L when there was a single visit to each herd, correlations with the control scenario ranged from r=0.74 to 0.83 for the AGEP4cat method, r=0.69 to 0.75 for the AGEP4av method, and r=0.83 to 0.87 for the AGEP4aug method. In contrast, for scenarios EM, ML and EL, when there were two visits to each herd, there were little differences across methods between the correlations with the control EML scenario (r=0.90 to 0.95), but none exceeded that of the AGEP4aug method.

Table 3.3 Correlations between estimated breeding values (EBVs) from scenario one relative to those from scenarios two to eight for the three different methods used to derive age at first blood plasma progesterone elevation (AGEP4) phenotypes. AGEP4cat: the number of the visit where the animal was first observed to have elevated BP4; AGEP4av: age in days at the visit the animal was first observed to have elevated BP4; and AGEP4aug: the continuous variable AGEP4 sampled from between the known lower and upper bounds. Correlations include within breed EBVs for sires that had >5 daughters with an AGEP4 phenotype (n = 103). Scenarios are described in Fig. 1, and analysis methods are described in Table 3.2.

Scenario		Correlations between EBVs relative to scenario one (EML)		
		AGEP4cat	AGEP4av	AGEP4aug
1	Early, mid and late visit (EML)	1.00	1.00	1.00
2	Early, mid or late visit (E/M/L)	0.67	0.65	0.75
3	Early visit only (E)	0.83	0.69	0.87
4	Mid visit only (M)	0.76	0.74	0.83
5	Late visit only (L)	0.74	0.75	0.83
6	Early and mid visit (EM)	0.94	0.95	0.95
7	Mid and late visit (ML)	0.90	0.95	0.94
8	Early and late visit (EL)	0.93	0.94	0.95

Breed Effect

We estimated the breed effect for each analysis (Table 3.4) as the difference in AGEP4 between Jersey and Holstein-Friesian animals. For the AGEP4cat and AGEP4aug methods, the breed difference was consistently negative (AGEP4cat on the one to four categorical scale: -1.67 to -0.87, AGEP4aug: -56 days to -25 days); whereas for the AGEP4av method, the credibility interval for breed difference spanned zero for the four scenarios based upon one herd visit (AGEP4av: -29 days to one day). For all methods, the size of the breed difference tended to decrease as phenotype censoring increased.

Table 3.4 Breed (Jersey relative to Holstein-Friesian) effect solutions and 95% credibility intervals from scenarios one to eight, for the three different methods used to analyze age at first blood plasma progesterone elevation (AGEP4) phenotypes. AGEP4cat: the number of the visit where the animal was first observed to have elevated BP4; AGEP4av: age in days at the visit the animal was first observed to have elevated BP4; and AGEP4aug: the continuous variable AGEP4 sampled from between the known lower and upper bounds. Scenarios are described in Fig. 1, and analysis methods are described in Table 3.2.

Scenario		Breed (Jersey) effect solution		
		AGEP4cat	AGEP4av	AGEP4aug
1	Early, mid and late visit (EML)	-1.62 (-1.26,-1.99)	-29 (-15,-43)	-56 (-34,-79)
2	Early, mid or late visit (E/M/L)	-1.31 (-0.87,-1.75)	0 (7,-7)	-40 (-18,-62)
3	Early visit only (E)	-1.58 (-1.09,-2.06)	-2 (5,-10)	-47 (-24,-72)
4	Mid visit only (M)	-1.47 (-1.01,-1.92)	-4 (3,-11)	-45 (-23,-67)
5	Late visit only (L)	-0.84 (-0.44,-1.26)	1 (8,-7)	-25 (-5,-44)
6	Early and mid visit (EM)	-1.67 (-1.26,-2.06)	-17 (-7,-27)	-54 (-34,-74)
7	Mid and late visit (ML)	-1.46 (-1.07,-1.85)	-16 (-6,-26)	-45 (-26,-64)
8	Early and late visit (EL)	-1.45 (-1.07,-1.83)	-24 (-11,-37)	-54 (-28,-78)

Herd Effects

We also estimated herd effects for each analysis (Table 3.5) based upon the expected phenotype of an average merit animal in that herd, where other fixed effects (in this case, proportion Jersey) were zero. Herd effect (n = 54) correlations between the control EML scenario and visit scenarios (E/M/L, E, M, L, EM, ML and EL) were positive and generally high across methods (AGEP4cat r = 0.45 to 0.99, AGEP4av r = 0.15 to 0.98, AGEP4aug r = 0.87 to 0.98). For all three methods, herd effect correlations decreased as phenotype censoring increased. The correlations of herd effect solutions between the control scenario and scenarios E, M and L, when only one visit was included per herd, were highest for the AGEP4aug method

(AGEP4cat r = 0.45 to 0.93, AGEP4av r = 0.15 to 0.80, AGEP4aug r = 0.87 to 0.95). The correlations of herd effect solutions between the control scenario and scenario E/M/L (which had just one randomly timed visit per herd) were lowest for the AGEP4av method (r = 0.15), and highest for the AGEP4aug method (r = 0.87). For scenarios EM, ML and EL, when two visits were included, the correlations of herd effect solutions from the control scenario were generally very high (AGEP4cat r = 0.51 to 0.99, AGEP4av r = 0.96 to 0.98, AGEP4aug r = 0.97 to 0.99).

Table 3.5 Correlations between herd (n = 54) effect solutions from scenario one relative to those from scenarios two to eight for the three different methods for analysing age at first blood plasma progesterone elevation (AGEP4) phenotypes. AGEP4cat: the number of the visit where the animal was first observed to have elevated BP4; AGEP4av: age in days at the visit the animal was first observed to have elevated BP4; and AGEP4aug: the continuous variable AGEP4 sampled from between the known lower and upper bounds. Scenarios are described in Fig. 1, and analysis methods are described in Table 3.2.

Scenario		Herd effect correlations relative to scenario one (EML)		
		AGEP4cat	AGEP4av	AGEP4aug
1	Early, mid and late visit (EML)	1.00	1.00	1.00
2	Early, mid or late visit (E/M/L)	0.47	0.15	0.87
3	Early visit only (E)	0.45	0.76	0.87
4	Mid visit only (M)	0.53	0.80	0.95
5	Late visit only (L)	0.93	0.76	0.93
6	Early and mid visit (EM)	0.51	0.97	0.98
7	Mid and late visit (ML)	0.97	0.96	0.97
8	Early and late visit (EL)	0.99	0.98	0.99

Heritability

The credibility intervals for heritabilities overlapped by scenario (rows) and methods (columns), as presented in Table 3.6. Nevertheless, the AGEP4cat method tended to produce the highest heritabilities across all scenarios, whereas the AGEP4av method produced the lowest heritabilities, with intermediate heritabilities resulting from the AGEP4aug method. Furthermore, heritabilities tended to be higher when scenarios included the ‘early’ observation (i.e., EML, E, EM, or EL scenarios).

Table 3.6 Heritabilities and 95% credibility intervals from scenarios one to eight for the three different methods used to analyze age at first blood plasma progesterone elevation (AGEP4) phenotypes. AGEP4cat: the number of the visit where the animal was first observed to have elevated BP4; AGEP4av: age in days at the visit the animal was first observed to have elevated BP4; and AGEP4aug: the continuous variable AGEP4 sampled from between the known lower and upper bounds. Scenarios are described in Fig. 1, and analysis methods are described in Table 3.2.

Scenario		Heritability		
		AGEP4cat	AGEP4av	AGEP4aug
1	Early, mid and late visit (EML)	0.39 (0.24,0.58)	0.23 (0.16,0.32)	0.32 (0.21,0.46)
2	Early, mid or late visit (E/M/L)	0.23 (0.13,0.38)	0.19 (0.13,0.28)	0.21 (0.11,0.35)
3	Early visit only (E)	0.36 (0.20,0.58)	0.29 (0.20,0.40)	0.31 (0.14,0.54)
4	Mid visit only (M)	0.23 (0.12,0.39)	0.19 (0.13,0.27)	0.19 (0.10,0.33)
5	Late visit only (L)	0.20 (0.10,0.35)	0.16 (0.10,0.22)	0.14 (0.05,0.26)
6	Early and mid visit (EM)	0.40 (0.24,0.62)	0.22 (0.15,0.30)	0.30 (0.19,0.45)
7	Mid and late visit (ML)	0.26 (0.15,0.42)	0.17 (0.11,0.23)	0.23 (0.11,0.36)
8	Early and late visit (EL)	0.33 (0.19,0.50)	0.19 (0.12,0.26)	0.28 (0.17,0.41)

Discussion

Sensitivity of sire EBV rankings to increased censoring depended on analysis method

In this study, we quantified the extent of sire re-ranking based on their AGEP4 EBVs across scenarios with different phenotype censoring using a Pearson correlation coefficient (Table 3.3). We determined that the sensitivity of sire re-ranking to increased censoring varied across the three analysis methods investigated. A correlation close to one between EBVs from two different phenotyping scenarios indicated that there was little re-ranking among sires, and sire selections would be similar irrespective of scenario. Conversely, a correlation close to 0 between EBVs from two different scenarios indicated that there was a large degree of re-ranking among sires. When there was large re-ranking of sires between scenarios, it follows that sire selection and thus genetic progress (Rendel and Robertson, 1950) will depend on the timing and frequency of observations. We determined that of the three analysis methods explored, the AGEP4aug method provided the most robust EBVs across the eight scenarios tested, implying

that reducing the number of herd visits was most feasible when using data augmentation to define AGEP4 phenotypes.

Sire EBV rankings were robust (r= >0.90) for all methods between our control scenario (EML) and scenarios with two observations included (i.e., EM, ML, and EL). These high correlations indicate that sire selections would be similar if offspring had two or three observations, and, in general, those two observations can be any combination of the early, mid-point or late herd visits. Our results indicate that for the purpose of determining sire rankings for genetic selection, there may be limited marginal gain in a third observation per animal. This finding could be useful for large-scale phenotype collection for routine genetic evaluations or for future trial design, as AGEP and other time-dependent binary traits can be logistically and economically difficult to measure.

The marginal gain of a second visit appears to be higher than that of a third visit, as greater re-ranking was apparent across all methods when scenarios included only one visit (i.e., E/M/L, E, M, or L). However, the extent of EBV re-ranking depended on the analysis method; EBVs produced by the AGEP4aug method were the most robust to this degree of phenotype censoring based upon a single observation. Furthermore, the timing of the single visit affected sire re-ranking. For the AGEP4cat and AGEP4aug methods, scenario E based on a single 'early' visit per herd resulted in the least re-ranking relative to our control scenario. In general, the AGEP4av method resulted in the most re-ranking when only a single visit was included, but the timing of a single visit did not appear to be as important. These results suggest that EBVs calculated using the AGEP4aug method are robust using a single observation per animal, potentially reducing the data measurement effort required and improving the scalability of phenotype collection. For example, a single observation per animal could enable a larger number of animals to be measured, which would improve the accuracy of sire EBVs, increasing the number of selection candidates, and thus the selection intensity for the trait.

Furthermore, the E/M/L scenario represents a likely practical measurement regime should a phenotype like AGEP4 be measured at scale. Seasonal calving dates tend to be aligned within regions of New Zealand and other countries that have pasture-based dairy systems, which means that the average ages of birth year groups are similar across herds. Hence, it may be infeasible to collect heifer BP4 status across large numbers of herds at a specific average age. Instead, it would be more reasonable to recommend that animals are measured on a single visit within a defined age window. We were not able to fully test this scenario using our current data, as our visits were scheduled to occur at only three average herd ages (297, 327, 357 days). However, if this phenotype was measured at scale, we would likely obtain BP4 status for a sire's offspring across a more diverse range of ages, which may minimize sensitivity to censoring. Nevertheless, it will be important to fully quantify the implications of random visit times, as it is also possible that under this constraint, a single observation does not provide

adequate data to inform sire ranking. That is, two or more observations may be required if the timing of observations cannot be aligned across herds.

Estimated breed effects depended on analysis method and phenotype censoring

The breed effects, which represent the estimated difference between breeds, depended on analysis method and scenario for phenotyping. The negative Jersey breed effect solutions when all three observation visits were included (scenario EML) indicated that Jersey animals experienced BP4 elevation before Holstein-Friesian animals, although the size of this difference varied by analysis method. Furthermore, within each analysis method, the estimated breed difference was smallest when the scenario did not include the 'early' visit (that is, when left censoring was increased). It has been previously reported that Jersey animals attained puberty 70 days earlier than Holstein-Friesian animals in a New Zealand system (Hickson et al., 2011). A breed difference of 70 days falls within the upper end of our credibility intervals under the AGEP4cat and AGEP4aug methods when the scenarios included the 'early' observation; however, using the AGEP4av method we did not estimate a plausible breed difference of up to 70 days under any scenario. Our results viewed alongside previous research indicate that the breed difference for AGEP4 is well estimated for the AGEP4cat and AGEP4aug methods when three observations are included but may become underestimated as the phenotype becomes more censored, especially left censored. Conversely, the breed difference under the AGEP4av method may be consistently underestimated across all phenotyping scenarios.

It is important that differences between breeds are well estimated. First, in a multi-breed analysis, the accuracy of breed effects will influence the dispersion parameters, and thus affect estimates of heritability. When breed is fitted as a fixed effect in a genetic analysis, the heritability should represent the proportion of phenotypic variance that is due to within breed additive genetic variance (that is, variance that cannot be attributed to residuals and relevant fixed effects, including breed). If the breed effect is poorly estimated, then variance due to breed can be incorrectly attributed to additive genetic variance, thus inflating the estimated heritability. Second, the inaccuracy of breed differences will systematically effect EBV rankings when animals are compared across breed. In dairy sectors with mixed-breed populations, such as New Zealand, farmers are often provided with 'across breed' EBVs (that is, the fixed breed effects are added to the within-breed EBV). This allows farmers to select the highest-ranking animals on an index, or for a given trait, irrespective of breed composition. If the breed difference for a trait is not accurate, this will result in systematic under- or over-estimation of a certain breed, leading to suboptimal genetic selection decisions. Third, the estimated breed differences may be useful in optimizing future phenotype collection. For example, our results indicate that the model solutions are most sensitive to left censoring of the phenotype. The

estimated breed difference can provide useful insight for reducing left censoring when measuring this trait across breed.

The Jersey breed was not well represented by pedigree Jerseys, as in this study as there were only 24 animals that were >90% Jersey, and they were all in the same contemporary group. However, around half of the animals included in this study were admixed crosses between the Holstein-Friesian and Jersey breeds, and these cross-bred animals were present in 53 of the 54 herds. The large number of cross-bred animals provided a reasonable basis for estimating a breed difference between Holstein-Friesians and Jerseys, as around half of the phenotypes included in the analysis will contribute to the solution for this fixed covariate. That said, we were not able to separate the effects of heterosis and breed, as heterosis coefficients and breed fractions were correlated in this population. It is possible that if we were to repeat this study in a population with greater representation of 100% Jersey animals, we would find that the estimation of breed differences behaved differently across method and across scenarios. Hence, further investigations including data from this breed are required.

Sensitivity of herd effects to increased censoring depended on analysis method

We quantified the extent that herds re-rank based on mean AGEP4 across the phenotype censoring scenarios using a Pearson correlation coefficient. Our results indicated a large degree of re-ranking of herds between our control scenario and the test scenarios with increased censoring for the AGEP4cat and AGEP4av method. In contrast, the herd rankings under the AGEP4aug method were remarkably consistent across various visit scenarios. Similar to the estimation of breed differences, the accuracy of the herd effects are important for the accurate estimation of dispersion parameters and EBVs. The stability of herd effects between scenarios for the AGEP4aug method will contribute to the stability of EBVs between scenarios.

Heritabilities were robust to method and phenotype censoring

Estimated heritabilities for AGEP4 varied little between different methods or scenarios. We did, however, observe a tendency for higher heritabilities when the ‘early’ visit was included in the analysis. This aligns with indications that breed and herd effects appear better estimated under scenarios that minimize left censoring. Including the early visit in the analysis differentiates animals with left-censored phenotypes and provides separation between their phenotypes and those of the remaining animals in the study. It would seem that this is an important distinction to make when estimating variance parameters, although the importance of including the early visit may be relative to the extent of left-censoring in the dataset. Across the methods and regimes analysed, the heritability for AGEP4 was estimated between 0.05 and

0.60, with a mean of around 0.25. This spread in heritabilities is comparable to those reported in current literature, which range from 0.10 to 0.56 (Smith et al., 1989; Fortes et al., 2012).

General Limitations

Using our method of AGEP4 phenotyping based on one to three herd visits to collect BP4, there will be some animals with false negatives for BP4 status. Concentrations of BP4 are cyclic in post-pubertal heifers and are not elevated for about one week of the three-week estrous cycle. Hence, these periods of naturally low BP4 in post-pubertal animals will have produced false negatives in our data (i.e., a pubertal animal will present with basal BP4 roughly 30% of the time). The four-week interval between herd visits means that natural BP4 depression in post-pubertal animals can only create a maximum of one false negative record per animal. False negatives will mean that some animals will be penalized incorrectly. False negatives may increase the residual variance associated with our AGEP4 phenotypes, and therefore reduce the estimated heritability of our phenotype. That said, we would expect false negatives to occur at random across our population, without bias towards the daughters of any particular sire. Therefore, we would not expect false negatives to have implications for sire rankings. This theory is supported by the findings of (Stephen et al., 2022b) where the authors tested the implications of phenotype censoring using AGEP4 phenotypes measured in a small population of some 500 cows measured weekly. In that study, AGEP phenotypes were compared under two levels of censoring. The least censored version of the phenotype involved an extended period of weekly blood testing, which would essentially eliminate the occurrence of false negatives in the phenotypes. The second, more censored version of the phenotype mirrored the phenotyping strategy that we have used in the present study. Their results indicated that phenotype censoring in this manner had minimal implications on animal EBV rankings.

The ‘control’ scenario presented here used only monthly measures and therefore was already censored, and this limits our ability to make inference about the implications of censoring. Our results indicate that the marginal gain of a third BP4 observation is not likely to justify the cost or effort associated with this measurement. The least censored version of this phenotype would be daily observations for each animal over the complete window where it was biologically possible for them to attain puberty; however, we were not able to test the implications of moving from no censoring (daily observations) to three observations per daughter. That said, Stephen et al. (2022a) recently investigated the implications of phenotype censoring using simulated AGEP phenotypes. In their study, the censoring scenarios mirrored most of those included in the present study (all except the E/M/L scenario), but the control scenario used uncensored daily phenotypes. The authors used a data augmentation approach and reported only minimal differences between either heritability estimates or EBVs across censoring scenarios.

Conclusions

It may be feasible for animal breeders with a given budget to increase the number of animals measured in a progeny test by reducing the number of observations per animal when collecting performance data for time-dependent traits such as AGEP. The three main findings of our study are, first, using the three analysis methods investigated, two observations per offspring may provide comparable sire rankings to three observations per offspring. Second, using a data augmentation approach, one observation per offspring may provide comparable sire rankings to three observations per offspring. Third, using any of the three methods investigated, one or two observations per offspring may provide comparable estimated heritabilities. However, it is worth noting that breed differences tended to decrease as phenotype censoring increased, and so care should be taken when applying these findings to an across-breed evaluation system.

Our findings have implications for the design of large-scale phenotyping initiatives as reducing the number of observations per animal for time-dependent traits could lead to a larger number of animals phenotyped, potentially improving accuracy and intensity of selection.

Abbreviations

AGEP: Age at puberty

AGEP4: Age at first elevation (> 1ng/mL) in blood plasma progesterone

AGEP4aug: Data augmentation method

AGEP4av: Age on visit method

AGEP4cat: Visit category method

BP4: Blood plasma progesterone

E: Early

EBVs: Estimated Breeding Values

EL: Early and Late

EM: Early and Late

E/M/L: Early, Mid or Late

HF: Holstein-Friesian

J: Jersey

L: Late

M: Mid

MCMC: Markov-chain Monte Carlo

ML: Mid and Late

N: No

XB: Cross-breed

Y: Yes

Declarations

Ethics approval and consent to participate.

The Ruakura Animal Ethics Committee (Hamilton, New Zealand) approved this study and all manipulations (AE application: 14448). Animals remained in the care of their owners for the duration of the study, and each owner provided informed consent regarding the involvement of their animals in this study.

Consent for publication

Not applicable

Availability of data and material

The datasets generated and/or analyzed during the present study are only available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was funded by New Zealand dairy farmers through DairyNZ Inc. and by the New Zealand Ministry of Business, Innovation and Employment (DRCX1302). Additional in-kind support was kindly received from LIC and CRV.

Authors' contributions

N.M. Steele, C.R. Burke, C.V.C. Phyn, S. Meier and M. A. Stephen designed and managed the animal trial. M.A. Stephen and D.J. Garrick conceived and designed the current analysis. M.A. Stephen undertook the analysis. M. A. Stephen wrote the paper in collaboration with D.J. Garrick, C.V.C. Phyn, J.E. Pryce and P.R. Amer.

Acknowledgements

This study was funded by New Zealand dairy farmers through DairyNZ Inc. and by the New Zealand Ministry of Business, Innovation and Employment (DRCX1302). Neither funder was involved in the design of this trial, or the collection, analysis, and interpretation of data. All authors declare no conflicts of interest. The authors gratefully acknowledge the support of LIC, CRV Ambreed, and New Zealand Animal Evaluation Ltd. (NZAEL) during herd enrolment into this study, particularly R. Bloxham (LIC), and J. Bryant and A. Young (formerly DairyNZ/NZAEL). The authors also kindly thank the DairyNZ technical team (especially S. Morgan, K. Watkins, A. Knight, N. Leach, and J. Brown) and the participating veterinary clinics and their staff for sampling, lab analyses, and data collection (especially E. Cuttance, VetEnt Te Awamutu; K. Goodare and S. McDougall, Anexa FVC Morrinsville; S. Bateman, Okato Vets; M. O'Sullivan, Oamaru Vets). This research would not have been possible without the generous support of the 54 herd owners and their graziers who provided access to their 2018-born dairy heifers for sampling and measurements.

References

Bezanson, J., A. Edelman, S. Karpinski, and V.B. Shah. 2017. Julia: A fresh approach to numerical computing. SIAM Review 59:65–98. doi:10.1137/141000671.

Cheng, H., R. Fernando, and D. Garrick. 2018. JWAS: Julia implementation of whole-genome analysis software. Page 859 in Proceedings of the world congress on genetics applied to livestock production, Auckland, New Zealand.

Dennis, N.A., K. Stachowicz, B. Visser, F.S. Hely, D.K. Berg, N.C. Friggens, P.R. Amer, S. Meier, and C.R. Burke. 2018. Combining genetic and physiological data to identify predictors of lifetime reproductive success and the effect of selection on these predictors on underlying fertility traits. J Dairy Sci 101:3176–3192. doi:10.3168/jds.2017-13355.

Donoghue, K.A., R. Rekaya, and J.K. Bertrand. 2004a. Comparison of methods for handling censored records in beef fertility data: Simulation study. J Anim Sci 82:351–356. doi:https://doi.org/10.2527/2004.822351x.

Donoghue, K.A., R. Rekaya, and J.K. Bertrand. 2004b. Comparison of methods for handling censored records in beef fertility data: Field data. J Anim Sci 82:357–361. doi:https://doi.org/10.2527/2004.822357x.

Fortes, M.R.S., S.A. Lehnert, S. Bolormaa, C. Reich, G. Fordyce, N.J. Corbet, V. Whan, R.J. Hawken, and A. Reverter. 2012. Finding genes for economically important traits: Brahman cattle puberty. Anim Prod Sci 52:143–150. doi:10.1071/AN11165.

Gianola, D., and J. Foulley. 1983. Sire evaluation for ordered categorical data with a threshold model. Genetics Selection Evolution 15:201. doi:https://doi.org/10.1186/1297-9686-15-2-201.

Grass, J.A., P.J. Hansen, J.J. Rutledge, and E.R. Hauser. 1982. Genotype x environmental interactions on reproductive traits of bovine females. I. Age at puberty as influenced by breed, breed of sire, dietary regimen and season.. J Anim Sci 55:1441–1457. doi:https://doi.org/10.2527/jas1982.5561441x.

Henderson, C.R. 1988. Theoretical basis and computational methods for a number of different animal models. J Dairy Sci 71:1–16. doi:http://dx.doi.org/10.1016/S0022-0302(88)79974-9.

Hickson, R.E., C.C. Balcomb, K.R. Fraser, N. Lopez-Villalobos, P.R. Kenyon, and S.T. Morris. 2011. The effect of breed on the onset of puberty in heifers. Pages 51–54 in Proceedings of the Association for the Advancement of Animal Breeding and Genetics, Perth, Australia.

Johnston, D.J., S.A. Barwick, N.J. Corbet, G. Fordyce, R.G. Holroyd, P.J. Williams, and H.M. Burrow. 2009. Genetics of heifer puberty in two tropical beef genotypes in northern Australia and associations with heifer- and steer-production traits. Anim Prod Sci 49:399–412. doi:10.1071/EA08276.

Luo, M.F., P.J. Boettcher, L.R. Schaeffer, and J.C.M. Dekkers. 2001. Bayesian inference for categorical traits with an application to variance component estimation. J Dairy Sci 84:694–704. doi:https://doi.org/10.3168/jds.S0022-0302(01)74524-9.

Macdonald, K.A., L.R. McNaughton, G.A. Verkerk, J.W. Penno, L.J. Burton, D.P. Berry, P.J.S. Gore, J.A.S. Lancaster, and C.W. Holmes. 2007. A comparison of three strains of Holstein-Friesian cows grazed on pasture: Growth, development, and puberty. J Dairy Sci 90:3993–4003. doi:10.3168/jds.2007-0119.

Meier, S., L.R. McNaughton, R.C. Handcock, P.R. Amer, P.R. Beatson, J.R. Bryant, K.G. Dodds, R. Spelman, J.R. Roche, and C.R. Burke. 2021. Heifers with positive genetic merit for fertility traits reach puberty earlier and have a greater pregnancy rate than heifers with negative genetic merit for fertility traits.. J Dairy Sci 104:3707–3721. doi:10.3168/jds.2020-19155.

Morris, C.A., J.A. Wilson, G.L. Bennett, N.G. Cullen, S.M. Hickey, and J.C. Hunter. 2000. Genetic parameters for growth, puberty, and beef cow reproductive traits in a puberty selection experiment. New Zealand Journal of Agricultural Research 43:83–91. doi:10.1080/00288233.2000.9513411.

Rendel, J.M., and A. Robertson. 1950. Estimation of genetic gain in milk yield by selection in a closed herd of dairy cattle. J Genet 50:1–8. doi:10.1007/BF02986789.

Smith, B.A., J.S. Brinks, and G.V. Richardson. 1989. Estimation of genetic parameters among reproductive and growth traits in yearling heifers. J Anim Sci 67:2886–2891. doi:10.2527/jas1989.00218812006700110007x.

Sorensen, D.A., D. Gianola, and I.R. Korsgaard. 1998. Bayesian mixed-effects model analysis of a censored normal distribution with animal breeding applications. Acta Agriculturae Scandinavica A: Animal Sciences 48:222–229. doi:10.1080/09064709809362424.

Stephen, M.A., H. Cheng, J.E. Pryce, C.R. Burke, N.M. Steele, C.V.C. Phyn, and D.J. Garrick. 2022a. Estimating heritabilities and breeding values from censored phenotypes using a data augmentation approach. Front Genet 13. doi:10.3389/fgene.2022.867152.

Stephen, M.A., S. Meier, M.D. Price, J.E. Pryce, C.R. Burke, Phyn. C.V.C., and D.J. Garrick. 2022b. Variance parameter estimation for age at puberty phenotypes under two levels of phenotype censorship. Journal of Dairy Science Communications. doi:10.3168/jdsc.2022-0218.

Tanner, M.A., and W.H. Wong. 1987. The calculation of posterior distributions by data augmentation. J Am Stat Assoc 82:528–540. doi:10.1080/01621459.1987.10478458.

CHAPTER 4. Estimating heritabilities and breeding values from censored phenotypes using a data augmentation approach.

Published in Frontier in Genetics Journal
https://doi.org/10.3389/fgene.2022.867152

Estimating Heritabilities and Breeding Values from Censored Phenotypes Using a Data Augmentation Approach

Melissa Stephen[1,5*], Hao Cheng[2], Jennie Pryce[3,4], Chris Burke[1], Nicole Steele[1], Claire Phyn[1], Dorian Garrick[5]

[1] DairyNZ Ltd, 605 Ruakura Road, Hamilton 3240, New Zealand

[2] Department of Animal Science, University of California Davis, Davis, CA 95616, USA

[3] Agriculture Victoria Research, AgriBio, Centre for AgriBioscience, Bundoora, Victoria 3083, Australia

[4] School of Applied Systems Biology, La Trobe University, Bundoora, Victoria 3083, Australia

[5] AL Rae Centre for Genetics and Breeding - Massey University, Ruakura, Hamilton 3214, New Zealand

* Corresponding Author
Melissa Stephen
DairyNZ Ltd, 605 Ruakura Road, Hamilton 3240, New Zealand
+6427 836 6294
melissa.stephen@dairynz.co.nz
ORCID: https://orcid.org/0000-0002-1210-5139

Highlight

- Estimated heritabilities and breeding values are remarkably robust to phenotype censoring when using a data augmentation approach.

Keywords: MCMC, Gibbs Sampling, Bayesian, Data Augmentation, Censored, Breeding

Abstract

Time-dependent traits are often subject to censorship, where instead of precise phenotypes, only a lower and/or upper bound can be established for some of the individuals. Censorship reduces the precision of phenotypes but can represent compromise between measurement cost and animal ethics considerations. This compromise is particularly relevant for genetic evaluation because phenotyping initiatives often involve thousands of individuals. This research aimed to: 1) demonstrate a data augmentation approach for analysing censored phenotypes, and 2) quantify the implications of phenotype censorship on estimation of heritabilities and predictions of breeding values. First, we simulated uncensored phenotypes, representing fine-scale 'age at puberty' for each individual in a population of some 5,000 animals across 50 herds. Analysis of these uncensored phenotypes provided a gold-standard control. We then produced seven 'test' phenotypes by superimposing varying degrees of left, interval, and/or right censorship, as if herds were measured on only one, two or three occasions, with a binary measure categorized for animals at each visit (either pre or post pubertal). We demonstrated that our estimates of heritabilities and predictions of breeding values obtained using a data augmentation approach were remarkably robust to phenotype censorship. Our results have important practical implications for measuring time-dependent traits for genetic evaluation. More specifically, we suggest that data collection can be designed with relatively infrequent repeated measures, thereby reducing costs and increasing feasibility across large numbers of animals.

Introduction

Maximizing the number of individuals contributing phenotypes to an analysis is particularly important in genetic evaluation and selection. Accuracy of evaluation and selection intensity are two key drivers of genetic improvement in a population (Rendel and Robertson, 1950). For any given trait, the accuracy of an individual's estimated breeding value (EBV) will improve as more of its immediate descendants have phenotypes measured. Response to selection depends on the EBV superiority of the individuals that are selected to become parents (Rendel and Robertson, 1950). In selection schemes that include individual phenotypes on selection candidates, selection intensity increases as more animals have phenotypes measured. However, precise measurement of phenotypes across large numbers of individuals can be problematic, especially when they are expensive to measure, require invasive procedures and/or measures must be repeated over time. Censored phenotypes are easier and cheaper to obtain, as fewer, and/or less specific observations are required. It follows that where resources are

limited, the strategic use of censorship can enable researchers to phenotype considerably more individuals.

There are several situations where animal breeders deliberately censor phenotypes. First, when a continuous trait, such as shoulder height, is measured using ordinal categories (for example a score of 1 to 9) instead of the underlying continuous variable. This type of censoring of continuous phenotypes makes them easier and faster to measure. Kizilkaya et al. (2014) reported that although EBV accuracy was compromised by this approach, it could be overcome by roughly doubling the number of animals phenotyped. A second situation is when a time-dependent trait is measured at relatively infrequent intervals. For example, Fortes et al. (2012) measured puberty status at intervals of four to six weeks, in preference to sustaining the cost and ethical issues of daily measures. In addition to interval censoring, left and right censoring are often introduced as a means to reduce observations. Left censoring occurs when the observation window begins after some animals have already expressed their phenotype, whereas right censoring occurs when animals express their phenotype after the observation window closes. Longevity is a phenotype that is subject to right-censoring, because individuals that are still alive at the time of data collection will only have a lower bound observation (Ducrocq et al., 1988).

Where time-dependent traits, such as age of puberty, are subject to left and interval censoring, individuals are often assigned a phenotype based on their age when they were first observed to have reached the threshold criterion (Fortes et al., 2012; Meier et al., 2021). That logic cannot be applied to right censoring, as there is essentially no upper bound on an animal's phenotype. A number of methods have been developed for handling right-censored phenotypes. Two common examples include adding an arbitrary penalty for right-censored phenotypes or predicting them using survival analysis techniques. Donoghue et al. (2004) analysed conception phenotypes by adding a 21 day penalty to right-censored phenotypes, while Ducrocq et al. (1988) analysed longevity phenotypes using survival analysis techniques such as the Cox proportional hazard model and the Weibull model to predict right-censored phenotypes. A data augmentation method Tanner and Wong (1987), where the phenotypes of any censored individuals are sampled from a truncated predictive distribution, provides an alternative approach for handling censored data. Donoghue et al. (2004) compared penalty and data augmentation approaches in their analysis of right-censored conception phenotypes and found that the results were similar.

It is likely that left, right and interval censorship of time-dependent traits may compromise the accuracy of EBVs. That said, high EBV concordance reported across varying degrees of right-censoring (Guo et al., 2001; Donoghue et al., 2004) indicates that this compromise may be minimal. It is difficult to investigate the implications of phenotype

censorship for traits that are commonly left, interval and right-censored, such as age of puberty, because the cost of obtaining precise phenotypes for an 'uncensored' comparison is prohibitive. Instead, we have simulated precise phenotypes, representing the trait 'age at puberty' (AGEP) and then applied a range of censorship scenarios to these phenotypes. These results can be used to make inferences about time-dependent traits that are challenging to measure precisely.

The aims of this study were to: 1) demonstrate a data augmentation approach for analysing left, interval and right-censored data, and 2) quantify the implications of varying phenotype censorship on estimates of heritabilities and predictions of EBVs, using a categorical, time-dependent trait. Our hypothesis was that the heritability and EBV rankings would be robust to phenotype censorship.

Methods

Simulated Phenotypes

We used the software XSim (Cheng et al., 2015), implemented in Julia (Bezanson et al., 2017) to simulate precise phenotypes representing the trait AGEP. The phenotypes were simulated using real single-nucleotide polymorphism (SNP) genotype data (Weatherbys Versa 50k SNP array) from 4,935 Holstein-Friesian, Holstein-Friesian cross Jersey cows. These 4,935 cows were born in 2018 and represent around 260 sires. We carried out quality control on these SNP genotypes prior to our simulation, disregarding unmapped SNP, as well as 2,120 SNP with minor allele frequency < 1%. This left around 47,000 SNP included in our analyses. We simulated a phenotype that represented AGEP, by specifying a genetic variance of 297 days, and heritability of 0.33 (Dennis et al., 2018). Animals were randomly assigned to one of 50 contemporary groups. The mean of each contemporary group was sampled at random from a normal distribution, with a mean of 342 days (Dennis et al., 2018) and a variance of 20. We assumed AGEP to be polygenic, with 500 SNP loci spread across the genome chosen to represent simulated additive QTL. The resultant precise phenotypes provided data for our 'gold-standard' (GOLD) control analyses.

We superimposed varying degrees of censorship to simulate these animals being observed at only 1, 2 or 3 herd visits for a seasonal window during which they would have been expected to attain puberty. In the first censored scenario, three herd visits (early, mid and late; EML) were simulated for each herd. The mid observation was on the day where 50% of the herd had attained puberty, and the early and late observations were 20 days either side of that day. This timing resulted in an even number of animals with left, interval and right censoring. In the second to fourth censored scenarios, herd visits were restricted to just the early and mid (EM), the mid and late (ML), or the early and late (EL) visits. In the fifth to seventh censored

scenarios, there was only one visit to each herd, with an early only (E), a mid only (M) or a late only (L) visit. Under censorship, the continuous variable GOLD was unobserved. Instead, the phenotype for each animal was only known to fall within a lower and/or upper bound (Figure 4.1).

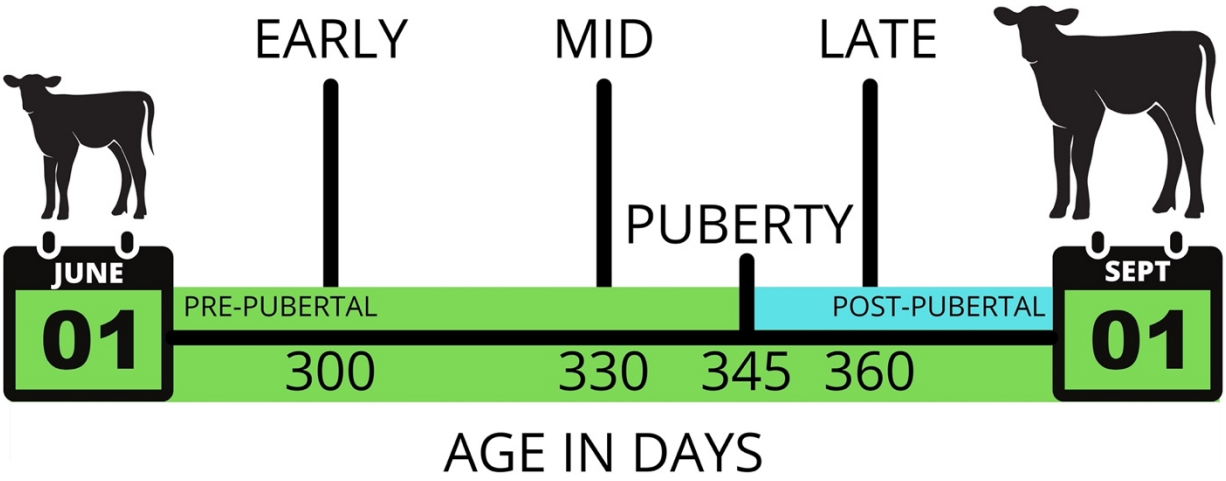


Figure 4.1. An example of interval censoring for 'age at puberty'. If this animal was observed daily, it would be recorded as attaining puberty at 345 days old. However, if the herd was observed only three times (early, mid and late), when this animal was 280, 300 and 330 days old, respectively, its phenotype would fall within the bounds of 330 to 360 days.

Data Augmentation

We used a Markov-chain Monte Carlo (MCMC) technique that included data augmentation (Tanner and Wong, 1987) to obtain posterior distributions for variance parameters and EBVs from censored phenotypes. The unobserved continuous variables representing the actual age that each animal attained puberty was treated as an unknown variable (hereinafter referred to as liabilities) whose value must fall between a known upper and lower bound. Plausible AGEP phenotypes were repeatedly sampled from a truncated predictive distribution for each animal. The sampled phenotypes were continuous variables representing a plausible value for each animal's AGEP, even though the observations on which they were based were binary (pre- or post-pubertal on a given herd visit). The mean and variance of these predictive distributions were determined by the simultaneous sampling of fixed herd effects and marker effects (mean) and residual variance (variance) within a single site Gibbs sampling approach. The truncation points for each predictive distribution were the known upper and/or lower bounds for each animal. This MCMC approach produced a posterior distribution of AGEP phenotypes for each animal (Figure 4.2).

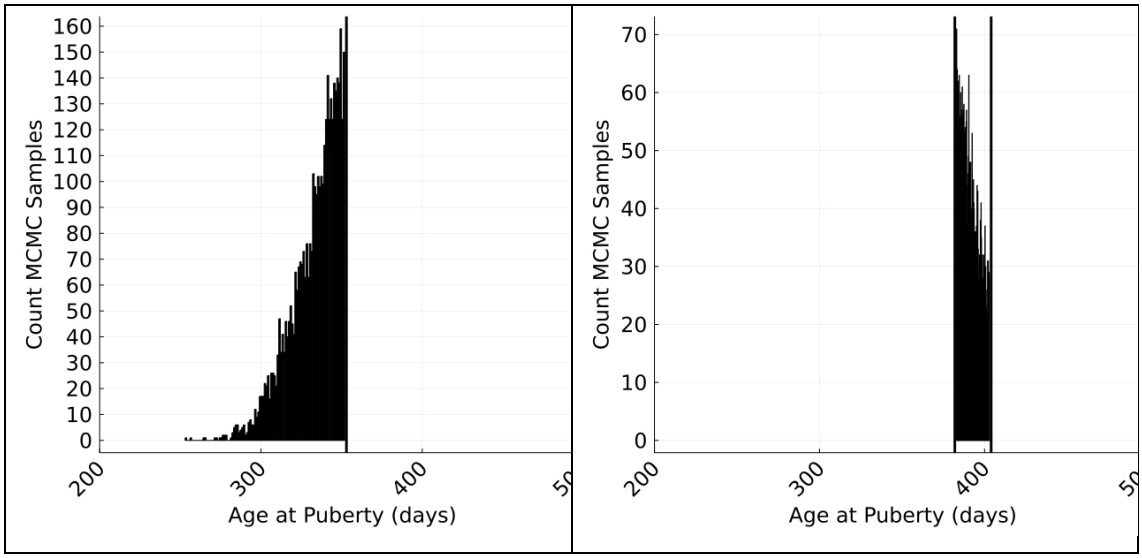


Figure 4.2. An example of the Markov-chain Monte Carlo (MCMC) sampled phenotypes for a single animal with a left- **(A)** or interval- **(B)** censored phenotype. Left censoring occurs where an animal has attained puberty on or before the first herd visit (i.e., sampled phenotypes are truncated by the age of the animal on the first visit). Interval censoring occurs when an animal reaches puberty between two visits (i.e., sampled phenotypes are truncated by the age of the animals on the flanking visits.

Model Equation

We fitted a mixed linear model using Bayesian methodology via single-site Gibbs sampling to construct a Markov chain of plausible values of unknowns. The model included random marker effects using BayesC priors (Garrick et al., 2014). Briefly, marker effects were assumed to follow a mixture distribution where their effects were either zero, with prior probability Pi=0.99, or normally distributed with prior probability (1-pi). Accordingly, at each iteration of the Gibbs sampler effects for about 4,700 markers were sampled from the non-zero distribution to collectively explain the breeding value or genetic component of the phenotypic differences between the 5,000 animals in our simulated dataset. We sampled the AGEP phenotypes conditional on the current sample values for all the effects in the linear model, and then fitted the linear model conditional on the sample of AGEP phenotypes, and so on.

The resultant MCMC samples of effects represented fixed herd effects, marker effects, and variance parameters. Matrix representation of the linear marker effects model equation is:

$$\mathbf{y}=X\mathbf{b}+\mathbf{Ma}+\mathbf{e} \qquad \text{Equation 1}$$

where **y** is a vector of phenotypes (one phenotype per study animal), **b** is a vector of herd effects, **a** is a vector of marker effects. The non-zero marker effects are assumed to be normally distributed and uncorrelated, with a variance equal to the genetic variance. The vector **e** represents residuals corresponding to each of the phenotypes. The residuals are assumed to be normally distributed and uncorrelated, with a variance equal to the residual variance.
The incidence matrix **X** relates each phenotype record to relevant fixed herd effects. The covariate matrix **M** relates each phenotype record to the number of one of the alleles at each SNP marker. The matrix **M** has a column for each SNP marker, and a row for each phenotype.

In all analyses, the unknowns include the vectors **b** and **a** and the scalars representing genetic and residual variances. Where phenotypes are censored with known lower or upper bounds, the vector **y** is also unknown, except for the bounds on each observation.

Software and solver

We used command line bash scripts and Julia (Bezanson et al., 2017) packages CSV, StatsPlots, and DataFrames to pre-process observation data into the vectors representing control or censored phenotypes. We performed the genetic analyses using the JWAS package (Cheng et al., 2018). The MCMC comprised 50,000 samples, with the first 10,000 samples disregarded as a burn-in and then every 10^{th} sample of the MCMC was retained. The Julia environment was used to post-process the results. We produced credibility intervals for genetic variance, residual variance, and heritability for the 5% (lower bound) and 95% (upper bound) percentiles based on all post-burn in samples. We used two methods to test for evidence of non-convergence of our MCMC chains. First, we undertook the diagnostic test described by (Geweke, 1992), and second, we observed trace plots to visually assess the convergence of posterior means of each parameter.

Comparisons across censorship scenario

We used Pearson's correlation coefficient to quantify the extent of re-ranking between EBVs obtained from each of our censorship scenarios. Correlations of EBVs included all animals with simulated phenotypes (n=4,935).

Results

Correlations between the posterior mean of MCMC phenotypes sampled for each animal and the control (GOLD) phenotype were strong and positive across all censorship scenarios (Table 4.1). Strong correlations were observed between EBVs estimated using phenotype bounds from each censorship scenario and EBVs estimated using GOLD phenotypes (Table 4.1). Unsurprisingly, these correlations decreased as censorship increased. Where the censorship scenario included at least two herd visits (EML, EM, ML, or EL), correlations between the GOLD and estimated phenotypes ranged from 0.90 to 0.95 and those between EBVs ranged from 0.92 to 0.96. Where the censorship scenario included just one herd visit (E, M or L), correlations between the GOLD and estimated (i.e., censored) phenotypes ranged from 0.81 to 0.85 and those between EBVs ranged from 0.85 to 0.88.

Correlations between the posterior means of sampled phenotypes from the EML censorship scenario and other censored scenarios ranged from 0.86 to 0.98 (Table 4.1). Likewise, correlations between EBVs from the EML censorship scenario and EBVs from the other more censored scenarios were all strong and positive, ranging from 0.88 to 0.96 (Table 4.1).

The posterior mean for the heritability of AGEP estimated using GOLD phenotypes was 0.29 (Table 4.1). The posterior mean of estimated heritabilities for different censorship scenarios also tended to be around 0.29 with 90% credibility intervals ranging from 0.22 to 0.34.

Table 4.1 Comparison across censorship scenarios for simulated 'age at puberty' phenotypes. Correlations between phenotypes (n = 4,935) (white shading, below diagonal), correlations between EBVs (n = 4,935) (grey shading, above the diagonal), and heritabilities with 90% credibility intervals (on the diagonal). In the control scenario (GOLD), the phenotypes represented those that would be obtained when animals were observed daily. Censored scenarios simulate if animals in a herd were observed at either one, two or three visits. In the first censored scenario, three herd visits (early, mid and late; EML) were simulated for each herd. In the second to fourth censored scenarios, herd visits were restricted to just the early and mid (EM), mid and late (ML), or early and late (EL) visits. In the fifth to seventh censored scenarios, herd visits were restricted to one per herd, with an early only (E), a mid only (M) or a late only (L) visit. 90% credibility intervals did not exceed 0.02 for any of the correlations.

.	GOLD	EML	EM	ML	EL	E	M	L
GOLD	0.29 (0.26,0.31)	0.96	0.92	0.93	0.94	0.85	0.88	0.85
EML	0.95	0.30 (0.27,0.33)	0.96	0.96	0.98	0.89	0.91	0.88
EM	0.90	0.95	0.29 (0.26,0.33)	0.93	0.91	0.92	0.95	0.78
ML	0.91	0.95	0.90	0.29 (0.26,0.33)	0.91	0.80	0.95	0.91
EL	0.93	0.98	0.90	0.90	0.30 (0.27,0.33)	0.90	0.83	0.89
E	0.81	0.86	0.9	0.71	0.88	0.27 (0.22,0.32)	0.80	0.72
M	0.85	0.90	0.95	0.94	0.80	0.73	0.29 (0.25,0.34)	0.79
L	0.81	0.86	0.70	0.90	0.88	0.58	0.72	0.27 (0.22,0.32)

Discussion

We determined that a data augmentation approach to analysing left-, interval- and right-censored data resulted in precisely estimated phenotypes for a time-dependent categorical trait, using simulated AGEP phenotypes as a case study. The extent of animal re-ranking, indicated by comparing correlations between censored phenotypes and their precise phenotypes (control), was relatively low even under extreme censorship scenarios, where animals only had a single

observation. Furthermore, EBVs were robust to phenotype censorship and animal rankings were largely consistent with our gold standard control scenario. In particular, the correlations between control EBVs and EBVs from any censored scenario where there were at least two visits per herd were greater than 0.90. We also determined that heritability estimates were relatively unaffected by phenotype censoring; across both control and censored scenarios, heritabilities tended to be around 0.29. Previous studies focusing on the implications of right censoring also indicated concordance of EBVs across varying degrees of censorship (Guo et al., 2001; Donoghue et al., 2004) and that a data augmentation approach produced minimal differences in variance parameters compared with uncensored phenotypes (Sorensen et al., 1998; Donoghue et al., 2004). Together, these outcomes support our hypothesis that heritabilities and EBVs can be robust to phenotype censorship. In the analysis presented here we have fit SNPs directly using a marker effects model. That said, our findings can be extended to a wide range of models including those analyses where a genomic or pedigree relationship matrix is used to describe the variance-covariance matrix between individuals.

Intentional phenotype censorship is useful for reducing cost and/or animal welfare concerns associated with phenotype collection. This is especially true for time-dependent traits, where repeated measurements are required to produce precise phenotypes. The trait AGEP provides a good case study as all animals begin prepubertal and, over time, reach sexual maturity and become post-pubertal. The timing of puberty varies between individuals and is influenced by a range of genetic and environmental factors. Puberty status can be determined through behavior monitoring, ovarian ultrasonography and/or blood testing for plasma progesterone concentrations (Morris et al., 2000; Macdonald et al., 2007; Hickson et al., 2011; Handcock et al., 2021); however, these measurements are labor intensive and therefore costly, in addition to being somewhat invasive, potentially compromising animal welfare or raising ethical issues. Hence, although daily observations would yield a precise phenotype, AGEP is often measured using as few observations as possible, resulting in censored phenotypes (Hickson et al., 2011; Fortes et al., 2013; Handcock et al., 2021). Here, our censored scenarios simulated relatively infrequent herd visits around the time that animals would be expected to attain puberty. Our results provide support for the strategic use of phenotype censoring, indicating that the effects on heritabilities and EBVs may be inconsequential for a time-dependent trait like AGEP and other commonly censored traits, such as longevity and/or other fertility phenotypes.

The current analysis has not exhaustively considered the implications of timing of observations. We timed our simulated herd visits to obtain about 25% of animals with left, interval E to M, interval M to L and right censoring using knowledge of the median AGEP for each herd. In reality, that information is not available in advance to plan the timing of

observations. As fewer herd visits are undertaken, timing may become more important. For example, if there is only one visit per herd, and it occurs earlier or later than the day of median AGEP there will be less variation among observations. In the worst case, all animals may be yet to reach puberty, or all animals may be post pubertal. Their phenotypes would not add value to genetic analysis, as any animal effects would be entirely confounded by the herd effect. Therefore, we have investigated the implications of visit timing within the bounds of our visit schedule. For example, our E scenario represents a single early visit, while our L scenario represents a single late visit. Our results indicate strong correlations between EBVs produced by all three scenarios with two herd visits. Therefore, when there are at least two visits, the timing is less important. Conversely, the correlations between EBVs produced by our three single visit scenarios are slightly weaker, indicating that when there is only one herd visit, animal selection decisions may be materially altered depending on visit timing. Further investigation is required to quantify the accuracy of EBVs produced using phenotypes from different visit timing before a recommendation on optimal timing can be made for a specific trait. If a visit was earlier than desirable, and most if not all animals were pre pubertal, there is still the option of visiting again to capture more information. A similar option is not available if a visit was later than desirable.

Based on the case study presented here, we conclude that heritability and EBV estimations for categorical, time-dependent traits are likely to be robust to left- interval- and right-censorship of phenotypes. In regard to the design of phenotyping strategies for specific traits, further simulation may be warranted. We assumed that once an animal attained puberty, she would be observed as pubertal thereafter with little measurement error. However, in reality, a trait like age at puberty could incur a relatively high incidence of false negative measures (where the animal has reached or exceeded the threshold but is observed to be under threshold). The extent of errors in allocating a phenotype to an animal would have implications on optimal measurement design. Further, we investigated only a single heritability, but the simulation could be applied to phenotypes with varied heritabilities, such that the implications of censorship on very low or very high heritability traits might also be quantified.

Conflict of Interest

MS, CB, NS and CP are employed by DairyNZ Limited. All authors declare no other competing interests.

Author Contributions

NS and CB managed the animal trial. MS and DG conceived and designed the current analysis. HC and MS developed the software required to complete the analyses. MS undertook the analysis. MS wrote the paper in collaboration with DG, CP, HC and JP.

Funding

This study received funding from New Zealand dairy farmers through DairyNZ Incorporated and from the New Zealand Ministry for Business, Innovation, and Employment (MBIE; contract #DRC1302). Additional in-kind support was kindly received from LIC and CRV. The funders were not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication. All authors declare no other competing interests.

Acknowledgments

We thank the 54 herd owners and their graziers who provided access to their 2018-born dairy heifers for genotyping, and gratefully acknowledge the contributions of the DairyNZ technical team, Anexa FVC - Morrinsville, VetEnt - Te Awamutu, Okato Vets, Whanganui Vets, and Oamaru Vets.

References

Bezanson, J., A. Edelman, S. Karpinski, and V.B. Shah. 2017. Julia: A fresh approach to numerical computing. SIAM Review 59:65–98. doi:10.1137/141000671.

Cheng, H., R. Fernando, and D. Garrick. 2018. JWAS: Julia implementation of whole-genome analysis software. Page 859 in Proceedings of the world congress on genetics applied to livestock production, Auckland, New Zealand.

Cheng, H., D. Garrick, and R. Fernando. 2015. Xsim: Simulation of descendants from ancestors with sequence data. G3: Genes, Genomes, Genetics 5:1415–1417. doi:10.1534/g3.115.016683.

Dennis, N.A., K. Stachowicz, B. Visser, F.S. Hely, D.K. Berg, N.C. Friggens, P.R. Amer, S. Meier, and C.R. Burke. 2018. Combining genetic and physiological data to identify predictors of lifetime reproductive success and the effect of selection on these predictors on underlying fertility traits. J Dairy Sci 101:3176–3192. doi:10.3168/jds.2017-13355.

Donoghue, K.A., R. Rekaya, and J.K. Bertrand. 2004. Comparison of methods for handling censored records in beef fertility data: Simulation study. J Anim Sci 82:351–356. doi:https://doi.org/10.2527/2004.822351x.

Ducrocq, V., R.L. Quaas, E.J. Pollak, and G. Casella. 1988. Length of Productive Life of Dairy Cows. 1. Justification of a Weibull Model. J Dairy Sci 71:3061–3070. doi:10.3168/jds.S0022-0302(88)79906-3.

Fortes, M.R.S., S.A. Lehnert, S. Bolormaa, C. Reich, G. Fordyce, N.J. Corbet, V. Whan, R.J. Hawken, and A. Reverter. 2012. Finding genes for economically important traits: Brahman cattle puberty. Anim Prod Sci 52:143–150. doi:10.1071/AN11165.

Fortes, M.R.S., Y. Li, E. Collis, Y. Zhang, and R.J. Hawken. 2013. The IGF1 pathway genes and their association with age of puberty in cattle. Anim Genet 44:91–95. doi:10.1111/j.1365-2052.2012.02367.x.

Garrick, D., J. Dekkers, and R. Fernando. 2014. The evolution of methodologies for genomic prediction. Livest Sci 166:10–18. doi:10.1016/j.livsci.2014.05.031.

Geweke, J. 1992. Evaluating the accuracy of sampling-based approaches to the calculation of posterior moments. Bayesian Statistics 169–193.

Guo, S.F., D. Gianola, R. Rekaya, and T. Short. 2001. Bayesian analysis of lifetime performance and prolificacy in Landrace sows using a linear mixed model with censoring. Livest Prod Sci 72:243–252. doi:10.1016/S0301-6226(01)00219-6.

Handcock, R.C., C.M.C. Jenkinson, R. Laven, L.R. McNaughton, N. Lopez-Villalobos, P.J. Back, and R.E. Hickson. 2021. Linear versus seasonal growth of dairy heifers decreased age at puberty but did not affect first lactation milk production. New Zealand Journal of Agricultural Research 64:83–100. doi:10.1080/00288233.2019.1607404.

Hickson, R.E., C.C. Balcomb, K.R. Fraser, N. Lopez-Villalobos, P.R. Kenyon, and S.T. Morris. 2011. The effect of breed on the onset of puberty in heifers. Pages 51–54 in Proceedings of the Association for the Advancement of Animal Breeding and Genetics, Perth, Australia.

Kizilkaya, K., R.L. Fernando, and D.J. Garrick. 2014. Reduction in accuracy of genomic prediction for ordered categorical data compared to continuous observations. Genetics Selection Evolution 46:1–13. doi:10.1186/1297-9686-46-37.

Macdonald, K.A., L.R. McNaughton, G.A. Verkerk, J.W. Penno, L.J. Burton, D.P. Berry, P.J.S. Gore, J.A.S. Lancaster, and C.W. Holmes. 2007. A comparison of three strains of Holstein-Friesian cows grazed on pasture: Growth, development, and puberty. J Dairy Sci 90:3993–4003. doi:10.3168/jds.2007-0119.

Meier, S., L.R. McNaughton, R.C. Handcock, P.R. Amer, P.R. Beatson, J.R. Bryant, K.G. Dodds, R. Spelman, J.R. Roche, and C.R. Burke. 2021. Heifers with positive genetic merit for fertility traits reach puberty earlier and have a greater pregnancy rate than heifers with negative genetic merit for fertility traits.. J Dairy Sci 104:3707–3721. doi:10.3168/jds.2020-19155.

Morris, C.A., J.A. Wilson, G.L. Bennett, N.G. Cullen, S.M. Hickey, and J.C. Hunter. 2000. Genetic parameters for growth, puberty, and beef cow reproductive traits in a puberty selection experiment. New Zealand Journal of Agricultural Research 43:83–91. doi:10.1080/00288233.2000.9513411.

Rendel, J.M., and A. Robertson. 1950. Estimation of genetic gain in milk yield by selection in a closed herd of dairy cattle. J Genet 50:1–8. doi:10.1007/BF02986789.

Sorensen, D.A., D. Gianola, and I.R. Korsgaard. 1998. Bayesian mixed-effects model analysis of a censored normal distribution with animal breeding applications. Acta Agriculturae Scandinavica A: Animal Sciences 48:222–229. doi:10.1080/09064709809362424.

Tanner, M.A., and W.H. Wong. 1987. The calculation of posterior distributions by data augmentation. J Am Stat Assoc 82:528–540. doi:10.1080/01621459.1987.10478458.

CHAPTER 5: Genome-Wide Association Study of age at puberty and its (co)variances with fertility and stature in growing and lactating Holstein-Friesian dairy cattle.

In review with the Journal of Dairy Science

Genome-Wide Association Study of age at puberty and its (co)variances with fertility and stature in growing and lactating Holstein-Friesian dairy cattle.

M.A. Stephen*[1,4], C.R. Burke[1], N. Steele[1], J.E. Pryce[2,3], S. Meier[1], P.R. Amer[5], C.V.C. Phyn[1], D.J. Garrick[1,4]

[1] DairyNZ Ltd, 605 Ruakura Road, Hamilton 3240, New Zealand

[2] Agriculture Victoria Research, AgriBio, Centre for AgriBioscience, Bundoora, Victoria 3083, Australia

[3] School of Applied Systems Biology, La Trobe University, Bundoora, Victoria 3083, Australia

[4] AL Rae Centre for Genetics and Breeding - Massey University, Ruakura, Hamilton 3214, New Zealand

[5]AbacusBio, 442 Moray Place, Dunedin 9016, New Zealand

* Corresponding Author
Melissa Stephen
DairyNZ Ltd, 605 Ruakura Road, Hamilton 3240, New Zealand
+6427 836 6294
melissa.stephen@dairynz.co.nz
ORCID: https://orcid.org/0000-0002-1210-5139

Highlights

- Heifer age at first progesterone elevation (AGEP4) is moderately heritable.
- The AGEP4 trait exhibits a moderate genetic correlation with pregnancy rate.
- A genomic region on chromosome 5 likely harbors a QTL for AGEP4.

Abstract

Reproductive performance is a key determinant of cow longevity in a pasture-based, seasonal dairy system. Unfortunately, direct fertility phenotypes such as inter-calving interval or pregnancy rate tend to have low heritabilities and occur relatively late in an animal's life. In contrast, age at puberty (AGEP) is a moderately heritable, early-in-life trait, that may be estimated using an animal's age at first measured elevation in blood plasma progesterone (AGEP4) concentrations. Understanding the genetic architecture of AGEP4 in addition to genetic relationships between AGEP4 and fertility traits in lactating cows is important, as is its relationship with body size in the growing animal. Thus, the objectives of this research were three-fold. First, to estimate the genetic and phenotypic (co)variances between AGEP4 and subsequent fertility during first and second lactations. Second, to quantify the associations between AGEP4 and height, length, and body weight (BW) measured when animals were around 1 yr of age. Third, to identify genomic regions that are likely to be associated with variation in AGEP4. We measured AGEP4, height, length, and BW in around 5,000 Holstein-Friesian or Holstein-Friesian x Jersey crossbred yearling heifers, across 54 pasture-based herds managed in seasonal calving farm systems. We also obtained calving rate (CR42: success or failure to calve within the first 42 d of the seasonal calving period), breeding rate (PB21: success or failure to be presented for breeding within the first 21 d of the seasonal breeding period) and pregnancy rate (PR42: success or failure to become pregnant within the first 42 d of the seasonal breeding period) phenotypes from their first and second lactations. The animals were genotyped using the Weatherby's Versa 50K SNP array (Illumina, USA). The estimated heritabilities of AGEP4, height, length, and BW were 0.34, 0.38, 0.31, and 0.33, respectively. In contrast, the heritabilities of CR42, PB21 and PR42 were all <0.05 in both first and second lactations. The genetic correlations between AGEP4 and these fertility traits were generally moderate ranging from 0.11 to 0.60, whereas genetic correlations between AGEP4 and yearling body conformation traits ranged from 0.02 to 0.28. Our GWAS highlighted a genomic window on chromosome 5 that was strongly associated with variation in AGEP4. We also identified 4 regions, located on chromosomes 14, 6, 1 and 11 (in order of decreasing importance), that exhibited suggestive associations with AGEP4. Our results show that AGEP4 is a reasonable predictor of estimated breeding values (EBVs) for fertility traits in lactating cows. While the GWAS provided insights into genetic mechanisms underpinning AGEP4, further work is required to test genomic predictions of fertility that use this information.

Key words: MCMC, BayesC, Heifer, Gibbs

Introduction

The profitability of seasonal, pasture-based dairy systems is substantially reduced by poor reproductive performance. Dairy producers desire a timely and condensed calving pattern as a strategy to align the feed demands of the herd to the seasonal feed supply from pasture grazed in situ (Macdonald and Roche, 2023). Holstein-Friesian and Jersey cattle have gestation lengths around 280 d (Norman et al., 2009); therefore to maintain an annual calving pattern, each cow must resume normal estrous cycles and initiate a pregnancy within 85 d of calving. Cows that extend that interval are more likely to be culled from the herd, truncating their potential lifetime production (Macdonald and Roche, 2023). Selection for fertility using direct measures of reproductive performance during lactation is now commonplace globally and has reportedly arrested historic declines in this important trait (Pryce et al., 2014). Nevertheless, genetic improvement in fertility traits has been, modest at best. Progress has been limited by the low heritability of reproduction phenotypes routinely measured during lactation, the age at which these phenotypes are expressed, and the antagonistic association that exists between fertility and milk production (Berry et al., 2003). For example, the fertility EBVs in New Zealand (NZ) represent a farmer-recorded calving rate trait, which is expressed relatively late-in-life and has a heritability of less than 0.10 (Harris et al., 2005; Bowley et al., 2015). While the value of fertility is recognized through its inclusion in many national breeding objectives around the world, milk production remains an important trait and, therefore, the antagonistic relationship between milk production and fertility continues to compromise genetic progress in fertility traits (Berry et al., 2014). This failure to realize genetic progress in fertility is exacerbated by the relative heritabilities of fertility and milk production traits, as the accuracy of selection for moderately heritable milk production traits exceeds that of fertility traits (Berry et al., 2014); hence, the rate of progress in milk production traits outweighs that of fertility. One approach for overcoming the constraints of low heritability on selection progress is to increase the number of phenotypes contributing to an analysis. Genomic evaluation can be used to leverage phenotypes measured on animals that do not necessarily share close pedigree relationships with selection candidates. That technology can provide high reliability EBVs for selection candidates despite them having relatively few direct or daughter phenotypes measured. Employing genomic evaluation might reduce the differential between the accuracy of EBVs for fertility, relative to other priority traits like milk production, provided there is a reference population of genotyped animals with fertility phenotypes.

Another strategy for improving the rate of genetic gain is to use a correlated predictor trait that either responds more readily to selection, or is expressed earlier in life, or both. Age at puberty (AGEP) is a candidate trait that may meet these requirements. In *Bos taurus* cattle, the onset of puberty occurs when animals are around 1 yr of age (Hickson et al., 2011; Meier et al.,

2021), and literature indicates that AGEP has a moderate heritability of around 0.30 (Smith et al., 1989; Fortes et al., 2012). Several authors have reported moderate genetic correlations of around 0.45 between AGEP and fertility traits measured during lactation (Morris et al., 2000; Mialon et al., 2001; Lefebvre et al., 2021). Furthermore, a predictor trait with higher heritability than the target trait, such as AGEP, can also add value in the context of genomic selection, as more accurate EBVs can be produced from smaller reference populations (Gonzalez-Recio et al., 2014). The improved accuracy of genomic EBVs for higher heritability traits provides evidence of improved estimation of marker effects, which may also extend to better QTL detection when using GWAS analysis. If two genetically correlated traits share QTL in common, a GWAS analysis of a higher heritability predictor trait may provide insight into the genetic architecture of a low heritability target trait, that would otherwise require a much larger population of phenotyped animals. Subsequent enrichment of a SNP chip in QTL regions can improve the accuracy of genomic prediction (Xiang et al., 2021), and, therefore, GWAS of a suitable predictor trait may contribute towards improving the accuracy of genomic EBVs for fertility.

A key limitation of AGEP as a predictor trait for fertility is the cost of measuring phenotypes, as a precise definition of onset of puberty might require confirmation of three events. First, behavioral estrus, second, ovulation and the formation of a corpus luteum (CL), and third, normal luteal function (Moran et al., 1989). That said, researchers who aim to measure AGEP at scale tend to simplify the definition of puberty, such that the phenotyping process becomes more economically and logistically feasible. For example, Meier et al. (2021) characterized AGEP as the age of first measured elevation in blood plasma progesterone (AGEP4), whereas Johnston et al. (2009) categorized animals as post-pubertal once a CL had been observed via rectal ultrasound scanning.

The objectives for this study were three-fold. First, to estimate the (co)variance parameters of AGEP4 and fertility traits expressed during lactation in a population of Holstein-Friesian and Holstein-Friesian x Jersey crossbred dairy cattle managed in seasonal, pasture-based systems in NZ. Second, to quantify the associations between AGEP4 and height, length, and body weight (BW) measured when animals were around 1 yr of age. Third, to identify genomic regions that are likely to be associated with variation in AGEP4. We hypothesized that AGEP4 would exhibit a non-zero genetic correlation with reproductive performance during lactation, and that our GWAS analysis would detect genomic regions associated with variance in AGEP.

Methods

Animals

The Ruakura Animal Ethics Committee (Hamilton, NZ) approved this study and all manipulations (AE applications: 14448 & 15004). Fifty-four herds were enrolled from a purposive selection of seasonal calving, pasture-based herds located in one of three regions (n = 35, Waikato; n = 15, Taranaki; and n = 4, Otago) of NZ, as described by Stephen et al. (2023). The herds were selected based on breed composition (herds with higher proportion of Holstein-Friesian animals were preferred), the quality of existing herd records, and regional location. The study focused on 2018-born cows, comprising 5,010 yearling heifers present when the AGEP phenotypes were measured. Some 322 of those animals were either not genotyped, had missing heifer phenotypes or had incomplete parentage records, leaving 4,688 animals representing 257 sires. Among those sires, 56 had at least 10 daughters that were spread over at least 3 herds. Binary fertility phenotypes were measured during first and second lactation, denoting an animal's success or failure to calve within the first 42 d of the herd's seasonal calving period (CR42_first, n = 4,327; CR42_second, n = 3,575), to be presented for breeding within the first 21 d of the seasonal breeding period (PB21_first, n = 4,111; PB21_second, n = 3,507), and to become pregnant within the first 42 d of the seasonal breeding period (PR42_first, n = 3,939; PR42_second, n = 3,353). Most animals enrolled in this study were Holstein-Friesian (>90% Holstein-Friesian; n = 2,340) or Holstein-Friesian x Jersey crossbreds (Holstein-Friesian or Jersey breed proportions are each <90%, but together sum to >90%; n = 2,276). The remaining animals were Jersey (>90% Jersey; n = 24) or other breeds (all breeds other than Holstein-Friesian, Jersey or Holstein-Friesian x Jersey crossbreed; n = 48). This reference population is hereafter referred to as the Puberty at Scale (PS) population (Figure 5.1). The PS animals were genotyped using the Weatherby's Versa 50K SNP array (Illumina, USA). Some 47,000 SNP were included in our analysis following the removal of unmapped or X-chromosome SNP (n = 806), and any SNP with minor allele frequency < 1% (n = 2,120). We used FImpute software (Sargolzaei et al., 2014) to impute the small proportion of missing SNP genotypes.

Age at puberty phenotypes

We used the trait AGEP4 as a practical proxy for age at puberty (AGEP), with animals phenotyped as described by Stephen et al. (2023). Briefly, animals were blood tested on three occasions when each herd cohort was approximately 10-, 11- and 12-mo of age, between May and August 2019. Blood was collected from the coccygeal vein into lithium heparin evacuated tubes (BD Vacutainers, BD New Zealand, Auckland, NZ), and immediately stored on ice. Samples were then centrifuged (at 4°C, 1,900 × g for 12 min) within 24 h of collection. Plasma was harvested and stored frozen at -20°C until a progesterone assay could be carried out using a

commercial radioimmune assay kit (ImmuChem Progesterone Double Antibody RIA, MP Biomedicals LLC, Irvine, CA) as described by Meier et al. (2021). Animals were categorized as having elevated blood plasma progesterone (BP4) once concentrations exceeded 1 ng/mL indicating that ovulation was likely to have occurred (Meier et al., 2021). The AGEP4 phenotype for each animal comprised a lower and upper bound, representing left-, interval- and right-censoring. The type of phenotype censoring present in our study is described elsewhere (Stephen et al., 2022a). Briefly, animals whose BP4 was already elevated in the first blood sample collected have left-censored phenotypes, whereby the lower bound of the phenotype is not known (and is set to 250 d in our analysis, Dennis et al., 2018), and the upper bound comprises their age on the day of their first blood test. Animals who had their first elevation in BP4 measured at visit 2 or visit 3 had interval-censored phenotypes. Their first BP4 elevation occurred at some stage between 2 blood test days, but the exact day is not known. The upper bound of their interval-censored phenotype is their age on the blood test day when their BP4 was first observed as >1 ng/mL. The lower bound is their age on the previous blood test day, approximately 30 d earlier. Animals that had not been measured with BP4 elevation before the final blood test had right-censored phenotypes. The lower bound of their phenotype is their age on the last blood test day and the upper bound is not known but is defined as 600 d in our analysis; we chose 600 d as we would expect all animals in this study to have reached puberty (and therefore have exhibited BP4 elevation) by that age, based on a phenotypic mean and standard deviation of 342 d and 30 d derived from a previous study (Dennis et al., 2018).

Height, Length, and BW phenotypes

We measured 3 yearling body conformation traits when the animals were approximately 1 year old (mean = 11 mo, SD = 0.5). These included height (distance in cm from the ground to the top of the shoulder), length (distance in cm from the base of the neck to the base of the tail) and BW (stationary weight in kg).

Calving and Breeding phenotypes

Animals were seasonally bred for the first time at around 15 mo old between September and December 2019, as per routine farm management. During first and second lactations, animals were assigned binary CR42 and PB21 phenotypes, denoting whether they calved within the first 42 d of their herd's seasonal calving period (score of 1) or not (score of 2), and whether they presented for breeding in the first 21 d of their herd's seasonal breeding period (score of 1) or not (score of 2), respectively. Therefore, a higher score for calving and breeding rate represented poorer performance. The start dates of the calving period and the breeding period in each herd were calculated as the mean calving date and the mean breeding date of the first 10%

of the animals in the herd to calve or be bred, respectively (Bowley et al., 2015). The animals in this study calved at an average age of 24 mo (SD = 0.75; between June and November 2020). Breeding in first lactation occurred between October and December 2020, using a combination of artificial insemination (AI) followed by natural breeding. The AI occurred over a period of 61 to 100 d (mean ± SD: 77 ± 9 d), as per routine farm management. Second calving occurred when the animals were an average age of 36 mo (SD = 0.80; between June and November 2021). Animals that had a calving recorded in first lactation but failed to become pregnant again were culled prior to second lactation. These missing animals were assigned a CR42 phenotype of 2 in second lactation, because if they had not been culled this would likely have been their phenotype. Breeding in second lactation occurred between October and December 2021, using a combination of AI and natural breeding. The AI occurred over a period of 28 to 95 d (mean ± SD: 61 ± 19 d). Calving and breeding data were recorded by farmers using commercial software, as is routine on NZ dairy farms. These data were accessed from the national Dairy Industry Good Animal Database (DIGAD) at the end of each lactation.

Pregnancy

Animals were assigned a binary PR42 phenotype in both first and second lactations denoting whether they became pregnant within the first 42 d of their herd's seasonal breeding period (score of 1) or not (score of 2). Therefore, as with the calving and breeding phenotypes, a higher score represented poorer reproductive performance. We used transrectal ultrasonography 11 to 14 weeks after the start of the herd's breeding period to diagnose pregnancy and determine conception date based on the size of the fetus, relative to the insemination records available for each cow. Ultrasonography and interpretation were carried out by qualified veterinary professionals. Pregnancy data were supplied by one or other of the two herd record providers in NZ (LIC, Hamilton, NZ; CRV, Hamilton, NZ).

Analysis

We used a combination of univariate, bivariate and trivariate marker effects models, depending on the parameters we were estimating, and the traits involved. All variances (genetic, residual, and phenotypic) were first estimated using univariate analyses. We estimated covariances between AGEP4 and each of the yearling body conformation traits using pair-wise bivariate analyses. Finally, we estimated covariances between AGEP4 and fertility traits recorded during first and second lactation using trivariate analyses, where AGEP4 was analysed alongside both the first and second lactation phenotype for CR42, PB21 or PR42. In all cases, the marker effects models can be represented by the following model equation:

$$\mathbf{y}=X\mathbf{b}+\mathbf{Ma}+\mathbf{e} \qquad \text{Equation 1}$$

In Equation 1, **y** is a vector of phenotypes. The vector **b** represents fixed effects, which comprise a herd effect for every analysis, and a measurement age in days effect for the analysis of yearling body conformation traits. Breed proportions were not included as fixed effects in our analyses, as the Bayesian multiple-regression analyses that we have implemented here have been shown to be robust to population structure (Toosi et al., 2018). The vector **a** represents additive marker effects and the vector **e** represents residual effects relating to each phenotype record. Phenotypes are related to each fixed effect using the incidence matrix **X**, and each marker effect using **M**, which is a matrix of genotype covariates (coded as 0,1,2). The number of rows in **M** is equal to the number of animals with both a genotype and a phenotype, and the number of columns of **M** is equal to the number of SNP markers included in the analysis. The vectors **b** and **a** are unknowns, as are the genetic and residual (co)variances. In addition, analyses that involve AGEP4 have another unknown, where the vector **y** that corresponds to AGEP4 phenotypes is itself unknown, except for upper and lower bounds. We applied BayesC methodology to estimate marker effects. The value of Pi, which is a scalar representing the proportion of markers that have no effect on the phenotypes, was fixed as 0.99 in univariate analyses, but sampled as an unknown parameter in our bivariate and trivariate analyses (Habier et al., 2011).

Software and solver

We generated phenotype files in the relevant formats required for analyses using command line bash scripts. We used the JWAS package (Cheng et al., 2018) implemented in Julia (Bezanson et al., 2017) to complete the genetic analyses, applying a Markov Chain Monte Carlo (MCMC) technique, using a single site Gibbs sampler to obtain samples of the unknown parameters. The first 20,000 MCMC samples were disregarded from each run as a burn in. A literature search to establish prior variance parameter values for each univariate analysis was undertaken (see supplementary material 1, Table S1.1). For example, our prior values for the genetic and residual variance for AGEP4 were 649 d^2 and 1202 d^2, respectively, and these were derived from a phenotypic variance of 1849 d^2 (Mialon et al., 2001) and a heritability of 0.35 (Mialon et al., 2000; Dennis et al., 2018). The posterior mean of variance parameters from univariate analyses were used as priors for the bivariate and trivariate analyses. Prior values for covariances were established from the literature (see supplementary material 1, Table S1.2).

We used two approaches to test for non-convergence of the Markov chain. First, we applied the method described by Geweke (1992). Second, we inspected box plots and trace plots of relevant statistics, such as heritabilities, to visually assess the MCMC chains for evidence of non-convergence of the posterior means and credibility intervals of the parameter estimated. To

construct the box plots, we grouped the MCMC chains into 4 groups according to sample number. For example, if the chain length was 100,000 samples: group 1 comprised samples 1 to 25,000, group 2 comprised samples 25,001 to 50,000, etc.). A parameter was considered converged if the 25th and 75th percentile of each group overlapped. We increased the chain lengths in models that were estimating more parameters, with chain lengths of 100,000, 300,000 and 600,000 for the univariate, bivariate and trivariate analyses. We selected these chain lengths by completing convergence testing on a series of test analyses with varied chain lengths. Longer chains were required to achieve convergence in the models that included larger numbers of (co)variance parameters.

GWAS analysis

In a post-processing step, the JWAS software was used to produce a WPPA (Window Posterior Probability of Association) for the set of markers that were located within predefined genomic windows. Each genomic window included approximately 20 contiguous SNPs, spanning an average of 1 cM. The WPPA represents the proportion of MCMC samples where a given window was associated with at least 1% of the variance in AGEP4 (Fernando et al., 2017). We used a WPPA threshold of 0.95 to identify regions that were associated with genetic variance in AGEP4. At that threshold, we would expect the proportion of false positives to be 0.05 (Fernando et al., 2017). We used the lower WPPA threshold of 0.70 to identify genomic windows that shared a suggestive association with AGEP4. We then validated each of the genomic regions with WPPA >0.70 using phenotypes measured in an independent validation population from a prior experiment (Meier et al., 2021). We used Ensembl (http://ensembl.org/) to investigate protein coding-genes within genomic regions of interest using the University of Maryland assembly of the *Bos taurus* 3.1 genome build (UMD 3.1, College Park, MD).

Validation method

We validated the estimated marker effects from the analysis of the PS population using an independent validation population from a prior experiment (Meier et al., 2021), consisting of 522 Holstein-Friesian cows, born in 2015. That herd (hereafter referred to as the Fertility Research Herd; FRH) was comprised of animals with extremely divergent parent average fertility EBVs based on re-calving rate within 42 d during second lactation (positive line: POS +5% fertility EBV; negative line: NEG -5% fertility EBV). That divergence in fertility EBVs was associated with phenotypic divergence in AGEP4, where POS animals were an average of 28 d younger than NEG animals when an elevation in BP4 concentrations was first detected (Meier et al., 2021). There were 21 animals in the validation population that were half-siblings to one or more PS animal; these half-siblings were removed, leaving 501 animals (n = 260 POS;

n = 241 NEG) contributing to our validation (Figure 5.1). In this smaller population, animals were blood tested more frequently (weekly intervals) and for a longer period of time compared with the approach taken in the PS population. The phenotyping method in the FRH population is described in detail by Meier et al. (2021). Briefly, animals were blood tested weekly from when they were approximately 190 kg BW (around 240 d old), through until 3 weeks after the start of their first seasonal breeding period (around 440-d old). An animal was deemed to have reached puberty once two of three consecutive weekly blood tests had elevated BP4 concentrations. Animals that failed to reach puberty by the end of the study were assigned an AGEP4 of their age on the final blood test, with the addition of a 7-d penalty.

All FRH animals were genotyped using the GeneSeek GGP Bovine 150K SNP Illumina array as described by Grala et al. (2021). Some 30K SNP on this array were common to SNP on the Weatherbys Versa 50K SNP array. We imputed the missing SNPs on the GGP array that were present on the Weatherbys array (using FImpute software, Sargolzaei et al., 2014), which enabled us to validate our marker effects directly derived from analysis of the PS population. The SNPs that were not present on the Weatherbys Versa 50K SNP array were not used in validation.

To produce validation EBVs for the FRH animals, we multiplied the vector of AGEP4 marker effects (number of SNP x 1) from the analysis of the phenotypes measured in the PS population by the matrix of observed and imputed SNP genotypes (number of animals x number of SNP) for the FRH animals. The multiplication yielded a vector of validation EBVs (number of animals x 1) for the FRH animals. The accuracy of the validation EBVs were represented as the correlation between the observed AGEP4 phenotypes (adjusted for fixed herd effects) and the EBVs obtained without the use of those phenotypes. We generated the mean and 90% credibility interval (90% CRI) for these correlations using a bootstrap (with replacement) method (Zhu, 1997) with a total of 1,000 bootstrap samples. We also used the FRH to validate the SNP effects within individual windows. To do this, we produced window EBVs where all SNP not located in the window of interest were disregarded from the EBV calculation.

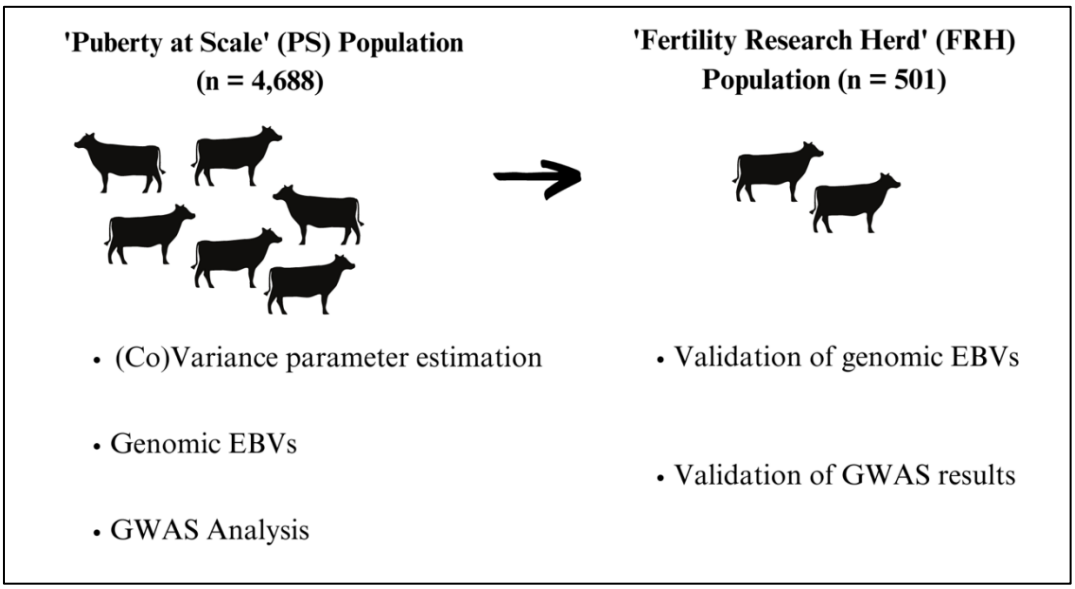


Figure 5.1. Phenotypes measured in the 'Puberty at Scale' (PS) population (n = 4,688) were used to undertake a marker effects analysis and a GWAS of 'Age at first elevation in blood plasma progesterone' (AGEP4). The marker effects and GWAS results were subsequently validated in the independent 'Fertility Research Herd' (FRH) population (n=501).

Results

Heritabilities

We estimated moderate single-trait heritabilities of 0.34, 0.28, 0.21 and 0.33 for AGEP4, height, length, and BW, respectively (Table 5.1). Estimated heritabilities of the six fertility traits measured during lactation (CR42, PB21 and PR42 in both first and second lactations) were much lower, ranging from 0.01 to 0.04 (Table 5.2).

Table 5.1. Heritabilities (diagonal), phenotypic correlations (below) and genetic (above) for age at puberty (AGEP4: defined as age at first measured elevation in blood plasma progesterone) and body confirmation traits including height, length, and BW. Credibility intervals representing the 5th and 95th percentiles of the MCMC samples are shown in parentheses.

Trait	AGEP4	Height	Length	BW
AGEP4	**0.34**	0.28	0.02	0.04
	(0.30, 0.37)	(0.19, 0.36)	(-0.08, 0.12)	(-0.04, 0.12)
Height	-0.04	**0.28**	0.63	0.67
	(-0.07, -0.01)	**(0.25, 0.31)**	(0.56, 0.71)	(0.61, 0.72)
Length	-0.14	0.32	**0.21**	0.82
	(-0.16, -0.11)	(0.29, 0.34)	**(0.18, 0.23)**	(0.76, 0.87)
BW	-0.24	0.47	0.48	**0.33**
	(-0.26, -0.21)	(0.45, 0.49)	(0.46, 0.50)	**(0.30, 0.36)**

Correlations among age at puberty and fertility traits

Overall, the genetic correlations (Table 5.2) between AGEP4 and fertility traits were positive and moderate. These correlations tended to be highest for PB21 (0.53, 0.60 in first and second lactations, respectively), and lowest for PR42 (0.34, 0.11 in first and second lactations respectively). The genetic correlations between AGEP4 and CR42 in first and second lactations were 0.45 and 0.58, respectively. Phenotypic correlations between AGEP4 and fertility traits were all near zero, ranging from 0.02 to 0.03.

Correlations among age at puberty and yearling body traits

The estimates of the genetic correlations between AGEP4 and the three body conformation traits are presented in Table 5.1. Height exhibited the highest genetic correlation with AGEP4 of 0.28, whereas length and BW exhibited near zero genetic correlations of 0.02 and 0.04, respectively. Phenotypic correlations between AGEP4 and the yearling body traits were -0.04 (height), -0.14 (length) and -0.24 (BW). Genetic correlations among height, length and BW were indicative of strong associations, ranging from 0.63 to 0.82, whereas phenotypic correlations were moderate, ranging from 0.32 to 0.48.

Table 5.2. Heritabilities (diagonal), phenotypic (below) and genetic (above) correlations for age at puberty (AGEP4: defined as age at first measured elevation in blood plasma progesterone) and fertility phenotypes (CR42: calving within 42 d; PB21: breeding within 21 d; PR42: pregnant within 42 d) from first and second lactation. Heritabilities were estimated using univariate analysis of each trait, while correlations were estimated from trivariate analyses including AGEP4 and both first and second lactation phenotypes for each of the calving, mating, and pregnancy traits. Credibility intervals representing the 5th and 95th percentiles of the MCMC samples are shown in parentheses. Fertility phenotypes are represented by binary scores, whereby a higher score indicates poorer fertility performance.

Trait	AGEP4	CR42_first	PB21_first	PR42_first	CR42_second	PB21_second	PR42_second
AGEP4	**0.34 (0.30, 0.37)**	0.45 (0.09, 0.73)	0.53 (0.27, 0.75)	0.34 (0.14, 0.53)	0.58 (0.29, 0.81)	0.60 (0.38, 0.79)	0.11 (-0.10, 0.36)
CR42_first	0.02 (-0.01, 0.05)	**0.01 (0.00, 0.02)**	0.78 (0.52, 0.94)	0.82 (0.56, 0.94)	0.78 (0.47, 0.94)	0.64 (0.01, 0.92)	0.71 (0.35, 0.92)
PB21_first	0.02 (-0.00, 0.05)	0.08 (0.05, 0.11)	**0.03 (0.01, 0.04)**	0.73 (0.39, 0.92)	0.77 (0.40, 0.95)	0.84 (0.68, 0.94)	0.82 (0.56, 0.94)
PR42_first	0.02 (-0.02, 0.05)	0.12 (0.09, 0.14)	0.19 (0.16, 0.21)	**0.04 (0.02, 0.06)**	0.91 (0.84, 0.96)	0.82 (0.55, 0.94)	0.88 (0.75, 0.97)
CR42_second	0.01 (-0.02, 0.04)	0.11 (0.08, 0.14)	0.19 (0.17, 0.22)	0.85 (0.84, 0.86)	**0.01 (0.00, 0.03)**	0.36 (-0.16, 0.71)	0.69 (0.39, 0.86)
PB21_second	0.03 (0.00, 0.07)	0.05 (0.02, 0.08)	0.14 (0.11, 0.17)	0.27 (0.23, 0.30)	0.26 (0.23, 0.29)	**0.03 (0.01, 0.05)**	0.48 (0.01, 0.77)
PR42_second	0.03 (-0.01, 0.06)	0.08 (0.05, 0.11)	0.09 (0.06, 0.12)	0.20 (0.16, 0.23)	0.20 (0.16, 0.23)	0.21 (0.18, 0.24)	**0.02 (0.01, 0.05)**

Note: A positive correlation between an AGEP trait and a fertility trait (CR42, PB21, PR42) indicates that selection for younger AGEP would result in improved fertility outcomes.

GWAS of age at puberty

Using a GWAS for AGEP4 in our reference PS population we identified 1 window with WPPA values greater than 0.95 (Figure 5.2). This window was located on chromosome 5 (UMD: 105, 337, 527 bp to 106, 432, 283 bp; WPPA: 1.00). Four other windows had WPPA greater than 0.70. These windows were located on chromosome 14 (UMD: 24, 482, 969 bp to 25, 731, 992 bp; WPPA: 0.93), chromosome 6 (UMD: 22, 273, 911 bp to 23, 352, 794 bp; WPPA: 0.84), chromosome 1 (UMD: 79,755,519 bp to 80,661,482 bp; WPPA: 0.75) and chromosome 11 (UMD: 39, 708, 839 bp to 41, 789, 973 bp; WPPA: 0.71). Collectively, these windows on chromosomes 5, 14, 6, 1 and 11 harbored between 2 and 16 protein-coding genes each, listed in Table 5.3.

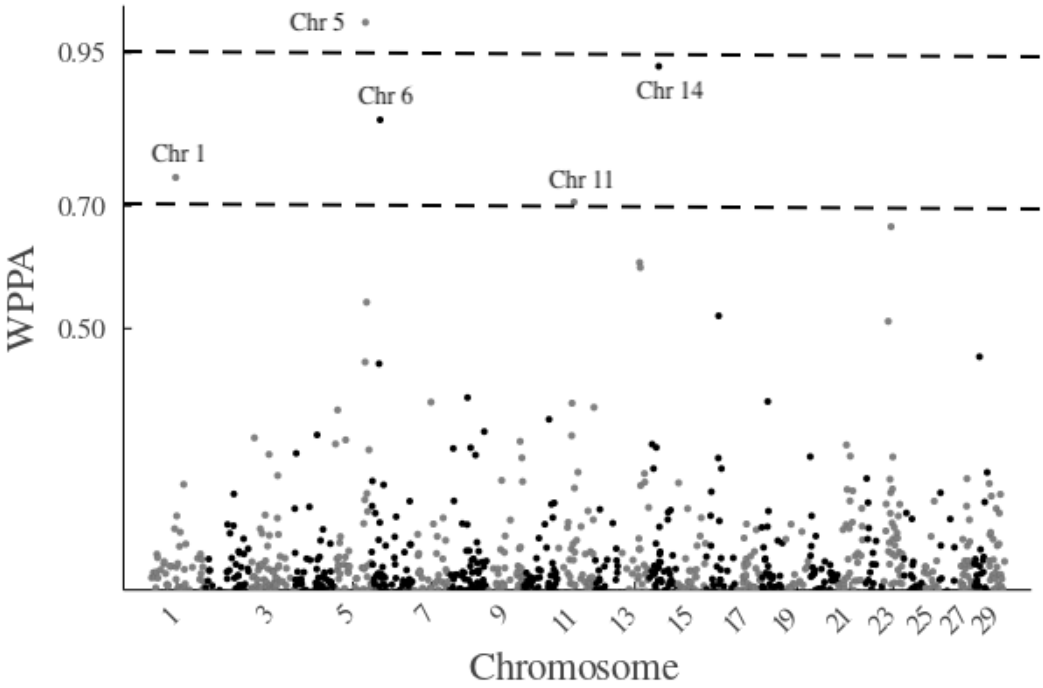


Figure 5.2. GWAS analysis of age at puberty. Five windows had a WPPA (Window Posterior Probability of Association) of 0.70 or greater. We used the UMD assembly to annotate our results, and these 5 windows were located on chromosomes 5 (105,337,527 bp to 106,432,283 bp), 14 (24, 482,969 bp to 25,731,992 bp), 6 (22,273,911 bp to 23,352,794 bp), 1 (79,755,519 bp to 80,661,482 bp) and 11 (39,708,839 bp to 41,789,973 bp).

Validation of marker effects

The mean AGEP4 phenotypes for the two fertility subgroups of the FRH population were 356 d (SD = 42.8) and 384 d (SD = 39.8) for POS and NEG, respectively (Meier et al., 2021). The mean gEBVs (Figure 5.3) for NEG and POS were 5.3 d (SD = 12.1) and -3.9 d (SD = 13.3) respectively. Therefore, the marker effects derived from the current study accounted for

9.2 d of the total 28-d phenotypic difference in AGEP4 between the two subgroups of our validation FRH population. The correlation between gEBVs and AGEP4 phenotypes in the FRH population was 0.41 (Table 5.4). The correlation between gEBVs and adjusted AGEP4 phenotypes within the POS and NEG fertility subgroups of the FRH population were 0.38 and 0.30, respectively.

The correlations between each of the 5 window EBVs and the validation AGEP4 phenotypes from the FRH are reported in Table 5.4. The posterior mean of all 5 correlations were positive, although the 90% CRI for the correlations involving the windows on chromosome 14 (24,482,969 bp to 25,731,992 bp) and chromosome 11 (39,708,839 bp to 41,789,973 bp) overlapped zero. The highest correlation between a window EBV and the validation phenotypes was observed for the genomic window on chromosome 5 (105,337,527 bp to 106,432,283 bp). This window on chromosome 5 also had the highest WPPA in our GWAS analysis of the PS population (Figure 5.2). The correlations between the 5 window EBVs were low, ranging from -0.05 to 0.11 (Table 5.4).

The minor allele frequencies of the largest effect SNP within high (>0.70) WPPA windows differed by between 0 and 0.16 (Table 5.5) between the PS and the FRH populations. The commonality across the Weatherby's Versa 50k SNP array (Illumina, USA) and the GeneSeek GGP Bovine 150K SNP Illumina array (Illumina, USA) was high across most of the high WPPA windows, ranging from 65 to 100%.

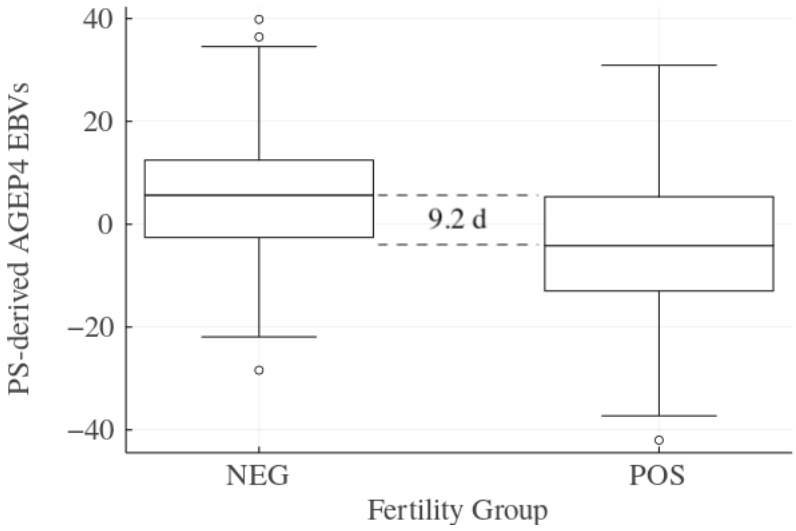


Figure 5.3. Distribution of EBVs for age at puberty (AGEP4: defined as age at first measured elevation in blood plasma progesterone) in the validation population (Fertility Research Herd; FRH). The EBVs were derived using SNP effects from the analysis of the animals in the PS (Puberty at Scale) population. The FRH animals are grouped according to the two sub-groups (positive line; POS +5% calving rate EBV, negative line; NEG -5% calving rate EBV).

Table 5.3. Candidate protein-coding genes located within genomic windows that had a WPPA >0.7 in our GWAS of age at puberty defined as age at first measured elevation in blood plasma progesterone (annotated using Ensembl UMD3.1). Top SNP refers to the SNP with the strongest effect within the genomic window.

BTA	Window Position	Top SNP (position)	Genes within region
5	105,337,527 bp – 106,432,283 bp	ARS-BFGL-NGS-54496 (106,178,425 bp)	KCNA5,KCNA6, ENSBTAG00000026522, GALNT8, NDUFA9, AKAP3, DYRK4, ENSBTAG00000047347, ENSBTAG00000048162, RAD51AP1, C5H12orf4, FGF23, TIGAR, CCND2
14	24,482,969 bp – 25,731,992 bp	BOVINEHD1400007259 (25,015,640 bp)	XKR4, TMEM68, TGS1, LYN, RPS20, RF01277, RF00003, MOS, PLAG1, CHCHD7, ENSBTAG00000039031, SDR16C5, SDR16C6, PENK, RF00026, IMPAD1
6	22,273,911 bp – 23,352,794 bp	HAPMAP1572-BTA-111558 (22,967,387 bp)	TACR3, ENSBTAG00000046850, CENPE, BDH2, SLC9B2, SLC9B1, CISD2
1	79,755,519 bp – 80,661,482 bp	HAPMAP41250-BTA-38429 (80,140,397 bp)	BCL6, RTP2, SST, RTP4, MASP1
11	39,708,839 bp – 41,789,973 bp	ARS-BFGL-NGS-36765 (41,379,246 bp)	VRK2, FANCL

Table 5.4. Correlations between age at puberty (AGEP4: defined as age at first measured elevation in blood plasma progesterone and adjusted for herd effect) phenotypes (n = 501) measured in an independent validation population (Fertility Research Herd; FRH) and genomic EBVs (derived using SNP effects from the analysis of the reference population in the Puberty at Scale (PS) study; n = 4,688 animals). Six EBVs were produced. The first EBV (All SNP) was derived using all SNP that were included in the analysis of the PS population (n = 46,792). The Ch5 EBV was restricted to SNP on chromosome 5 within the window of 105,337,527 bp to 106,432,283 bp. The Ch14 EBV was restricted to SNP on chromosome 14 within the window of 24,482, 969 bp to 25,731,992 bp. The Ch6 EBV was restricted to SNP on chromosome 6 within the window of 22,273,911 bp to 23,352,794 bp. The Ch1 EBV was restricted to SNP on chromosome 1 within the window of 79,755,519 bp to 80,661,482 bp. The Ch11 EBV was restricted to SNP chromosome 11 within the window of 39,708,839 bp to 41,789,973 bp.

Parameter	AGEP4	All SNP	Ch5	Ch14	Ch6	Ch1	Ch11
AGEP4 Phenotype	1.00						
All SNP	0.41 (0.34,0.47)	1.00					
Ch5	0.34 (0.27,0.40)	0.48 (0.42,0.53)	1.00				
Ch14	0.08 (-0.00,0.15)	0.26 (0.18,0.33)	0.10 (0.03,0.17)	1.00			
Ch6	0.11 (0.03,0.18)	0.27 (0.20,0.35)	0.04 (-0.03,0.12)	-0.01 (-0.08,0.05)	1.00		
Ch1	0.17 (0.10,0.24)	0.29 (0.23,0.35)	0.00 (-0.07,0.08)	0.10 (0.03,0.17)	-0.03 (-0.10,0.05)	1.00	
Ch11	0.06 (-0.00,0.13)	0.12 (0.05,0.19)	0.11 (0.04,0.17)	0.02 (-0.06,0.09)	-0.01 (-0.09,0.07)	-0.05 (-0.13,0.02)	1.00

Table 5.5. Minor allele frequencies (MAF) for selected SNP in the Puberty at Scale (PS) population compared with the Fertility Research Herd (FRH) used for independent validation. The selected SNP are the largest effect SNP within high (>0.70) WPPA windows in the analysis of age at puberty in the PS population. SNP array commonality describes the proportion of SNP within the given genomic window that were common across the two arrays used to genotype the PS population (Weatherby's Versa 50k SNP array; Illumina, USA) and the FRH population (GeneSeek GGP Bovine 150K SNP Illumina array [Illumina, USA]). The SNPs that were on the PS array, but not the FRH array were imputed using FImpute software (Sargolzaei et al., 2014).

Chromosome (window position)	SNP ID (UMD 3.1 location)	MAF PS/FRH	SNP array commonality. Top SNP present (percent commonality)
5 (105,337,527 bp – 106,432,283 bp)	ARS-BFGL-NGS-54496 (106,178,425 bp)	0.35/0.37	Yes (72%)
14 (24,482,969 bp – 25,731,992 bp)	BOVINEHD1400007259 (25,015,640 bp)	0.29/0.13	Yes (100%)
6 (22,273,911 bp – 23,352,794 bp)	HAPMAP1572-BTA-111558 (22,967,387 bp)	0.40/0.40	Yes (72%)
1 (79,755,519 bp – 80,661,482 bp)	HAPMAP41250-BTA-38429 (80,140,397 bp)	0.18/0.26	Yes (82%)
11 (39,708,839 bp – 41,789,973 bp)	ARS-BFGL-NGS-36765 (41,379,246 bp)	0.37/0.21	No (65%)

Discussion

In this study, we measured AGEP4 in a population of predominantly Holstein-Friesian and Holstein-Friesian x Jersey crossbred dairy cattle to investigate its suitability as a predictor for genetic merit for fertility in a seasonal management system. We determined a moderate heritability for AGEP4 and detected moderate genetic associations with key fertility traits that represent reproductive success during lactation. Conversely, genetic associations between AGEP4 and body conformation traits were low. Our results indicate that incorporating AGEP4 as a predictor of fertility EBVs will result in an accelerated rate of genetic gain in fertility.

Heritability

We estimated the heritability of AGEP4 to be approximately 0.34, which aligns well with existing literature where the heritabilities of AGEP and various related substitute phenotypes such as AGEP4 have been reported (Fortes et al., 2012; Lefebvre et al., 2021). The heritability of a trait provides an indication of how readily it might respond to direct selection, and heritability is an important factor when considering the value of a AGEP4 in the context of a predictor of fertility EBVs. In particular, if the heritability of a target trait (for example, fertility during lactation) is very low, it would be advantageous to select for a higher heritability trait that exhibits a moderate to high genetic correlation with the target trait, if such a trait exists. In general, fertility traits measured during lactation have heritabilities of <0.10 as determined in the current study (where CR42, PB21 and PR42 traits had heritabilities between 0.01 and 0.04) and reported previously (Brotherstone et al., 2002; Harris et al., 2006; Bowley et al., 2015). Hence, with a moderate heritability of 0.34, AGEP4 has potential as a useful predictor of fertility EBVs.

Phenotyping strategy

In our study, the phenotypes for AGEP4 were subject to left, interval and right censoring. This censorship was the result of our strategic trade-off between precision of the AGEP4 phenotypes and the number of animals we could practically and cost-effectively include in the study. It was a priority for us to maximize the number of animals involved in the study as this was a key parameter that would dictate the stability of our model solutions for marker effects and (co)variance parameters. That is, we would expect the credibility intervals surrounding our parameter estimates to decrease as animal numbers with phenotypes increase. Importantly, an analysis of low heritability traits like CR42, PB21 or PR42 requires more phenotypes to achieve the same level of certainty (that is, width of credibility intervals) of estimated parameters when compared to higher heritability traits like AGEP4. Thus, we decided to compromise the precision of the AGEP4 phenotypes so that we could measure a larger

number of animals and, therefore, improve the certainty of our (co)variance parameters that involve calving, breeding, and pregnancy phenotypes. The phenotyping strategy that we applied to AGEP4 was supported by previous studies. Stephen et al. (2022a) used simulated AGEP phenotypes to demonstrate that EBV and variance parameter estimation are robust to left-, right- and interval-phenotype censoring. Furthermore, Stephen et al. (2022b) used real AGEP4 phenotypes measured in a population of some 500 Holstein-Friesians heifers (i.e., the FRH) to demonstrate the robustness of AGEP4 variance parameter estimation to phenotype censoring.

Correlation between age at puberty and fertility traits measured during lactation

We detected favorable genetic correlations between AGEP4 and calving, breeding, and pregnancy phenotypes, indicating that earlier onset of puberty is genetically associated with earlier calving, breeding, and pregnancy dates. This is an important result because it confirms the value of AGEP4 as a predictor trait for fertility EBVs representing reproductive success during lactation. Although the genetic correlations between AGEP4 and calving, breeding and pregnancy phenotypes were generally moderate, there was some suggestion that the strength of the association depended on the specific fertility phenotype of interest. For example, AGEP4 tended to exhibit the strongest genetic correlation with PB21, and that tendency was evident in both first and second lactations. Our results align well with several existing studies, in which authors have reported genetic correlations between AGEP and fertility during lactation ranging from 0.36 to 0.58 in both beef (Mialon et al., 1999; Morris et al., 2000) and dairy cattle populations (Lefebvre et al., 2021). Lefebvre et al. (2021) and Mialon et al. (2001) used postpartum anestrus interval (PPAI) to represent fertility during lactation. That phenotype would be most similar to our PB21 phenotype, as cows with shorter PPAI would be more likely to present for breeding earlier. Morris et al. (2000) used calving date (continuous) and pregnancy rate (binary) traits to represent fertility during lactation, and those traits are very comparable to our CR42 and PR42 phenotypes. In contrast to these three studies, Patterson et al. (1992) reported low genetic correlations between AGEP and PPAI of 0.05 (Angus x Hereford, n = 148) and 0.12 (Brahman x Hereford, n = 148). However, their very small population sizes limited their ability to detect an association.

We have reported phenotypic correlations of near zero between AGEP4 and fertility traits measured during lactation. These results indicate that an animal's own phenotype for AGEP does not provide any real insight into her reproductive performance later in life once the herd effect is removed. Our results align with those published by Lefebvre et al. (2021) who reported a phenotypic association of 0.08 between AGEP and PPAI.

In our study population, animals that failed to become pregnant during the seasonal breeding period in first lactation were removed from the herd prior to the next season, and so do not have calving, breeding and pregnancy phenotypes recorded for a second lactation. Culling

on reproductive failure is common practice in seasonal calving, pasture-based systems; however, this fertility-driven preselection could have been a source of bias in our estimation of (co)variance parameters. The removed animals represent the poorest performing group from first lactation. These animals would be likely to perform poorly in second lactation, but this was not captured in the second lactation phenotype measurements, and so can never be known with certainty.

We mitigated the effects of this preselection by analyzing each of the three fertility traits in a trivariate context, with each analysis including AGEP4, the first lactation phenotype and the second lactation phenotype for each animal. This approach ensured that the missing animals were accounted for when estimating the relationship between AGEP4 and second lactation traits. In addition, we were also able to take a second approach to mitigate the effects of fertility-driven preselection on the calving rate trait, as we could identify the animals that were culled due to late pregnancies or failure to attain pregnancy in first lactation, and accurately assign them a penalty calving phenotype in second lactation. The genetic relationships between AGEP4 and both the CR42 and the PB21 traits were stable across first and second lactations, suggesting that analyzing them in a trivariate context sufficiently mitigated the effects of fertility-driven preselection on (co)variance parameter estimation. Conversely, the PR42 trait was not as consistent, and the positive association between AGEP4 and PR42 in first lactation (older AGEP genetically associated with later pregnancy) was not apparent in second lactation. Given our approach to account for fertility-driven preselection, it is possible that this difference reflects a real interaction of parity on the genetic association between AGEP4 and PR42, but further work is required to confirm this premise.

Correlation between age at puberty and yearling body conformation traits

Our results indicate that, in this population, AGEP4 was not genetically associated with yearling length or BW. Conversely, there was a positive moderate genetic association between AGEP4 and yearling height, whereby taller height was associated with older AGEP4. Our result of a near zero genetic correlation between AGEP4 and BW is consistent with the null to moderate correlations reported in the existing literature, which range from -0.30 and 0 (Smith et al., 1989; Gregory et al., 1995; Mialon et al., 2001; Wolcott et al., 2014). The genetic associations between AGEP4 and height reported in existing literature vary widely from -0.24 to 0.36 (Gregory et al., 1995; Wolcott et al., 2014), and so our result of 0.28 is consistent with this range. However, to the best of our knowledge, the genetic association between AGEP4 and length has not been previously reported. We also determined that AGEP4 exhibited negative phenotypic correlations with yearling height, length, and BW. That is, larger animals at yearling age tended to attain puberty at a younger age. These results aligned well with existing literature, wherein negative phenotypic correlations have been reported between AGEP and yearling

height (Gregory et al., 1995) and AGEP and yearling BW (Martin et al., 1992). It is well established that the onset of puberty is linked to an animal's nutrition, and faster growing animals are likely to reach puberty before their slower growing contemporaries (Patterson et al., 1992b). The effects of different feeding levels across herds were accounted for in our analysis (through inclusion of herd as a fixed effect in the model) and should not be reflected in our phenotypic correlations. That said, feeding levels within herd can also vary, and even in a grazing context, the more dominant animals may consume more feed. Therefore, differences in feed intake may be a driver of the phenotypic association between AGEP and yearling body traits.

Validation of genomic EBVs for age at puberty

We validated our gEBVs for AGEP4 (derived from analysis of the PS population) in an independent FRH population, which consisted of two subgroups with divergent calving rate EBVs and significantly different phenotypic expression of AGEP4 (Meier et al., 2021). Our gEBVs predicted a mean difference of 9.2 d in AGEP4 between the POS and NEG groups in the FRH population, which corresponds to approximately 33% of the observed 28-d phenotypic difference. This result indicates that the marker effects estimated using the PS population are relevant to other populations; however, the effects may be underestimated, perhaps because the PS population exhibited less extreme phenotypes and, therefore, had proportionally fewer genotypes from very low and very high fertility animals than the divergently selected FRH population. Another explanation could be the number of phenotypes contributing to the marker effects analysis. The estimated marker effects may have exhibited less shrinkage with a larger reference population, perhaps resulting in a larger mean difference between the validation gEBVs for the POS and NEG groups. The mean reliability of gEBVs in the PS population was 0.59, indicating that the expected correlation between those gEBVs and true BVs in the PS population was 0.77. The true BVs of the FRH animals used in our validation are unknown, and so we assessed the prediction accuracy of the gEBVs in relation to phenotypes on FRH animals adjusted for herd effects. The correlation of 0.41 between the gEBVs and FRH adjusted phenotypes is a promising validation, indicating that about 17% of the phenotypic variation in that population was being explained.

Daetwyler et al. (2012) analyzed traits with similar heritabilities to our AGEP4 trait and they reported slightly lower correlations between gEBVs and validation phenotypes; however, their reference populations were smaller than ours, ranging from 500 to 2,500 animals. The accuracy of gEBVs increases relative to the size of the reference population (Daetwyler et al., 2012). Hence, the higher accuracy in our analysis relative to the study by Daetwler et al. (2012) is not unexpected. Furthermore, the accuracy of our gEBVs for AGEP4 would likely have been higher if our reference population had included more animals. Nevertheless, both of our

approaches for validation indicate that the results of our gEBVs have predictive value outside of our reference population.

Validation of genomic windows for age at puberty

Several genomic windows of interest were identified in our initial GWAS of AGEP4 in the PS reference population, and window gEBVs produced for these genomic windows were then compared with AGEP4 phenotypes in our validation FRH population. The window located on chromosome 5, spanning 105,337,527 bp – 106,432,283 bp, had the highest WPPA value in our GWAS of AGEP4 in the PS population. The WPPA was close to 1, indicating that the window explained at least 1% of the variation in AGEP4 phenotypes in almost every MCMC sample. Further validation of this region indicated that gEBVs produced using only the SNP within this window (n = 20) explained around 11% of the variance in AGEP4 phenotypes in the independent FRH validation population. The high WPPA of this genomic window on chromosome 5 along with its successful validation in an independent population provide compelling evidence that this region harbors a QTL that is associated with variation in AGEP4. There are 14 candidate genes within this region (Table 5.3). Several of these candidate genes have been implicated in fertility. For example, the *CCND2* gene is differentially expressed in the granulosa cells of bovine follicles in varying developmental stages (Shimizu et al., 2013). The expression of *CCND2* has been shown to be regulated by FSH, and female mice knockouts for the *CCDN2* gene exhibited infertility, while their male counterparts had hypoplastic testes (Sicinski et al., 1996). Similarly, the *TIGAR* gene, which encodes the TP53-induced glycolysis and apoptosis regulator protein, has been associated with oocyte quality in mice (Wang et al., 2018), and both *CCND2* and *TIGAR* genes have been implicated in BW and stature in cattle (Hardie et al., 2017; Bouwman et al., 2018).

The window on chromosome 6, spanning 22,273,911 bp – 23,352,794 bp, was also validated in the FRH. This window harbors 7 candidate genes (Table 5.3). A particularly promising candidate within this genomic window is *TACR3,* which encodes for the neurokinin B-neurokinin receptor (NK3R). Neurokinin and NK3R are upstream regulators of kisspeptin, a hypothalamic neuropeptide that stimulates GnRH secretion initiating the pulsatile luteinizing hormone (LH) surges that precede the ovulation of a dominant follicle. Nakamura et al. (2017) reported dose-dependent responses in mean LH concentrations, the amplitude and frequency of pulsatile LH, and the timing of first postpartum ovulation when a NK3R selective agonist was administered to lactating cattle. Cattle administered the highest dose of the NK3R agonist had the shortest interval from calving to ovulation, indicating that loss of function in the *TACR3* gene would likely compromise fertility performance. The AGEP trait and postpartum resumption of cyclicity share a moderate genetic correlation (Lefebvre et al., 2021), and so it is possible that variants in the *TACR3* gene could also affect the onset of puberty. Moreover,

Clarke et al. (2022) directly investigated the expression of the kisspeptin gene (*Kiss1*) and the neurokinin B gene (*TAC3*) in a subset of the FRH animals. They reported greater *Kiss1* and *TAC3* expression in the arcuate nucleus of cows in the POS fertility group relative to NEG cows. Given that POS cows have an earlier onset of puberty (Meier et al., 2021) and greater ability to resume cycling postpartum (Meier et al., 2022), these findings indicate variants in the *TACR3* gene and other genes associated with the kisspeptin signaling pathway could play a role in the regulation of these fertility traits. This premise is also supported by the direct association between loss-of-function mutations in the *TAC3* or *TACR3* gene that cause hypothalamic hypogonadism and pubertal failure in humans (Topaloglu et al., 2009).

Further windows of interest identified from our GWAS included genomic regions on chromosomes 1, 14, and 11. The window located on chromosome 1, spanning 79,755,519 bp – 80,661,482 bp, validated successfully in the FRH population; however, to our knowledge, none of the 5 candidate genes (Table 5.3) within this window have been directly associated with cattle fertility. The region on chromosome 14 had the second highest WPPA of 0.93 in our GWAS using the PS population and harbors 16 candidate genes. The *PLAG1* gene is located at 25,007,291 bp - 25,009,296 bp, which is approximately 6 kb from the highest effect SNP within this window. The *PLAG1* gene is well documented to affect stature and BW in cattle (Karim et al., 2011; Littlejohn et al., 2012; Fink et al., 2017), and has previously been reported to share an association with variation in the fertility traits 'age of first calving' and 'age of first corpus luteum' (Fortes et al., 2016). Given the association between AGEP and stature, it seems likely that the *PLAG1* gene was driving the association between this genomic window and our AGEP4 phenotypes in the PS dataset. The final region on chromosome 11 harbors 2 candidate genes, but neither have previously been directly associated with fertility in cattle.

The EBVs corresponding to the two windows on chromosomes 14 and 11 were positively correlated with AGEP4 phenotypes measured in the FRH; however, in both cases, these correlations were relatively weak (<0.10) and lower bound of the 90% CRI overlapped 0 (Table 5.4). We cannot be confident that the association observed between SNP in these two regions and AGEP4 phenotypes in the PS dataset will extend to other populations. However, the poor validation of these two windows in the FRH may be explained by differences in the structure of the PS and FRH populations. The FRH was a single herd of 100% Holstein-Friesian cows with extreme divergence in fertility EBVs based on re-calving rate, whereas the larger PS population consisted of 54 herds that included a normal range of fertility EBVs and Holstein-Friesian and Holstein-Friesian x Jersey cows. Differences in breed composition and animal selection will result in some differences in allele frequencies, and this might explain how a genomic region could be legitimately associated with variance in AGEP4 in the PS population yet fail validation in the FRH. For example, the *PLAG1* gene, which is located within the genomic window identified on chromosome 14, segregates very differently between Holstein-

Friesian and Jersey cows. That is, the allele associated with smaller BW and stature is almost fixed in Jersey populations (Littlejohn et al., 2012), while the alternate allele that is associated with larger stature and BW is represented at a frequency of around 85% in Holstein-Friesians (Littlejohn et al., 2012); therefore, this gene is likely to segregate to a greater extent in a mixed-breed population, relative to a purebred population. Moreover, the selection strategy used to establish the FRH actively minimized variance in a range of traits such as BW and milk production, while maximizing variance in fertility traits. This selection process would likely result in the FRH having a unique population structure.

We gained some insight into how the population structure of the reference (PS) and validation (FRH) populations may differ by comparing the minor allele frequencies of the highest effect SNP within these windows (Table 5). These analyses showed that the top SNP within the genomic windows on chromosomes 14 and 11 were not segregating uniformly across PS and FRH populations. In both cases, the minor allele frequencies were 0.16 lower in the FRH, when compared with the PS population. Conversely, the top SNP in the other 3 windows with WPPA >0.70 (on chromosomes 5, 6 and 1) had uniform segregation across the FRH and PS populations. The accuracy of imputation may also contribute to the poor validation of the window on chromosome 11. The commonality between the arrays used to genotype the PS and FRH population is only 65% within this window, and furthermore, the SNP with the highest effect in the analysis of the PS animals was not common across the two arrays. Conversely, the top SNP in each of the other 4 genomic windows were present on both SNP arrays, and the SNP commonality was at least 72%. Missing SNPs were imputed, and error association with this imputation may compromise our validation of the window on chromosome 11. Further research into the two regions that failed validation in this study (located on chromosome 14 and 11) is required to validate or invalidate their association with AGEP4.

Conclusion

Moderate genetic correlations between AGEP4 and breeding, calving and pregnancy rate traits indicate that selection for earlier AGEP4 will improve genetic merit for fertility during lactation. We suggest that AGEP could add value as an early predictor of fertility EBVs, especially given the large differential between the heritabilities of AGEP4 and breeding, calving and pregnancy rate traits. Genomic EBVs for AGEP4 exhibited a correlation of 0.41 with independent validation phenotypes adjusted for fixed effects. Furthermore, there was 1 genomic region in our GWAS analysis, located on chromosome 5 that is likely to harbor a QTL for the AGEP4 trait. Window gEBVs produced for this region on chromosome 5 explained around 11% of the variance in our validation phenotypes. Our results contribute to a growing understanding of the genetic architecture of AGEP, and may also offer insight into correlated traits, such as

fertility traits measured during lactation. Further investigation of the association between our identified genomic regions and variance in key fertility traits such as calving, breeding and pregnancy performance is warranted.

Acknowledgments

This study was conducted as part of the “Pillars of New Dairy System” research programme, which was funded by a partnership between New Zealand dairy farmers through DairyNZ Incorporated and by the New Zealand Ministry of Business, Innovation and Employment (contract DRCX1302). Additional funding was provided under the DairyNZ-funded Resilient Dairy project – part of the LIC, DairyNZ, and the New Zealand Ministry for Primary Industries’ “Innovative breeding for a sustainable future” programme. The authors gratefully acknowledge the support of LIC, CRV, and New Zealand Animal Evaluation Ltd. (NZAEL) during herd enrolment into the PS study, particularly R. Bloxham (LIC), and J. Bryant and A. Young (formerly DairyNZ/NZAEL). We also thank these herd record providers for provision of animal data including calving, breeding, and pregnancy phenotypes and for valuable assistance in sire verification (especially K. Carnie, D. Chen, R. Sherlock, LIC; E. O’Connor, J Broekhuizen, CRV; and S. Maritz, B. Wickham, A. Fear, NZAEL). The authors kindly thank the DairyNZ technical team (especially S. Morgan, K. Watkins, A. Knight, N. Leach, and J. Brown) and the participating veterinary clinics and their staff for sampling, lab analyses, and data collection (especially E. Cuttance, VetEnt Te Awamutu; K. Goodare and S. McDougall, Anexa FVC Morrinsville; S. Bateman, Okato Vets; M. O’Sullivan, Oamaru Vets). This research would not have been possible without the generous support of the 54 herd owners and their graziers who provided access to their 2018-born dairy heifers for sampling and measurements.

I wish to acknowledge the use of New Zealand eScience Infrastructure (NeSI) high performance computing facilities as part of this research. New Zealand's national facilities are provided by NeSI and funded jointly by NeSI's collaborator institutions and through the Ministry of Business, Innovation & Employment's Research Infrastructure programme.

Conflict of interest

M A Stephen, C R Burke, N Steele, S Meier, and C V C Phyn are employed by DairyNZ Limited. All authors declare no other competing interests.

References

Berry, D.P., F. Buckley, P. Dillon, R.D. Evans, M. Rath, and R.F. Veerkamp. 2003. Genetic relationships among body condition score, body weight, milk yield, and fertility in dairy cows. J Dairy Sci 86:2193–2204. doi:10.3168/jds.S0022-0302(03)73809-0.

Berry, D.P., E. Wall, and J.E. Pryce. 2014. Genetics and genomics of reproductive performance in dairy and beef cattle. Animal 8:105–121. doi:10.1017/S1751731114000743.

Bezanson, J., A. Edelman, S. Karpinski, and V.B. Shah. 2017. Julia: A fresh approach to numerical computing. SIAM Review 59:65–98. doi:10.1137/141000671.

Bouwman, A.C., H.D. Daetwyler, A.J. Chamberlain, C.H. Ponce, M. Sargolzaei, F.S. Schenkel, G. Sahana, A. Govignon-Gion, S. Boitard, M. Dolezal, H. Pausch, R.F. Brøndum, P.J. Bowman, B. Thomsen, B. Guldbrandtsen, M.S. Lund, B. Servin, D.J. Garrick, J. Reecy, J. Vilkki, A. Bagnato, M. Wang, J.L. Hoff, R.D. Schnabel, J.F. Taylor, A.A.E. Vinkhuyzen, F. Panitz, C. Bendixen, L.E. Holm, B. Gredler, C. Hozé, M. Boussaha, M.P. Sanchez, D. Rocha, A. Capitan, T. Tribout, A. Barbat, P. Croiseau, C. Drögemüller, V. Jagannathan, C. vander Jagt, J.J. Crowley, A. Bieber, D.C. Purfield, D.P. Berry, R. Emmerling, K.U. Götz, M. Frischknecht, I. Russ, J. Sölkner, C.P. van Tassell, R. Fries, P. Stothard, R.F. Veerkamp, D. Boichard, M.E. Goddard, and B.J. Hayes. 2018. Meta-analysis of genome-wide association studies for cattle stature identifies common genes that regulate body size in mammals. Nat Genet 50:362–367. doi:10.1038/s41588-018-0056-5.

Bowley, F.E., R.E. Green, P.R. Amer, and S. Meier. 2015. Novel approaches to genetic analysis of fertility traits in New Zealand dairy cattle. J Dairy Sci 98:2005–2012. doi:10.3168/jds.2014-8266.

Brotherstone, S., G. Banos, and M.P. Coffey. 2002. Evaluation of yield traits for the development of a UK fertility index for dairy cattle. Pages 1–28 in World Congress on Genetics Applied to Livestock Production.

Cheng, H., R. Fernando, and D. Garrick. 2018. JWAS: Julia implementation of whole-genome analysis software. Page 859 in Proceedings of the world congress on genetics applied to livestock production, Auckland, New Zealand.

Clarke, I.J., C.B. Reed, C.R. Burke, Q. Li, and S. Meier. 2022. Kiss1 expression in the hypothalamic arcuate nucleus is lower in dairy cows of reduced fertility. Biol Reprod 106:802–813. doi:10.1093/biolre/ioab240.

Daetwyler, H.D., A.A. Swan, J.H. Van Der Werf, and B.J. Hayes. 2012. Accuracy of pedigree and genomic predictions of carcass and novel meat quality traits in multi-breed sheep data assessed by cross-validation. Genetics Selection Evolution 44:1–11. doi:10.1186/1297-9686-44-33.

Dennis, N.A., K. Stachowicz, B. Visser, F.S. Hely, D.K. Berg, N.C. Friggens, P.R. Amer, S. Meier, and C.R. Burke. 2018. Combining genetic and physiological data to identify predictors of lifetime reproductive success and the effect of selection on these predictors on underlying fertility traits. J Dairy Sci 101:3176–3192. doi:10.3168/jds.2017-13355.

Fernando, R., A. Toosi, A. Wolc, D. Garrick, and J. Dekkers. 2017. Application of Whole-Genome Prediction Methods for Genome-Wide Association Studies: A Bayesian Approach. J Agric Biol Environ Stat 22:172–193. doi:10.1007/s13253-017-0277-6.

Fink, T., K. Tiplady, T. Lopdell, T. Johnson, R.G. Snell, R.J. Spelman, S.R. Davis, and M.D. Littlejohn. 2017. Functional confirmation of PLAG1 as the candidate causative gene underlying major pleiotropic effects on body weight and milk characteristics. Sci Rep 7:1–8. doi:10.1038/srep44793.

Fortes, M.R.S., S.A. Lehnert, S. Bolormaa, C. Reich, G. Fordyce, N.J. Corbet, V. Whan, R.J. Hawken, and A. Reverter. 2012. Finding genes for economically important traits: Brahman cattle puberty. Anim Prod Sci 52:143–150. doi:10.1071/AN11165.

Fortes, M.R.S., L.T. Nguyen, L.R. Porto Neto, A. Reverter, S.S. Moore, S.A. Lehnert, and M.G. Thomas. 2016. Polymorphisms and genes associated with puberty in heifers. Theriogenology 86:333–339. doi:10.1016/j.theriogenology.2016.04.046.

Geweke, J. 1992. Evaluating the accuracy of sampling-based approaches to the calculation of posterior moments. Bayesian Statistics 169–193.

Gonzalez-Recio, O., M.P. Coffey, and J.E. Pryce. 2014. On the value of the phenotypes in the genomic era. J Dairy Sci 97:7905–7915. doi:10.3168/jds.2014-8125.

Grala, T.M., M.D. Price, B. Kuhn-Sherlock, C.R. Burke, and S. Meier. 2021. Investigating anogenital distance and antral follicle count as novel markers of fertility within a herd of cows with positive or negative genetic merit for fertility traits. J Dairy Sci 104:12939–12952. doi:10.3168/jds.2020-19948.

Gregory, K.E., L. v. Cundiff, and R.M. Koch. 1995. Genetic and phenotypic (co)variances for production traits of female populations of purebred and composite beef cattle.. J Anim Sci 73:2235–2242. doi:10.2527/1995.7382235x.

Habier, D., R.L. Fernando, K. Kizilkaya, and D.J. Garrick. 2011. Extension of the bayesian alphabet for genomic selection. BMC Bioinformatics 12. doi:10.1186/1471-2105-12-186.

Hardie, L.C., M.J. VandeHaar, R.J. Tempelman, K.A. Weigel, L.E. Armentano, G.R. Wiggans, R.F. Veerkamp, Y. de Haas, M.P. Coffey, E.E. Connor, M.D. Hanigan, C. Staples, Z. Wang, J.C.M. Dekkers, and D.M. Spurlock. 2017. The genetic and biological basis of feed efficiency in mid-lactation Holstein dairy cows. J Dairy Sci 100:9061–9075. doi:10.3168/jds.2017-12604.

Harris, B.L., J.E. Pryce, Z.Z. Xu, and W.A. Montgomerie. 2005. Fertility Breeding Values in New Zealand, the Next Generation. Interbull Bulletin 33:47–50.

Harris, B.L., J.E. Pryce, Z.Z. Xu, and W.A. Montgomerie. 2006. Development of new fertility breeding values in the dairy industry. Pages 107–112 in Proceedings of the New Zealand Society of Animal Production. New Zealand Society of Animal Production, Napier.

Hickson, R.E., C.C. Balcomb, K.R. Fraser, N. Lopez-Villalobos, P.R. Kenyon, and S.T. Morris. 2011. The effect of breed on the onset of puberty in heifers. Pages 51–54 in Proceedings of the Association for the Advancement of Animal Breeding and Genetics, Perth, Australia.

Johnston, D.J., S.A. Barwick, N.J. Corbet, G. Fordyce, R.G. Holroyd, P.J. Williams, and H.M. Burrow. 2009. Genetics of heifer puberty in two tropical beef genotypes in northern Australia and associations with heifer- and steer-production traits. Anim Prod Sci 49:399–412. doi:10.1071/EA08276.

Karim, L., H. Takeda, L. Lin, T. Druet, J.A.C. Arias, D. Baurain, N. Cambisano, S.R. Davis, F. Farnir, B. Grisart, B.L. Harris, M.D. Keehan, M.D. Littlejohn, R.J. Spelman, M. Georges, and W. Coppieters. 2011. Variants modulating the expression of a chromosome domain encompassing PLAG1 influence bovine stature. Nat Genet 43:405–415. doi:10.1038/ng.814.

Lefebvre, R., H. Larroque, S. Barbey, Y. Gallard, J.J. Colleau, A.L. Laine, D. Boichard, and P. Martin. 2021. Genome-wide association study for age at puberty and resumption of cyclicity in a crossbred dairy cattle population. J Dairy Sci 104:5794–5804. doi:10.3168/jds.2020-18228.

Littlejohn, M., T. Grala, K. Sanders, C. Walker, G. Waghorn, K. MacDonald, W. Coppieters, M. Georges, R. Spelman, E. Hillerton, S. Davis, and R. Snell. 2012. Genetic variation in PLAG1 associates with early life body weight and peripubertal weight and growth in Bos taurus. Anim Genet 43:591–594. doi:10.1111/j.1365-2052.2011.02293.x.

Macdonald, K.A., and J.R. Roche. 2023. Farm systems research at Ruakura–a 60-year legacy underpinning profitable and sustainable pasture-based dairy systems. New Zealand Journal of Agricultural Research 1–118. doi:10.1080/00288233.2023.2168707.

Martin, L.C., J.S. Brinks, R.M. Bourdon, and L. V. Cundiff. 1992. Genetic effects on beef heifer puberty and subsequent reproduction. J Anim Sci 70:4006–4017. doi:10.2527/1992.70124006x.

Meier, S., L.R. McNaughton, R.C. Handcock, P.R. Amer, P.R. Beatson, J.R. Bryant, K.G. Dodds, R. Spelman, J.R. Roche, and C.R. Burke. 2021. Heifers with positive genetic merit for fertility traits reach puberty earlier and have a greater pregnancy rate than heifers with negative genetic merit for fertility traits.. J Dairy Sci 104:3707–3721. doi:10.3168/jds.2020-19155.

Mialon, M.M., G. Renand, D. Krauss, and F. Ménissier. 1999. Puberty of Charolais heifers in relation to growth rate. 1. Phenotypic variability. Animal Research 48:413–426. doi:10.1051/animres:19990601.

Mialon, M.M., G. Renand, D. Krauss, and F. Ménissier. 2000. Genetic variability of the length of postpartum anoestrus in Charolais cows and its relationship with age at puberty. Genetics Selection Evolution 32:403–414. doi:10.1051/gse:2001119.

Mialon, M.M., G. Renand, D. Krauss, and F. Ménissier. 2001. Genetic relationship between cyclic ovarian activity in heifers and cows and beef traits in males. Genetics Selection Evolution 33:273–287. doi:10.1186/1297-9686-33-3-273.

Moran, C., J.F. Quirke, and J.F. Roche. 1989. Puberty in heifers: a review. Anim Reprod Sci 18:167–182. doi:10.1016/0378-4320(89)90019-5.

Morris, C.A., J.A. Wilson, G.L. Bennett, N.G. Cullen, S.M. Hickey, and J.C. Hunter. 2000. Genetic parameters for growth, puberty, and beef cow reproductive traits in a puberty selection experiment. New Zealand Journal of Agricultural Research 43:83–91. doi:10.1080/00288233.2000.9513411.

Nakamura, S., Y. Wakabayashi, T. Yamamura, S. Ohkura, and S. Matsuyama. 2017. A neurokinin 3 receptor-selective agonist accelerates pulsatile luteinizing hormone secretion in lactating cattle. Biol Reprod 97:81–90. doi:10.1093/biolre/iox068.

Norman, H.D., J.R. Wright, M.T. Kuhn, S.M. Hubbard, J.B. Cole, and P.M. VanRaden. 2009. Genetic and environmental factors that affect gestation length in dairy cattle. J Dairy Sci 92:2259–2269. doi:10.3168/jds.2007-0982.

Patterson, D.J., L.R. Corah, J.R. Brethour, J.J. Higgins, G.H. Kiracofe, and J.S. Stevenson. 1992a. Evaluation of reproductive traits in Bos taurus and Bos indicus crossbred heifers: relationship of age at puberty to length of the postpartum interval to estrus.. J Anim Sci 70:1994–1999. doi:10.2527/1992.7071994x.

Patterson, D.J., R.C. Perry, H. Kiracofe, R.A. Bellows, R.B. Staigmiller, and L.R. Corahg. 1992b. Management Considerations in Heifer Development and Puberty. J Anim Sci 70:4018–4035. doi:10.2527/1992.70124018x.

Pryce, J.E., R. Woolaston, D.P. Berry, E. Wall, M. Winters, R. Butler, and M. Shaffer. 2014. World trends in dairy cow fertility. Pages 1–10 in 10th World Congress of Genetics Applied to Livestock Production.

Sargolzaei, M., J.P. Chesnais, and F.S. Schenkel. 2014. A new approach for efficient genotype imputation using information from relatives. BMC Genomics 15. doi:10.1186/1471-2164-15-478.

Shimizu, T., Y. Hirai, and A. Miyamoto. 2013. Expression of cyclins and cyclin-dependent kinase inhibitors in granulosa cells from bovine ovary. Reproduction in Domestic Animals 48. doi:10.1111/rda.12177.

Sicinski, P., J.L. Donaher, Y. Geng, S.B. Parker, H. Gardner, M.Y. Park, R.L. Robker, J.S. Richards, L.K. McGinnis, J.D. Biggers, J.J. Eppig, R.T. Bronson, S.J. Elledge, and R.A.

Weinberg. 1996. Cyclin D2 is an FSH-responsive gene involved in gonadal cell proliferation and oncogenesis. Nature 384:470–474. doi:10.1038/384470a0.

Smith, B.A., J.S. Brinks, and G.V. Richardson. 1989. Estimation of genetic parameters among reproductive and growth traits in yearling heifers. J Anim Sci 67:2886–2891. doi:10.2527/jas1989.00218812006700110007x.

Stephen, M.A., C.R. Burke, N. Steele, J.E. Pryce, S. Meier, P.R. Amer, C.V.C. Phyn, and D.J. Garrick. 2023. Genome-Wide Association Study of anogenital distance and its (co)variances with fertility in growing and lactating Holstein-Friesian dairy cattle. J Dairy Sci In Press.

Stephen, M.A., H. Cheng, J.E. Pryce, C.R. Burke, N.M. Steele, C.V.C. Phyn, and D.J. Garrick. 2022a. Estimating heritabilities and breeding values from censored phenotypes using a data augmentation approach. Front Genet 13. doi:10.3389/fgene.2022.867152.

Stephen, M.A., S. Meier, M.D. Price, J.E. Pryce, C.R. Burke, Phyn. C.V.C., and D.J. Garrick. 2022b. Variance parameter estimation for age at puberty phenotypes under two levels of phenotype censorship. Journal of Dairy Science Communications. doi:10.3168/jdsc.2022-0218.

Toosi, A., R.L. Fernando, and J.C.M. Dekkers. 2018. Genome-wide mapping of quantitative trait loci in admixed populations using mixed linear model and Bayesian multiple regression analysis. Genetics Selection Evolution 50. doi:10.1186/s12711-018-0402-1.

Topaloglu, A.K., F. Reimann, M. Guclu, A.S. Yalin, L.D. Kotan, K.M. Porter, A. Serin, N.O. Mungan, J.R. Cook, M.N. Ozbek, S. Imamoglu, N.S. Akalin, B. Yuksel, S. O'Rahilly, and R.K. Semple. 2009. TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. Nat Genet 41:354–358. doi:10.1038/ng.306.

Wang, H., Q. Cheng, X. Li, F. Hu, L. Han, H. Zhang, L. Li, J. Ge, X. Ying, X. Guo, and Q. Wang. 2018. Loss of TIGAR induces oxidative stress and meiotic defects in oocytes from obese mice. Molecular and Cellular Proteomics 17:1354–1364. doi:10.1074/mcp.RA118.000620.

Wolcott, M.L., D.J. Johnston, and S.A. Barwick. 2014. Genetic relationships of female reproduction with growth, body composition, maternal weaning weight and tropical adaptation in two tropical beef genotypes. Anim Prod Sci 54:60–73. doi:10.1071/AN13012.

Xiang, R., I.M. MacLeod, H.D. Daetwyler, G. de Jong, E. O'Connor, C. Schrooten, A.J. Chamberlain, and M.E. Goddard. 2021. Genome-wide fine-mapping identifies pleiotropic and functional variants that predict many traits across global cattle populations. Nat Commun 12:1–13. doi:10.1038/s41467-021-21001-0.

Zhu, W. 1997. Making bootstrap statistical inferences. Res Q Exerc Sport 68:44–55. doi:10.1080/02701367.1997.10608865.

CHAPTER 6. Genome-Wide Association Study of anogenital distance and its (co)variances with fertility in growing and lactating Holstein-Friesian dairy cattle.

Published in the Journal of Dairy Science
https://doi.org/10.3168/jds.2023-23427

Genome-Wide Association Study of anogenital distance and its (co)variances with fertility in growing and lactating Holstein-Friesian dairy cattle.

M.A. Stephen*[1,4], C.R. Burke[1], N. Steele[1], J.E. Pryce[2,3], S. Meier[1], P.R. Amer[5], C.V.C. Phyn[1], D.J. Garrick[1,4]

[1] DairyNZ Ltd, 605 Ruakura Road, Hamilton 3240, New Zealand

[2] Agriculture Victoria Research, AgriBio, Centre for AgriBioscience, Bundoora, Victoria 3083, Australia

[3] School of Applied Systems Biology, La Trobe University, Bundoora, Victoria 3083, Australia

[4] AL Rae Centre for Genetics and Breeding - Massey University, Ruakura, Hamilton 3214, New Zealand

[5]AbacusBio, Dunedin, New Zealand

* Corresponding Author

Melissa Stephen

DairyNZ Ltd, 605 Ruakura Road, Hamilton 3240, New Zealand

+6427 836 6294

melissa.stephen@dairynz.co.nz

ORCID: https://orcid.org/0000-0002-1210-5139

Highlights

- Anogenital distance (AGD) is moderately heritable.
- The AGD trait exhibits a moderate genetic correlation with pregnancy rate.
- There is a high genetic correlation between AGD traits measured at two ages.
- A genomic region on chromosome 20 likely harbors a QTL for AGD.

Abstract

Anogenital distance (AGD) is a moderately heritable trait that can be measured at a young age that may provide an opportunity to indirectly select for improved fertility in dairy cattle. In this study, we characterized AGD and its genetic and phenotypic relationships with a range of body stature and fertility traits. We measured AGD, shoulder height, body length and body weight in a population of 5,010 Holstein-Friesian and Holstein-Friesian x Jersey crossbred heifers at approximately 11 mo of age (AGD1). These animals were born in 2018 across 54 seasonal calving, pasture-based dairy herds. A second measure of AGD was collected in a subset of herds (n=17; 1,956 animals) when the animals averaged 29 mo of age (AGD2). Fertility measures included age at puberty (AGEP), then time of calving, breeding, and pregnancy during the first and second lactations. We constructed binary traits reflecting the animal's ability to calve during the first 42 d of their herd's seasonal calving period (CR42), be presented for breeding during the first 21 d of the seasonal breeding period (PB21) and become pregnant during the first 42 d of the seasonal breeding period (PR42). The posterior mean of sampled heritabilities for AGD1 was 0.23, with 90% of samples falling within a credibility interval (90% CRI) of 0.20 to 0.26, whereas the heritability of AGD2 was 0.29 (90% CRI 0.24 to 0.34). The relationship between AGD1 and AGD2 was highly positive, with a genetic correlation of 0.89 (90% CRI 0.82 to 0.94). Using a GWAS analysis of 2,460 genomic windows based on 50k genotype data, we detected a region on chromosome 20 that was highly associated with variation in AGD1, and a second region on chromosome 13 that was moderately associated with variation in AGD1. We did not detect any genomic regions associated with AGD2 which was measured in fewer animals. The genetic correlation between AGD1 and AGEP was 0.10 (90% CRI 0.00 to 0.19), whereas the genetic correlation between AGD2 and AGEP was 0.30 (90% CRI 0.15 to 0.44). The timing of calving, breeding, and pregnancy (CR42, PB21 and PR42) during first or second lactations exhibited moderate genetic relationships with AGD1 (0.19 to 0.52) and AGD2 (0.46 to 0.63). Genetic correlations between AGD and body stature traits were weak (≤0.16). We conclude that AGD is a moderately heritable trait, which may have value as an early-in-life genetic predictor for reproductive success during lactation.

Introduction

The reproductive success of a dairy cow is a crucial determinant of its economic value within a farm system (Verkerk, 2003). This is especially true in grazing-based systems, where cows are generally required to maintain a strict annual seasonal calving cycle to coincide feed demand with pasture growth and availability (Macdonald and Roche, 2023). Cows with superior reproductive performance calve earlier in the calving season and thus have more time for postpartum recovery before the start of the next seasonal breeding period. The former leading to

longer lactations and the latter to an increased chance of becoming pregnant in a timely manner, and therefore having longer productive lives in the herd.

The accuracy of fertility EBVs may be improved though selection for traits that are genetically correlated to the breeding objective. For example, Sun et al. (2010) reported improved model stability and predictive ability when using milk production traits as predictors for fertility EBVs. However, large ranges in estimated genetic correlations between fertility traits and potential predictor traits such as milk production have been reported (Berry et al., 2014). Furthermore, milk production traits may not be suitable predictors of fertility, because antagonistic genetic correlations have been reported between milk production and fertility traits (Berry et al., 2014). Milk production is a trait that is under direct selection in most countries and using milk production traits as predictors of fertility would make it difficult to identify bulls with superior EBVs for both production and fertility. It is therefore preferable to focus on more suitable predictor traits that do not exhibit a strong antagonistic association with traits that are already under direct selection.

One possible candidate for an early in life and moderately heritable (Gobikrushanth et al., 2019) predictor of fertility is anogenital distance (AGD). In females, AGD can be defined as the distance between the anus and the clitoris (Figure 6.1; Gobikrushanth et al., 2017) and it has been associated with fertility performance in mice (Zehr et al., 2001; Welsh et al., 2008), humans (Mendiola et al., 2012) and cattle (Gobikrushanth et al., 2017; Carrelli et al., 2021; Grala et al., 2021; Madureira et al., 2022). The process for measuring AGD is straightforward, and this trait could be measured by farmers in the future. There is some evidence, however, that the association between AGD and fertility performance in cattle is not consistent across populations and parities. Gobikrushanth et al. (2019) were not able to detect an association in an Irish population of mixed-parity dairy cattle and an interaction between the association of AGD with fertility performance and parity has been reported (Gobikrushanth et al., 2017; Madureira et al., 2022). Hence, it is of interest to understand not only the phenotypic and genetic associations between AGD and fertility traits in cattle, but also the potential interactions of age or parity on these associations. Furthermore, a GWAS of a predictor trait may improve understanding of the genetic architecture of a low heritability target trait. Some genomic regions that are associated with a predictor trait will also be associated with the target trait, and enrichment of SNP chips in these regions might improve the accuracy of genomic EBVs for the target trait (Xiang et al., 2021).

The present study had 3 objectives. First, to calculate (co)variances of AGD measured relatively early in life, at approximately 11 mo of age (AGD1) around the time Holstein-Friesian and Jersey heifers reach puberty (Hickson et al., 2011; Meier et al., 2021), and at approximately 29-mo of age (AGD2) during first lactation. Second, to identify regions of the genome associated with variation in AGD. Third, to estimate the covariances between AGD,

yearling body stature traits and fertility phenotypes. We hypothesized that AGD1 would exhibit a high genetic correlation with AGD2, and that AGD at either age would exhibit moderate phenotypic and genetic relationships with reproductive phenotypes.

Figure 6.1. Photo demonstrating the method used to measure anogenital distance. The calipers were positioned to measure the distance from the centre of the anus to the base of the clitoris, as previously defined by Gobikrushanth et al. (2017).

Methods

Animals

The Ruakura Animal Ethics Committee (Hamilton, New Zealand) approved the design of this study and all manipulations (AE application: 14448). Heifer phenotypes (age at puberty [AGEP], shoulder height, body length, BW and AGD) were collected from 5,010 animals born between July and September 2018 that had been reared on 54 seasonal calving, pasture-based herds located across 3 regions (Waikato: n = 35 herds, 3,197 animals; Taranaki: n = 15 herds, 1,277 animals; Otago: n = 4 herds, 536 animals) of New Zealand. On average, there were 92 (SD = 49) heifers in each herd. The herds were selected based on the quality of existing data records, the proportion of the heifer cohort that was >90% Holstein-Friesian, their ability to enhance the diversity of sires contributing to the final pool of animals and the willingness of the herd owners to make animals available for measurements. Most of the animals in this study were >90% Holstein-Friesian (n = 2,340) and Holstein-Friesian x Jersey crossbred (n = 2,276). A small number of were >90% Jersey (n = 24). There were 48 animals could not be assigned to the breed categories of Holstein-Friesian, Jersey or Holstein-Friesian x Jersey, these animals

were predominantly Ayrshire cross Holstein-Friesian. Some 322 animals were excluded from our analysis due to incomplete parentage records or missing genotypes or both.

A total of 257 sires were represented in the dataset, and 56 of those sires had at least 10 daughters distributed over at least 3 of the 54 herds. Most of these heifers were followed through to the completion of their second lactation. First lactation calving (n = 4,327), breeding (n = 4,111) and pregnancy (n = 3,939) phenotypes were recorded between 1st June 2020 and 31st May 2021. Second lactation calving (n = 3,575), breeding (n = 3,507) and pregnancy (n = 3,353) phenotypes were recorded between 1st June 2021 and 31st May 2022. Animals with missing phenotypes in first or second lactation were either culled, sold or deceased. A subset of herds (n=17; Waikato: n = 9 herds, 1015 animals; Taranaki: n = 6 herds, 629 animals; Otago: n = 2 herds, 312 animals) comprising 1,956 animals had AGD measured for a second time during first lactation. Some 131 of these animals were excluded from our analysis due to incomplete parentage records, missing genotypes, or both. Herds were selected to be included in the second measure of AGD based on their quality of recording and the willingness of their owners to make animals available for measurement. A total of 128 sires were represented in the subset of animals with AGD2 phenotypes, and 33 of those sires had at least 10 daughters distributed over at least 3 of the 17 herds.

Anogenital Distance

We defined AGD as the distance between the center of the anus and the base of the clitoris (Figure 6.1), measured using stainless steel digital calipers as described by Gobikrushanth et al. (2017). The AGD1 phenotype was measured when the average age of heifers was 11 mo (SD = 0.5 mo). The AGD2 phenotype was measured approximately 11 to 14 wks after the herd's planned start of the seasonal breeding period during the first lactation, when the animals were, on average, 29 mo old (SD = 0.65 mo).

Age at puberty

We used the time at which blood plasma concentrations of progesterone (BP4) first exceeded 1 ng/mL (Meier et al. (2021) to represent the trait AGEP. This phenotype was based on 3 herd visits at monthly intervals between May and August 2019. The BP4 levels of a post-pubertal heifer follow a 3 wk cycle, and generally BP4 will be basal for 1 wk, followed by 2 weeks of elevation (Williams and Cardoso, 2021). Cyclic BP4 levels will result in some false negatives when using BP4 concentration measured at monthly intervals to represent pubertal status. Some animals will happen to be in a basal phase of their 3 wk cycle in the first visit following their onset of puberty, and so they will not be identified as post-pubertal until the following herd visit, around 1 mo later. Therefore, as well as the reduced precision arising from

a monthly interval between visits, the monthly visit schedule implemented here will also introduce some upward bias in mean AGEP phenotypes. Although our phenotyping approach yields an AGEP phenotype that lacks some precision, reducing visit frequency is an established strategy to manage costs and enable phenotype collection across thousands of animals (Johnston et al., 2009; Wolcott et al., 2018). Furthermore, Stephen et al. (2022a) have previously used simulated AGEP phenotypes to demonstrate that estimated variance parameters and breeding values are remarkably robust to infrequent herd visits. The average age of these 2018-born heifers was 10, 11 and 12 mo at these visits. The visits were timed to target 45% of animals being post-pubertal on the second visit, which was predicted according to the stochastic model of Dennis et al. (2018). Blood samples were collected from a coccygeal vessel into blood tubes containing lithium heparin (BD Vacutainers, BD New Zealand, Auckland, New Zealand). These samples were placed on ice for transport and then centrifuged (at 4 °C, 1,900 × *g* for 12 min) within 24 h of collection. The blood plasma was stored at -20 °C. The BP4 concentrations were determined using a commercial radioimmune assay kit (ImmuChem Progesterone Double Antibody RIA, MP Biomedicals LLC, Irvine, CA), following the same protocol described by Meier et al. (2021).

Each animal's AGEP phenotype was censored, in that it is not known precisely, but rather determined to fall within an upper and lower bound. This type of phenotype censoring is described in detail by Stephen et al. (2022a) who used simulated data to produce an identical phenotyping strategy to that used here. Briefly, the lower bound for an animal that attained puberty during the period of measurement was their age at the visit immediately prior to the visit when the animal was first observed to have BP4 >1 ng/mL. For example, the lower bound for an animal with first BP4 elevation on the second visit was their age on the first visit, because we observed them as prepubertal (that is, they did not have BP4 elevation) at that age, and so we assumed that they did not reach puberty any younger than this. The lower bound for an animal that attained puberty prior to the period of measurement was its birth date as we do not have information about how old the animal was when BP4 first became elevated. The upper bound for an animal that attained puberty during the measurement period was the age on the visit when the animal was first observed to have BP4 >1 ng/mL. For example, the upper bound for an animal with elevated BP4 on the second visit was the age at the day of the second visit. The upper bound for an animal that attained puberty after the measurement period was 600 d in the current analysis. This represents an age at which we would expect every animal to have attained puberty (Dennis et al., 2018).

Height, Length and BW

Animals had their height, length and BW measured on the same day as AGD1 (at approximately 11-mo old). Height was defined as the distance (cm) from the ground to the top

of the shoulder. Length was defined as the distance (cm) between the shoulders and the base of the tail. Body weight (kg) was measured using calibrated electronic weigh scales, while animals were stationary.

Calving and breeding in first and second lactation

Animals were seasonally bred to calve for the first time between June and November 2020 when they were, on average, 24 mo old (SD = 0.75). The mean calving date across all herds was 31st July 2020 and 95% of cows calved before 4th September 2020. These cows were then re-bred using either artificial insemination or natural breeding between October and December of the same year as per routine farm management under a seasonal calving, pasture-based grazing system. The length of the breeding periods (artificial inseminations [AI] only) in each herd ranged from 61 to 100 d (mean ± SD: 77 ± 9 d). Animals calved for the second time between June and November 2021 when they were, on average, 36 mo old (SD = 0.80). The mean calving date across all herds was 10th August 2021 and 95% of cows calved before 26th October 2021. These cows were then re-bred between October and December of the same year using either artificial insemination or natural breeding. The length of the 2021 breeding periods (AI only) for the 2018-born animals in each herd ranged from 28 to 95 d (mean ± SD: 61 ± 19 d). Hormone-based fertility interventions to advance estrous are a routine management tool in the New Zealand dairy industry and were used strategically on later calving cows in most herds involved in this study; however, producer recording of these interventions was incomplete and therefore these were ignored in the current analysis. Calving and breeding dates were recorded by herd managers. All available first lactation data were downloaded from the New Zealand national dairy database in June 2021, at the completion of that cohort's first lactation. Second lactation data was downloaded in June 2022. In both lactations, calving and breeding rates were defined as binary scores. We assigned animals that calved within the first 42 d of their herd's calving season (CR42) a score of 1, and those who calved later than this a score of 2. Animals that were removed from the herd due to reproductive failure (late pregnancy or failure to become pregnant) in first lactation were assigned a score of 2 for CR42 in second lactation. This phenotype recognizes that if they had not been removed from the herd, they would have either calved after the first 42 d of the herd's calving season, or they would have failed to calve. The start of calving for each herd was defined as the mean calving date of the first 10% of 2018 born cows to calve. This method of deriving a calving phenotype is consistent with national evaluations in New Zealand (Bowley et al., 2015). We assigned animals that were presented for breeding within the first 21 d of their herd's seasonal breeding period (PB21) a score of 1, and those who were mated later than this, or did not present for breeding, a score of 2. The start of breeding for each herd was defined as the mean breeding date of the first 10% of 2018 born cows to be mated (Bowley et al., 2015).

Pregnancy in first and second lactation

All 2018-born animals underwent pregnancy testing for fetal-age using ultrasonography as per standard practice offered commercially by veterinary providers. Pregnancy testing was carried out 11 to 14 wks after the herd's planned start of the seasonal breeding period. Animals were pregnancy tested in both first and second lactations. Conception dates were confirmed by verifying that the size of the fetus was consistent with the predicted age based on insemination records. Pregnancy rate was defined as a binary score. We assigned cows that attained pregnancy within the first 42 d of their herd's seasonal breeding period (PR42) a score of 1, whereas cows that did not attain pregnancy within this timeframe were assigned a score of 2. Animals that were removed from the herd in the first lactation were not assigned a phenotype for PB21 or PR42 in the second lactation.

Genotypes

All animals were genotyped using the Weatherbys Versa 50k SNP array (Illumina, USA). The SNP call rates were consistently high, and only 12 samples were excluded from our analysis due to low call rates (<90%). We imputed a small proportion of missing genotypes using FImpute software (Sargolzaei et al., 2014). We disregarded SNP located on the X chromosome, unmapped SNP, as well as 2,120 SNP with minor allele frequency < 1%. This left around 47,000 SNP to be included in our analyses.

Analyses

We first estimated genetic, residual, and phenotypic variances for each trait using univariate analyses. We used trivariate analyses to estimate the covariances between body traits (height, length, body weight, AGD1 and AGD2) and fertility traits in first and second lactations (CR42, PB21 and PR42). For example, the covariances between AGD1, CR42 in first lactation and CR42 in second lactation were estimated using a trivariate analysis to lessen the implications of fertility-driven selection occurring between first and second lactation, where animals that do not become pregnant during the seasonal breeding period in first lactation are removed from the herd. We used bivariate analyses to estimate the covariances between the pair-wise combinations of height, length, BW, AGEP, AGD1 and AGD2. We used bivariate analyses to estimate covariances between fertility traits using the pair-wise combinations of CR42, PB21 and PR42 during first and second lactations.

Model Equation

We fitted a marker effects model (Garrick et al., 2014) to estimate random additive marker effects, fixed herd effects, genetic and residual variance parameters, and, in the case of bivariate and trivariate analyses, covariance parameters. Univariate analyses of AGD1 and AGD2 were used to undertake a GWAS. Matrix representation of the marker effects model equation is:

$$\mathbf{y}=X\mathbf{b}+\mathbf{Ma}+\mathbf{e} \qquad \text{Equation 1}$$

where **y** is a vector of phenotypes (1 phenotype per study animal), **b** is a vector of fixed effects. Fixed effects for AGD1, height, length and BW were herd and age (in d), whereas only herd was fitted as a fixed effect for AGD2, AGEP and fertility measured during lactation. Bayesian multiple-regression analysis is robust to population structure (Toosi et al., 2018), therefore we did not fit breed proportions as fixed effects. The vector **a** contains additive marker effects. The vector **e** represents residuals corresponding to each of the phenotypes. The incidence matrix **X** relates each phenotype record to relevant fixed herd effects, and in the case of AGD1, height, length or LWT analyses, the fixed effects of age as a covariate. The covariate matrix **M** is a matrix of genotype covariates (coded as 0, 1 or 2) and relates each phenotype record to the number of 1 of the alleles at each SNP marker position. **M** has a column for each SNP marker, and a row for each genotyped animal with a phenotype. The unknowns sampled in each analysis include the vectors **b** and **a** and the scalars representing genetic and residual (co)variances. For the AGEP trait, where phenotypes are censored, only a lower and upper bound is available. Therefore, for the analysis involving AGEP, the vector **y** is also unknown, and this variable is sampled within the Gibbs sampling process, alongside other unknowns.

Software and solver

Genetic analysis was performed using the JWAS package (Cheng, 2019) implemented in Julia (Bezanson et al., 2017). A Markov Chain Monte Carlo (MCMC) technique was applied using a single site Gibbs sampler to obtain samples from the posterior distributions of variance parameters and fixed and marker effects. BayesC methodology was used, where Pi, the scalar proportion of markers with no effect, was set to 0.99 for univariate analyses. In bivariate and trivariate analyses, the vector Pi was sampled along with other unknowns (Habier et al., 2011).

For the analysis of AGEP, where phenotypes were censored, we used a data augmentation approach, implemented within the Gibbs sampler to obtain plausible values for each animal's phenotype. The AGEP phenotypes were re-sampled at each iteration at the Gibbs sampler, along with each of the other unknown variables (Stephen et al., 2022a).

We tested the chains of MCMC samples in our analyses for evidence of non-convergence of parameters, we made inference on using the method described by (Geweke, 1992). In addition, we assessed MCMC convergence by grouping post burn-in samples consecutively in quartile groups. That is, group 1 included the first 25% of MCMC samples, group 2 included next 25% of MCMC samples and so forth). We compared the mean and distribution of unknown variance parameters calculated from each of the 4 MCMC sample quartiles. Each analysis was considered converged when there was consistent alignment in the 25% and 75% percentile (50% credibility interval) for parameters across the consecutive quartiles of MCMC samples. The univariate, bivariate and trivariate analyses comprised 100,000, 300,000 and 600,000 samples, respectively. We selected suitable chain lengths by undertaking several test analyses where the number of MCMC iterations was varied and tested for evidence of non-convergence. The first 2,000 samples were disregarded as a burn-in.
Prior values for genetic and residual variances estimated using univariate analysis were obtained from existing literature (see Supplemental Material 1, Table S1.1). Prior values for genetic and residual variances estimated using bivariate or trivariate analysis were obtained from our univariate analysis, while prior values for covariances were obtained from the literature (see Supplementary Material 1, Table S1.2).

The Julia packages CSV, StatsPlots, DataFrames were used to post-process the results. We produced credibility intervals (CI) based on thresholds for the 5% (lower bound) and 95% (upper bound) percentiles (90CRI).

Genome-Wide Association Study (GWAS)

We defined genomic windows as spanning 20 contiguous SNP, which resulted in 2,460 windows, that were generally 1-cM long. The JWAS software computes WPPA (Window Posterior Probability of Association) for each of the 2,460 genomic windows, which represents the probability that a given region is associated with variance in a trait (Fernando et al., 2017). The WPPA metric can be used as a proxy for the probability that a region harbors a QTL. We considered windows that explained at least 1% of the total genetic variance and used a WPPA threshold of 0.8 to identify regions that were associated with genetic variance in AGD. At that threshold, we would expect the proportion of false positives to be restricted to 0.20 (Fernando et al., 2017). We used Ensembl (http://ensembl.org/) to view the University of Maryland assembly of the *Bos taurus* 3.1 genome build (UMD 3.1, College Park, MD) and to search for protein-coding genes within genomic regions that exhibited a WPPA above threshold.

Results

Heritabilities

We estimated heritabilities of 0.23 and 0.29 for AGD1 and AGD2, respectively (Table 6.1). Estimated heritabilities for pre-lactation traits measured as heifers were 0.28 for height, 0.21 for length, 0.33 for BW (Table 6.1) and 0.34 for AGEP (Table 6.2). The estimated heritabilities of CR42, PB21 and PR42 in first lactation were 0.01, 0.03 and 0.04, and in second lactation were 0.01, 0.03 and 0.02 (Table 6.3).

Table 6.1. Genetic (above the diagonal) and phenotypic (below the diagonal) correlations between AGD measured at 2 ages (**AGD1**: approximately 11-mo of age; **AGD2**: approximately 29 mo of age) and heifer (pre-lactation) traits including height, length and BW. Heritabilities are displayed on the diagonal. Correlations were calculated using pair-wise bivariate analyses. Heritabilities were calculated using univariate analyses. Values in parentheses represent 90% credibility intervals.

	AGD1	**AGD2**	**HEIGHT**	**LENGTH**	**BW**
AGD1	0.23 (0.20,0.26)	0.89 (0.82,0.94)	0.16 (0.07,0.25)	0.06 (-0.04,0.16)	0.13 (0.05,0.22)
AGD2	0.33 (0.30,0.37)	0.29 (0.24,0.34)	0.15 (-0.03,0.32)	-0.10 (-0.33,0.11)	0.12 (-0.04,0.26)
HEIGHT	0.06 (0.03,0.08)	0.04 (0.00,0.08)	0.28 (0.26,0.31)	0.63 (0.56,0.71)	0.67 (0.61,0.72)
LENGTH	0.06 (0.03,0.08)	0.03 (-0.01,0.07)	0.32 (0.29,0.34)	0.21 (0.18,0.23)	0.82 (0.76,0.87)
BW	0.12 (0.10,0.15)	0.04 (0.00,0.08)	0.47 (0.45,0.49)	0.48 (0.46,0.50)	0.33 (0.30,0.36)

Table 6.2. Genetic (above the diagonal) and phenotypic (below the diagonal) correlations between AGD measured at 2 ages (**AGD1**: approximately 11-mo of age; **AGD2**: approximately 29 mo of age) and age at puberty (**AGEP**). Heritabilities are displayed on the diagonal. Correlations were calculated using pair-wise bivariate analyses. Heritabilities were calculated using univariate analyses. Values in parentheses represent 90% credibility intervals.

	AGD1	AGD2	AGEP
AGD1	0.23 (0.20,0.26)	0.89 (0.82,0.94)	0.10 (0.00,0.19)
AGD2	0.33 (0.30,0.37)	0.29 (0.24,0.34)	0.30 (0.15,0.44)
AGEP	-0.02 (-0.04,0.01)	0.09 (0.05,0.13)	0.34 (0.30,0.37)

Table 6.3. Genetic (above the diagonal) and phenotypic (below the diagonal) correlations between AGD measures at 2 ages (**AGD1**: approximately 11-mo of age; **AGD2**: approximately 29 mo of age) and fertility phenotypes recorded during first and second lactation. Heritabilities are displayed on the diagonal. **CR42:** A binary score denoting whether a cow calved within the first 42 d of their herd's seasonal calving period (success = 1, failure = 2) during her first (L1) or second lactation (L2). **PB21:** A binary score denoting whether a cow was mated within the first 21 d of their herd's seasonal breeding period (success = 1, failure = 2) during her first (L1) or second lactation (L2). **PR42:** A binary score denoting whether a cow became pregnant within the first 42 d of their herd's seasonal breeding period (success = 1, failure = 2) during her first (L1) or second (L2) lactation. Correlations involving CR42, PB21 and PR42 were calculated using trivariate analyses so that both L1 and L2 phenotypes were included. All other correlations were calculated using pair-wise bivariate analyses. Heritabilities were calculated using univariate analyses. Values in parentheses represent 90% credibility intervals.

	AGD1	AGD2	CR42 L1	PB21 L1	PR42 L1	CR42 L2	PB21 L2	PR42 L2
AGD1	**0.23 (0.20,0.26)**	0.89 (0.82,0.94)	0.24 (-0.22,0.66)	0.52 (0.21,0.74)	0.28 (0.06,0.50)	0.32 (-0.09,0.72)	0.40 (0.08,0.68)	0.19 (-0.09,0.50)
AGD2	0.33 (0.30,0.37)	**0.29 (0.24,0.34)**	0.58 (0.33,0.82)	0.57 (0.29,0.82)	0.46 (0.23,0.69)	0.63 (0.32,0.85)	0.52 (0.25,0.78)	0.48 (0.21,0.73)
CR42 L1	-0.01 (-0.04,0.01)	0.07 (0.04,0.11)	**0.01 (0.00,0.02)**	0.78 (0.52,0.94)	0.82 (0.56,0.94)	0.80 (0.58,0.95)	0.64 (0.01,0.92)	0.71 (0.35,0.92)
PB21 L1	0.02 (-0.00,0.05)	0.04 (-0.01,0.08)	0.08 (0.05,0.11)	**0.03 (0.01,0.04)**	0.73 (0.39,0.92)	0.77 (0.40,0.95)	0.81 (0.57,0.93)	0.82 (0.56,0.94)
PR42 L1	0.02 (-0.01,0.05)	0.10 (0.06,0.14)	0.12 (0.09,0.14)	0.19 (0.16,0.21)	**0.04 (0.02,0.06)**	0.91 (0.84,0.96)	0.82 (0.55,0.94)	0.90 (0.78,0.96)
CR42 L2	0.00 (-0.02,0.03)	0.11 (0.07,0.15)	0.11 (0.08,0.14)	0.19 (0.17,0.22)	0.85 (0.84,0.86)	**0.01 (0.00,0.03)**	0.36 (-0.16,0.71)	0.69 (0.39,0.86)
PB21 L2	0.02 (-0.01,0.05)	0.03 (-0.01,0.08)	0.05 (0.02,0.08)	0.14 (0.11,0.17)	0.27 (0.23,0.30)	0.26 (0.23,0.29)	**0.03 (0.01,0.05)**	0.48 (0.01,0.77)
PR42 L2	0.03 (0.01,0.06)	0.03 (-0.01,0.07)	0.08 (0.05,0.11)	0.09 (0.06,0.12)	0.20 (0.16,0.23)	0.20 (0.16,0.23)	0.21 (0.18,0.24)	**0.02 (0.01,0.05)**

Note: A positive correlation between an AGD trait and a fertility trait (CR42, PB21, PR42) indicates that selection for shorter AGD would result in improved fertility outcomes.

Correlations – AGD with fertility traits measured during first and second lactation

The genetic and phenotypic correlations between AGD measured at 2 ages and fertility traits during first and second lactations are presented in Table 6.3. The genetic correlations between AGD1 and CR42 in first and second lactations were 0.24 and 0.32, respectively. The genetic correlations between AGD1 and PB21 in first and second lactations were 0.52 and 0.40, respectively. The genetic correlation was 0.28 for AGD1 and PR42 in first lactation, whereas it was 0.19 for AGD1 and PR42 in second lactation. The phenotypic correlations between AGD1 and fertility traits measured during lactation ranged from -0.01 to 0.03. The genetic correlations between AGD2 and fertility traits measured during first and second lactations were 0.58 and 0.63 for CR42, 0.57 and 0.52 for PB21, and 0.46 and 0.48 for PR42, respectively. Phenotypic correlations between AGD2 and fertility traits measured during lactation were weakly positive, ranging from 0.03 to 0.11.

AGD Correlations with Pre-lactation Heifer Traits

The genetic and phenotypic correlations between AGD measured at 2 ages with pre-lactation heifer traits are presented in Table 6.1 and Table 6.2. The AGD1 and AGD2 traits exhibited a strong positive genetic correlation of 0.89, whereas the phenotypic correlation was weaker at 0.33. In general, both genetic and phenotypic correlations between AGD and body stature traits were weak (<0.16). Genetic and phenotypic correlations were 0.16 and 0.06 between AGD1 and height, 0.06 and 0.06 between AGD1 and length, and 0.13 and 0.12 between AGD1 and BW, respectively. The genetic and phenotypic correlations were 0.15 and 0.04 between AGD2 and height, -0.10 and 0.03 between AGD2 and length, and 0.12 and 0.04 between AGD2 and BW, respectively. The genetic relationship between AGD and AGEP varied depending on the timing of the AGD measure. The genetic correlation between AGD1 and AGEP was weak at 0.10. Conversely, the genetic correlation between AGD2 and AGEP was moderate at 0.3. The phenotypic relationship between AGD and AGEP was also dependent on the timing of the AGD measure. The phenotypic correlation between AGD1 and AGEP was -0.02, whereas the phenotypic correlation between AGD2 and AGEP was 0.09.

Body Stature Correlations with Pre-lactation Heifer Traits

Genetic and phenotypic correlations between pre-lactation body stature phenotypes were consistently positive, and relationships ranged from moderate to high (Table 6.1). Height exhibited a genetic correlation of 0.63 with length and 0.67 with BW, and length and BW exhibited a genetic correlation of 0.82. Phenotypically, height exhibited a correlation of 0.32 with length and 0.47 with BW, and length and BW exhibited a phenotypic correlation of 0.48.

Correlations between first and second lactation fertility traits

The genetic and phenotypic correlations between fertility traits measured during lactation are presented in Table 6.3. Genetic correlations among first lactation fertility phenotypes were high and positive, ranging from 0.73 to 0.78, whereas genetic correlations among second lactation fertility phenotypes were moderately to highly positive, ranging from 0.36 to 0.69. Genetic correlations between first lactation and second lactation fertility phenotypes were also high and positive, ranging from 0.64 to 0.91. Phenotypic correlations between fertility phenotypes were weakly positive, ranging from 0.08 to 0.19 between first lactation traits and from 0.20 to 0.26 between second lactation traits. Phenotypic correlations between first lactation and second lactation fertility phenotypes also tended to be weakly positive; however, PR42 in first lactation exhibited a highly positive phenotypic correlation with CR42 in second lactation (0.85).

GWAS of AGD1 and AGD2

We identified 1 genomic region in our GWAS of AGD1 with WPPA values above our nominal threshold of 0.8 (Figure 6.2). This region was on chromosome 20, spanning 34,158,865 to 35,034,032 bp, and had a WPPA of 0.87. The SNP with the largest effect within this window was BTA-50400-NO-RS (location: 34,474,873 bp). There was 1 protein coding gene within this region on Ensembl (Table 6.4). A second noteworthy region was identified on chromosome 13, with a WPPA of 0.78, just below our threshold of 0.80. This genomic region spanned 9,276,071 to 9,996,039 bp (Table 6.4). The SNP with the largest effect within this window was BOVINEHD1300002589 (location: 9,656,579 bp). There was 1 protein coding gene recorded within this region on Ensembl (Table 6.4). The GWAS of AGD2, based on only about a third of the number of animals, did not yield any regions associated with variation in the AGD trait (Figure 6.3).

Table 6.4 Genomic regions of suggestive association with anogenital distance measured at 11-mo of age in some 5,000 Holstein-Friesian and Holstein-Friesian x Jersey crossbreed dairy cattle (Annotated using Ensembl UMD3.1, Collage Park, MD [http://ensembl.org/])

BTA	Position	SNP ID (SNP position)	Genes within region
13	9,276,071 bp - 9,996,039 bp	BOVINEHD1300002589 (9,656,579 bp)	MACROD2
20	34,158,865 bp - 35,034,032 bp	BTA-50400-NO-RS (34,474,873 bp)	dab2

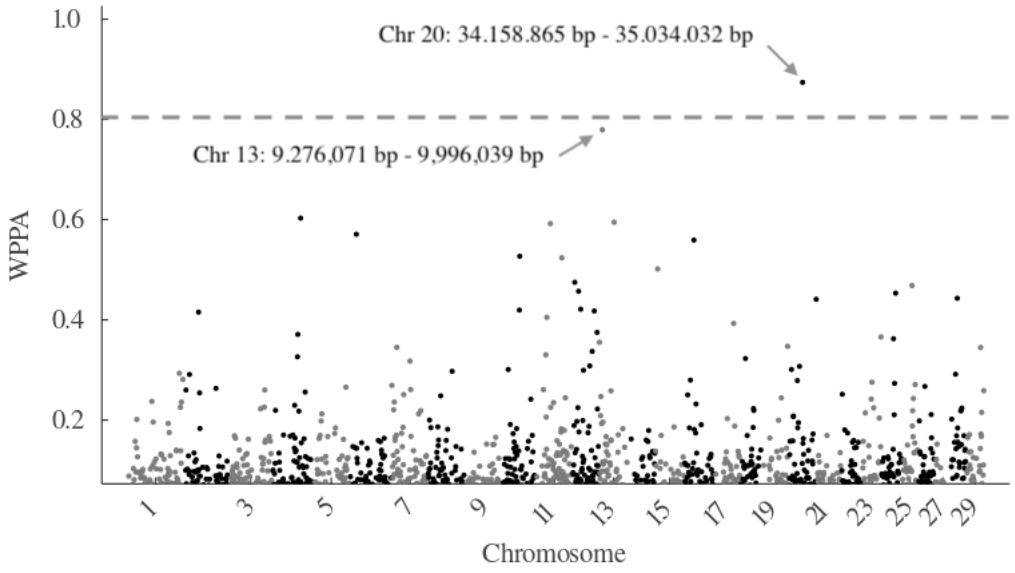


Figure 6.2 Genome-Wide Association Study for anogenital distance measured at approximately 11-mo old, in some 5,000 Holstein-Friesian or Holstein-Friesian x Jersey crossbred heifers. WPPA: Window Posterior Probability of Association for each 20-marker (approximately 1 Mb) genomic window, Chr: Chromosome.

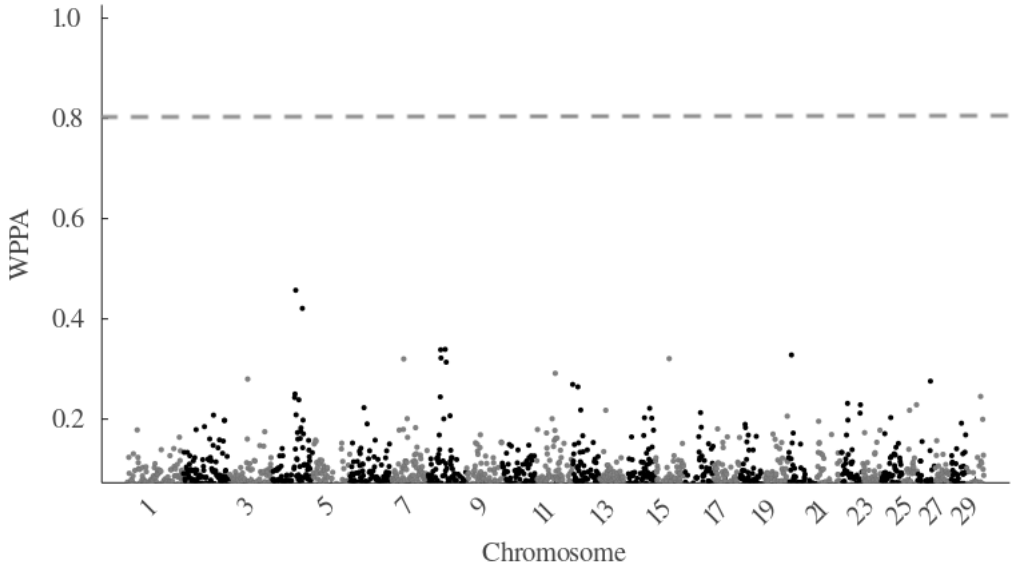


Figure 6.3 Genome-Wide Association Study for anogenital distance measured at approximately 29-mo old, in some 2,000 Holstein-Friesian, Holstein-Friesian x Jersey crossbred heifers during their first lactation. WPPA: Window Posterior Probability of Association for each 20-marker (approximately 1 Mb) genomic window.

Discussion

We investigated AGD and its genetic and phenotypic relationships with fertility traits in a population of predominantly Holstein-Friesian sired dairy cattle managed in a seasonal calving, pasture-based system. Our analysis determined that AGD measured at approximately 11 and 29 mo old were moderately heritable and highly genetically correlated traits, indicating that AGD has promise as a novel predictor trait that can be measured relatively early in life. Most importantly, AGD exhibited a moderate genetic correlation with a range of low heritability fertility traits that are measured during lactation and often used for genetic selection for fertility. Together, these results indicate that inclusion of AGD as a predictor trait could improve the accuracy of fertility EBVs, much earlier in the life of cows (and their sires) than conventional fertility phenotypes allow. Furthermore, we identified 2 genomic regions (chromosome 20 and 13) that were associated with AGD1. Investigation of these genomic regions could improve our understanding of the genetic architecture of calving-, breeding- and pregnancy-related fertility traits and offer opportunities to accelerate genetic improvement.

Heritability of AGD

Anogenital distance was a moderately heritable trait when measured at either age, but there was a suggestion that measuring AGD later in life may yield a higher heritability. An increased heritability in older animals could reflect their greater maturity. This logic is consistent with Rajesh et al. (2022), who reported that AGD became more repeatable as animals got older, possibly due to an interaction between AGD and maturity. Animals in the present study were born over a 3-mo period, as is typical for replacement heifers in a seasonal, pasture-based system, which means animal age and, therefore, maturity varied on the day that we measured AGD. We attempted to account for the maturity-related variance in AGD by fitting age as a fixed covariate within our analysis. For our analysis of AGD1, the effect of age was nearly 0.01 cm per day, which represents a difference between the oldest and youngest animals in this study of nearly 1 phenotypic standard deviation in AGD1. Conversely, the age effect was negligible when analysing AGD2 phenotypes, and it was subsequently removed from the model.

The heritability of AGD has not been widely published; however, Gobikrushanth et al. (2019) reported a heritability of 0.37 (± 0.08) in a population of dairy cows 2 to 4 yrs old, which is slightly higher than the heritability of 0.29 for AGD when measured at 29-mo old in our study. In the context of fertility-related traits, a candidate predictor trait with a moderate heritability is of high interest, as target fertility traits that tend to be measured during lactation generally have very low heritabilities. In New Zealand, the target fertility trait is a combination of calving rate (CR42) during second, third and fourth lactation. Calving rate phenotypes have

previously been reported to have heritabilities of less than 0.10 (Harris et al., 2006; Bowley et al., 2015) and, in the present study, we have also found low heritabilities for fertility phenotypes measured during the first and second lactations. For any given trait, response to selection is dependent on the reliability of EBVs and that is a function of heritability and number of observations. One strategy for improving the response to selection in a low heritability trait is to make use of traits with a non-zero genetic correlation. The large differential between the heritability of AGD and fertility traits measured during lactation means that AGD may add value as a predictor trait.

Correlation between AGD and fertility measured during lactation

To our knowledge, this is the first time that genetic associations between AGD and reproductive traits have been reported in dairy cattle. Our analysis indicates that the genetic relationships between AGD and calving, breeding and pregnancy rate phenotypes are moderately positive, indicating that shorter AGD is associated with earlier calving, breeding, and pregnancy dates in a seasonal calving system. Therefore, the use of AGD as a predictor trait would increase the reliability of fertility EBVs, particularly among animals that have an AGD phenotype, but do not have direct fertility phenotypes. In New Zealand, the fertility EBV represents a combination of calving rate in first, second, third and fourth parity. We have only considered first and second parity in our analysis, but we did not detect an interaction of parity on the genetic association between AGD and fertility traits measured during lactation. The consistency of our results across first and second lactation suggests that, in this population, AGD is predictive of fertility performance regardless of parity.

Our analysis indicates that the genetic correlation between AGD and target fertility traits are highest when AGD is measured in older animals. In this study, our second measure of AGD coincided with early pregnancy testing during the seasonal breeding period in first lactation, which would not confer a timing advantage over using pregnancy rate directly in fertility evaluations and would actually be available later than calving rate measured in first lactation. For both AGD1 and AGD2, however, our estimated genetic associations were accompanied by large 90CRIs. The lack of precision in these estimates makes it difficult to know if the genetic correlations truly depend on the age that AGD is measured, especially as AGD1 and AGD2 were themselves highly genetically correlated (0.89) with a tight 90CRI. We recommend that AGD and fertility phenotypes are measured on further cohorts of animals, as additional data will improve the precision of our estimated correlations. If the genetic correlation between AGD and fertility does depend on the timing of the AGD measure, then it would be useful to understand this dependency in more detail. It is likely that there is an age for measuring AGD when the trade-off between the strength of the genetic correlation and the potential earlier timing advantage are optimized; a longitudinal study of AGD is therefore

required. Ideally, this would involve repeat measurements of AGD at least bi-monthly in a population of animals from birth through to 30 mo of age so that the genetic correlations between AGD measured at many ages and fertility during lactation can be estimated directly.

In the population of dairy cattle used in the present study, the phenotypic associations between AGD and fertility traits were consistently close to 0; however, they tended to be weakly positive when animals were older at the time of the AGD measurement (AGD2). That is, shorter AGD2 exhibited a weak phenotypic association with superior fertility outcomes in our population. Our results indicate that there may be an interaction of age at the time of AGD measure, on the phenotypic association between AGD and fertility. This potential interaction is an important consideration for those who aim to predict fertility using AGD phenotypes. Like the genetic associations discussed previously, we did not detect an interaction of parity on the phenotypic associations we determined. Our results are supported by a general consensus in the literature that shorter AGD is associated with superior fertility (Gobikrushanth et al., 2017; Akbarinejad et al., 2019; Grala et al., 2021; Carrelli et al., 2022; Madureira et al., 2022).

Gobikrushanth et al. (2017) measured AGD in a population of North American dairy cattle managed in continuous calving, housed systems. They identified moderate phenotypic associations between AGD and reproductive traits, whereby a shorter AGD in first and second parity cows was associated with a greater pregnancy rate to first artificial insemination (P/AI) and a greater likelihood of pregnancy by 250 DIM. The authors noted, however, the possibility of an interaction between parity and AGD, as they did not observe an association between AGD and these fertility outcomes in third parity cows and greater. These higher parity groups are likely comprised of cows with superior genetic merit for fertility, as poorer fertility cows are generally culled from the herd. The work of Gobikrushanth et al. (2017) was later repeated in a mixed-parity population that included first, second and third plus parity Irish dairy cows managed in a seasonal calving, pasture-based system (Gobikrushanth et al., 2019). In that second study, authors reported a null association between AGD and fertility phenotypes, which included the PB21 and PR42 traits reported in the current study. Gobikrushanth et al. (2019) considered that aggressive selection for reproductive traits in their population of Irish cattle may have altered the phenotypic relationship between AGD and reproduction traits. However, more recently Grala et al. (2021) reported a moderate phenotypic association between AGD and CR42 in second lactation (shorter AGD was associated with earlier calving) in a herd of around 475 seasonally managed, grazing Holstein-Friesian cows in New Zealand. Fertility is a priority trait in New Zealand, and it has been directly incorporated into the national selection index since 2005 (Pryce et al., 2014). Instead, the null associations reported by Gobikrushanth et al. (2019) may be due to the greater representation of third parity and older animals in their population, as there is some evidence that the association between AGD and fertility phenotypes is dependent on parity (Gobikrushanth et al., 2017). Madureira et al. (2022) and Carrelli et al. (2022) also

grouped the cows in their studies by parity and reported that shorter AGD was phenotypically associated with improved performance for a range of fertility-related phenotypes in both their younger (first parity) and older (second parity and greater) cow groups. They also detected an interaction between AGD and parity for 1 of the phenotypes they measured (the relative increase in activity during estrus) and their results suggested a stronger relationship between AGD and estrus activity change in later parity cows. Gobikrushanth et al. (2019) analyzed cows from multiple parities together, and it is possible they would have detected an association between AGD and fertility if they had partitioned animals by parity and accounted for the fact that the least fertile animals were likely to have been culled before their third parity. Nevertheless, it is also possible that the association between AGD and fertility traits is population specific, which supports the need to investigate this novel phenotype across different cow populations.

The drivers of genetic variance in AGD and the physiological mechanisms underpinning its genetic and phenotypic association with fertility are not well understood; however, it has been established that AGD is a marker for pre-natal exposure to androgens, and dysfunction in pre-natal androgen exposure can have long standing effects on the health and fertility outcomes of mammals. For example, exposure to excess pre-natal testosterone concentrations has been shown to masculinize external genitalia of female rats (Hotchkiss et al., 2007) and sheep (Lamm et al., 2012). Conversely, androgen deficiencies, engineered by the administration of androgen or androgen receptor antagonists, resulted in a significantly reduced AGD in male rats (Welsh et al., 2008; MacLeod et al., 2010). Furthermore, inappropriate pre-natal androgen exposure is associated with a number of metabolic and reproductive disorders in mammals, including insulin resistance (Bruns et al., 2004), polycystic ovaries (Abbott et al., 1998), and permanent deformation of reproductive organs (Welsh et al., 2008; Lamm et al., 2012). It is likely that both AGD and fertility traits are influenced by pre-natal androgen exposure, and this is the basis of the associations exhibited between the two traits.

GWAS

Our GWAS of AGD1 identified 1 genomic region on chromosome 20 associated with variance in the trait, and a second region on chromosome 13 that was borderline using our WPPA threshold of 0.8. As a WPPA threshold is lowered, the chance of false positives in a GWAS is increased (Fernando et al., 2017). Conversely, using a higher WPPA threshold introduces risk that true positives will be disregarded. We attempted to balance these risks by setting our WPPA threshold as 0.80. To our knowledge, this is the largest dataset examined to date for genomic regions or SNP associated with AGD, but an even larger population size for phenotyping AGD1 would improve the statistical power of the GWAS presented here and perhaps lead to the identification of additional genomic regions above this WPPA threshold.

Further, neither of these regions were associated with AGD2 and our GWAS of AGD2 did not identify any additional genomic regions of interest. The discordance of our results across the 2 AGD phenotypes may simply be due to population size, as fewer animals had AGD2 phenotypes measured. In future, it would be beneficial to measure AGD2 in a larger population and repeat our GWAS analysis for this later phenotype. Enriching SNP chips across genomic regions associated with variation in AGD would potentially increase the prediction accuracy of genomic EBVs for AGD, improving its utility as a predictor trait for fertility. Identifying specific gene effects on AGD could improve the utility of AGD as a predictor trait for fertility in 2 ways. First, if a gene that is associated with AGD is also associated with fertility, then enriching a SNP chip in the region of that gene could directly improve genomic the prediction of fertility. Second, if a gene that is associated with AGD is not associated with fertility then removing genetic variation in AGD caused by this genomic region could ultimately improve the predictive ability of the adjusted ADG trait. The genomic region that we identified on chromosome 20 has been previously identified in a GWAS of AGD in 908 mixed-parity Irish Holstein-Friesian cows (Gobikrushanth et al., 2019). Although Gobikrushanth et al. (2019) did not identify any SNP significantly associated with variance in AGD after adjustment for multiple testing; they did highlight 6 SNP of suggestive association on chromosomes 6, 15, 20 and 26. The SNP identified on chromosome 20 in their study is approximately 10Mb from the boundary of our region of interest on chromosome 20. Therefore, our GWAS provides some support of an association between a QTL in this region, and variance in AGD, although given the distance of 10 Mb it is possible that we have identified an entirely different QTL in the present study. One candidate gene is located within this region, named *dab2*. The human analog of the *dab2* gene (*DAB2*) has been previously established as a tumor suppressant (Zhang et al., 2014). Morris et al. (2002) investigated the prenatal function of this gene using a knockout mouse model and reported that embryos that lacked the gene failed to progress past a very early development stage. In particular, the authors determined that the *dab2* gene was critical for normal development of the visceral endoderm, an extraembryonic cell layer that is involved with early embryo development. It is well established that AGD is permanently influenced during pre-natal development, and so it is plausible that a gene associated with normal early embryo development and general growth via regulation of cell proliferation could be associated with AGD. That said, further work is required to validate the association between *dab2* and AGD in dairy cattle. The second genomic region that we identified in our GWAS of AGD1 is located on chromosome 13. To our knowledge, this is the first time that this region has been associated with variance in AGD. There is 1 candidate gene within this region, called *MACROD2,* a gene associated with a reduction in antral follicle count (AFC), and anti-Mullerian hormone (AMH) in humans (Schuh-Huerta et al., 2012). Although these associations have not been validated in cattle, both AFC, which is a count of the number of antral follicles on

a female's ovaries, and AMH, which is a hormonal marker for AFC, have been established as predictors of reproductive competence in cattle (Alward and Bohlen, 2020). Given that the region of the *MACROD2* gene has now been associated with variance in AGD, and the gene itself has been associated with AFC and AMH, it is plausible that this gene is responsible for some of the covariance between AGD and fertility traits we determined. The *MACROD2* gene has also been associated with survival in dairy cattle (Shabalina et al., 2020), resistance to bovine tuberculosis (González-Ruiz et al., 2019), and cell growth mediation and proliferation in humans (Mohseni et al., 2014). Although this region on chromosome 13 exhibited a borderline WPPA in our GWAS analysis, the previous associations identified between *MACROD2* and fertility, immunity and cell growth mean that further investigation into the *MACROD2* gene is warranted.

Two previously published GWAS on AGD have detected associations between regions on chromosome 26, and variance in AGD (Gobikrushanth et al., 2019; Grala et al., 2021). In particular, Grala et al. (2021) used a small population of 538 first parity Holstein-Friesian cows managed in a seasonal calving, pasture-based dairy system in New Zealand (Grala et al., 2021); our dataset did not validate their result in a population of animals with similar genetic selection history and managed under a similar grazing-based dairy system. However, this different outcome may reflect the ad-mixed Holstein-Friesian and Jersey breed composition of our population, as it is possible that the QTL previously identified on chromosome 26 segregates to a lesser extent in the Jersey population, relative to Holstein-Friesians, and thus our ability to detect an effect at this locus may be reduced. We tested the effect of the 24 purebred Jersey animals on our GWAS results by excluding them and repeating the GWAS analysis. Excluding these Jersey animals did not meaningfully change our GWAS results.

AGD1 and AGD2 share a highly positive genetic correlation

We detected a highly positive genetic correlation between AGD1 and AGD2. This is important because AGD is more valuable as a predictor trait for fertility if it can be measured relatively early in an animal's life. The phenotypic correlation between the 2 AGD traits was moderate, and this aligns well with the results presented by Rajesh et al. (2022) who reported that AGD measures taken roughly 12 mo apart had a phenotypic correlation of around 0.30. Rajesh et al. (2022) concluded that animals must be at least 6 mo old before their AGD becomes phenotypically repeatable. The genetic correlations between AGD at ages younger than 6 mo old, and AGD at breeding age was not reported in this previous study. Here, we report a high genetic correlation, and a moderate phenotypic correlation between AGD measured at 11 mo and 28 mo of age. Thus, a low phenotypic correlation between AGD measured in animals <6 mo old is not necessarily indicative of a low genetic correlation. It would be beneficial to establish the genetic correlations between fertility and AGD at various early stages of life (<6

mo) to assist with designing large-scale phenotyping strategies if AGD is included as a predictor trait for fertility in routine genetic evaluations.

Correlations between AGD and body stature traits

Both phenotypic and genetic correlations between AGD and body stature (height, length, BW) phenotypes were weakly positive, demonstrating that longer AGD is associated with increased height, length, and BW. To our knowledge, this is the first time that genetic correlations between AGD and body confirmation traits have been reported for dairy cattle. The weak positive phenotypic correlations presented here align with these previous cattle studies (Gobikrushanth et al., 2017, 2019; Rajesh et al., 2022). Correlations reported for humans are varied, ranging from weak to moderate. For example, (Sathyanarayana et al., 2010) determined a moderate correlation of 0.4 to 0.5 between AGD and birth weight and birth length in human infants, whereas (Thankamony et al., 2009) reported only weak associations between AGD and weight and length in humans. Weak correlations between AGD and body confirmation traits has important implications for the use of AGD as a marker of reproductive success, as it indicates that the AGD trait is largely independent of these routinely measured phenotypes. As such, AGD represents a truly novel phenotype.

Correlation between AGD and AGEP

In our population, AGD1 exhibited a low genetic correlation with AGEP. This genetic correlation improved slightly when AGD was measured at an older age, but still remained low to moderate. Age at puberty has previously been identified as a candidate trait to predict reproductive success (Morris et al., 2000; Mialon et al., 2001; Lefebvre et al., 2021). It is useful to know that AGD and AGEP do not share a high genetic covariance as this suggests that they could have complementary value as predictor traits for reproductive success. Anogenital distance is more straightforward to measure then AGEP, and it has the advantage of being easily measured by farmers. It is worth noting that AGEP was measured in this population using a phenotyping strategy designed to be cost effective and practical at a large scale, and as a result, measurement precision was compromised. We have previously established that the reduced precision of our AGEP phenotype is unlikely to have implication for our genetic analyses presented here (Stephen et al., 2022b; a)

Conclusion

Our results indicate that female AGD can provide value as a predictor for fertility EBVs in dairy cattle. The AGD trait can be measured much earlier in a cow's life than economically important fertility traits such as calving, breeding and pregnancy rates, and using AGD to

predict fertility EBVs may increase the reliability of fertility EBVs. This increase in reliability may improve the accuracy of selection for fertility, and thus increase the rate of genetic gain. Further, the strength of the genetic correlation between AGD and fertility may depend on the timing of the AGD measure.

Acknowledgments

This study was funded by New Zealand dairy farmers through DairyNZ Inc. and by the New Zealand Ministry of Business, Innovation and Employment (DRCX1302). The authors gratefully acknowledge the support of LIC, CRV Ambreed, and New Zealand Animal Evaluation Ltd. (NZAEL) during herd enrolment into this study, particularly R. Bloxham (LIC), and J. Bryant and A. Young (formerly DairyNZ/NZAEL). The authors also kindly thank the DairyNZ technical team (especially S. Morgan, K. Watkins, A. Knight, N. Leach, and J. Brown) and the participating veterinary clinics and their staff for sampling, lab analyses, and data collection (especially E. Cuttance, VetEnt Te Awamutu; K. Goodare and S. McDougall, Anexa FVC Morrinsville; S. Bateman, Okato Vets; M. O'Sullivan, Oamaru Vets). This research would not have been possible without the generous support of the 54 herd owners and their graziers who provided access to their 2018-born dairy heifers for sampling and measurements.

I wish to acknowledge the use of New Zealand eScience Infrastructure (NeSI) high performance computing facilities as part of this research. New Zealand's national facilities are provided by NeSI and funded jointly by NeSI's collaborator institutions and through the Ministry of Business, Innovation & Employment's Research Infrastructure programme.

References

Abbott, D.H., D.A. Dumesic, J.R. Eisner, R.J. Colman, and J.W. Kemnitz. 1998. Insights into the development of Polycystic Ovary Syndrome (PCOS) from studies of prenatally androgenized female Rhesus monkeys. Trends in Endocrinology & Metabolism 9:1043–2760. doi:10.1016/S1043-2760(98)00019-8.

Akbarinejad, V., F. Gharagozlou, M. Vojgani, E. Shourabi, and M.J.M. Makiabadi. 2019. Inferior fertility and higher concentrations of anti-Müllerian hormone in dairy cows with longer anogenital distance. Domest Anim Endocrinol 68:47–53. doi: 10.1016/j.domaniend.2019.01.011.

Alward, K.J., and J.F. Bohlen. 2020. Overview of Anti-Müllerian hormone (AMH) and association with fertility in female cattle. Reproduction in Domestic Animals 55:3–10. doi:10.1111/rda.13583.

Berry, D.P., E. Wall, and J.E. Pryce. 2014. Genetics and genomics of reproductive performance in dairy and beef cattle. Animal 8:105–121. doi:10.1017/S1751731114000743.

Bezanson, J., A. Edelman, S. Karpinski, and V.B. Shah. 2017. Julia: A fresh approach to numerical computing. SIAM Review 59:65–98. doi:10.1137/141000671.

Bowley, F.E., R.E. Green, P.R. Amer, and S. Meier. 2015. Novel approaches to genetic analysis of fertility traits in New Zealand dairy cattle. J Dairy Sci 98:2005–2012. doi:10.3168/jds.2014-8266.

Bruns, C.M., S.T. Baum, R.J. Colman, J.R. Eisner, J.W. Kemnitz, R. Weindruch, and D.H. Abbott. 2004. Insulin resistance and impaired insulin secretion in prenatally androgenized male rhesus monkeys. Journal of Clinical Endocrinology and Metabolism 89:6218–6223. doi:10.1210/jc.2004-0918.

Carrelli, J.E., M. Gobikrushanth, M. Corpron, I. Rajesh, W. Sandberg, M.G. Colazo, A. Ahmadzadeh, M. Oba, and D.J. Ambrose. 2021. Relationship of anogenital distance with fertility in nulliparous Holstein heifers. J Dairy Sci 104:8256–8264. doi:10.3168/jds.2020-19940.

Carrelli, J.E., M. Gobikrushanth, M. Corpron, W. Sandberg, I. Rajesh, A. Ahmadzadeh, M. Oba, and D.J. Ambrose. 2022. Associations between anogenital distance and measures of fertility in lactating North American Holstein cows: A validation study. J Dairy Sci 105:6339–6352. doi:10.3168/jds.2021-20827.

Cheng, H. 2019. JWAS.

Dennis, N.A., K. Stachowicz, B. Visser, F.S. Hely, D.K. Berg, N.C. Friggens, P.R. Amer, S. Meier, and C.R. Burke. 2018. Combining genetic and physiological data to identify predictors of lifetime reproductive success and the effect of selection on these predictors on underlying fertility traits. J Dairy Sci 101:3176–3192. doi:10.3168/jds.2017-13355.

Fernando, R., A. Toosi, A. Wolc, D. Garrick, and J. Dekkers. 2017. Application of Whole-Genome Prediction Methods for Genome-Wide Association Studies: A Bayesian Approach. J Agric Biol Environ Stat 22:172–193. doi:10.1007/s13253-017-0277-6.

Garrick, D., J. Dekkers, and R. Fernando. 2014. The evolution of methodologies for genomic prediction. Livest Sci 166:10–18. doi:10.1016/j.livsci.2014.05.031.

Geweke, J. 1992. Evaluating the accuracy of sampling-based approaches to the calculation of posterior moments. Bayesian Statistics 169–193.

Gobikrushanth, M., T.C. Bruinjé, M.G. Colazo, S.T. Butler, and D.J. Ambrose. 2017. Characterization of anogenital distance and its relationship to fertility in lactating Holstein cows. J Dairy Sci 100:9815–9823. doi:https://doi.org/10.3168/jds.2017-13033.

Gobikrushanth, M., D.C. Purfield, J. Kenneally, R.C. Doyle, S.A. Holden, P.M. Martinez, E.R. Canadas, T.C. Bruinjé, M.G. Colazo, D.J. Ambrose, and S.T. Butler. 2019. The relationship between anogenital distance and fertility, and genome-wide associations for anogenital distance in Irish Holstein-Friesian cows. J Dairy Sci 102:1702–1711. doi:10.3168/jds.2018-15552.

González-Ruiz, S., M.G. Strillacci, M. Durán-Aguilar, G.J. Cantó-Alarcón, S.E. Herrera-Rodríguez, A. Bagnato, L.F. Guzmán, F. Milián-Suazo, and S.I. Román-Ponce. 2019. Genome-wide association study in mexican holstein cattle reveals novel quantitative trait loci regions and confirms mapped loci for resistance to bovine tuberculosis. Animals 9:1–15. doi:10.3390/ani9090636.

Grala, T.M., M.D. Price, B. Kuhn-Sherlock, C.R. Burke, and S. Meier. 2021. Investigating anogenital distance and antral follicle count as novel markers of fertility within a herd of cows with positive or negative genetic merit for fertility traits. J Dairy Sci 104:12939–12952. doi:10.3168/jds.2020-19948.

Habier, D., R.L. Fernando, K. Kizilkaya, and D.J. Garrick. 2011. Extension of the bayesian alphabet for genomic selection. BMC Bioinformatics 12. doi:10.1186/1471-2105-12-186.

Harris, B.L., J.E. Pryce, Z.Z. Xu, and W.A. Montgomerie. 2006. Development of new fertility breeding values in the dairy industry. Pages 107–112 in Proceedings of the New Zealand Society of Animal Production. New Zealand Society of Animal Production, Napier.

Hickson, R.E., C.C. Balcomb, K.R. Fraser, N. Lopez-Villalobos, P.R. Kenyon, and S.T. Morris. 2011. The effect of breed on the onset of puberty in heifers. Pages 51–54 in Proceedings of the Association for the Advancement of Animal Breeding and Genetics, Perth, Australia.

Hotchkiss, A.K., C.S. Lambright, J.S. Ostby, L. Parks-Saldutti, J.G. Vandenbergh, and L.E. Gray. 2007. Prenatal testosterone exposure permanently masculinizes anogenital distance, nipple development, and reproductive tract morphology in female sprague-dawley rats. Toxicological Sciences 96:335–345. doi:10.1093/toxsci/kfm002.

Johnston, D.J., S.A. Barwick, N.J. Corbet, G. Fordyce, R.G. Holroyd, P.J. Williams, and H.M. Burrow. 2009. Genetics of heifer puberty in two tropical beef genotypes in northern Australia and associations with heifer- and steer-production traits. Anim Prod Sci 49:399–412. doi:10.1071/EA08276.

Lamm, C.G., P.M. Hastie, N.P. Evans, and J.E. Robinson. 2012. Masculinization of the distal tubular and external genitalia in female sheep with prenatal androgen exposure. Vet Pathol 49:546–551. doi:10.1177/0300985811419533.

Lefebvre, R., H. Larroque, S. Barbey, Y. Gallard, J.J. Colleau, A.L. Laine, D. Boichard, and P. Martin. 2021. Genome-wide association study for age at puberty and resumption of cyclicity in a crossbred dairy cattle population. J Dairy Sci 104:5794–5804. doi:10.3168/jds.2020-18228.

Macdonald, K.A., and J.R. Roche. 2023. Farm systems research at Ruakura–a 60-year legacy underpinning profitable and sustainable pasture-based dairy systems. New Zealand Journal of Agricultural Research 1–118. doi:10.1080/00288233.2023.2168707.

MacLeod, D.J., R.M. Sharpe, M. Welsh, M. Fisken, H.M. Scott, G.R. Hutchison, A.J. Drake, and S. Van Den Driesche. 2010. Androgen action in the masculinization programming

window and development of male reproductive organs. Int J Androl 33:279–287. doi:10.1111/j.1365-2605.2009.01005.x.

Madureira, A.M.L., T.A. Burnett, J.E. Carrelli, M. Gobikrushanth, R.L.A. Cerri, and D.J. Ambrose. 2022. Anogenital distance is associated with postpartum estrous activity, intensity of estrous expression, ovulation, and progesterone concentrations in lactating Holstein cows. J Dairy Sci. doi:10.3168/jds.2022-21897.

Meier, S., L.R. McNaughton, R.C. Handcock, P.R. Amer, P.R. Beatson, J.R. Bryant, K.G. Dodds, R. Spelman, J.R. Roche, and C.R. Burke. 2021. Heifers with positive genetic merit for fertility traits reach puberty earlier and have a greater pregnancy rate than heifers with negative genetic merit for fertility traits.. J Dairy Sci 104:3707–3721. doi:10.3168/jds.2020-19155.

Mendiola, J., M. Roca, L. Mínguez-Alarcón, M.P. Mira-Escolano, J.J. López-Espín, E.S. Barrett, S.H. Swan, and A.M. Torres-Cantero. 2012. Anogenital distance is related to ovarian follicular number in young Spanish women: a cross-sectional study. Environ Health 11:1–8. doi:10.1186/1476-069X-11-90.

Mialon, M.M., G. Renand, D. Krauss, and F. Ménissier. 2001. Genetic relationship between cyclic ovarian activity in heifers and cows and beef traits in males. Genetics Selection Evolution 33:273–287. doi:10.1186/1297-9686-33-3-273.

Mohseni, M., J. Cidado, S. Croessmann, K. Cravero, A. Cimino-Mathews, H.Y. Wong, R. Scharpf, D.J. Zabransky, A.M. Abukhdeir, J.P. Garay, G.M. Wang, J.A. Beaver, R.L. Cochran, B.G. Blair, D.M. Rosen, B. Erlanger, P. Argani, P.J. Hurley, J. Lauring, and B.H. Park. 2014. MACROD2 overexpression mediates estrogen independent growth and tamoxifen resistance in breast cancers. Proc Natl Acad Sci U S A 111:17606–17611. doi: 10.1073/pnas.140865011.

Morris, C.A., J.A. Wilson, G.L. Bennett, N.G. Cullen, S.M. Hickey, and J.C. Hunter. 2000. Genetic parameters for growth, puberty, and beef cow reproductive traits in a puberty selection experiment. New Zealand Journal of Agricultural Research 43:83–91. doi:10.1080/00288233.2000.9513411.

Morris, S.M., M.D. Tallquist, C.O. Rock, and J.A. Cooper. 2002. Dual roles for the Dab2 adaptor protein in embryonic development and kidney transport. EMBO Journal 21:1555–1564. doi:10.1093/emboj/21.7.1555.

Pryce, J.E., R. Woolaston, D.P. Berry, E. Wall, M. Winters, R. Butler, and M. Shaffer. 2014. World trends in dairy cow fertility. Pages 1–10 in 10th World Congress of Genetics Applied to Livestock Production.

Rajesh, I., M. Gobikrushanth, J.E. Carrelli, M. Oba, and D.J. Ambrose. 2022. Repeatability of anogenital distance measurements from birth to maturity and at different physiological states in female Holstein cattle. J Dairy Sci 86. doi:10.3168/jds.2021-21419.

Sargolzaei, M., J.P. Chesnais, and F.S. Schenkel. 2014. A new approach for efficient genotype imputation using information from relatives. BMC Genomics 15. doi:10.1186/1471-2164-15-478.

Sathyanarayana, S., L. Beard, C. Zhou, and R. Grady. 2010. Measurement and correlates of ano-genital distance in healthy, newborn infants. Int J Androl 33:317–323. doi:10.1111/j.1365-2605.2009.01044.x.

Schuh-Huerta, S.M., N.A. Johnson, M.P. Rosen, B. Sternfeld, M.I. Cedars, and R.A. Reijo Pera. 2012. Genetic markers of ovarian follicle number and menopause in women of multiple ethnicities. Hum Genet 131:1709–1724. Doi:10.1007/s00439-012-1184-0.

Shabalina, T., T. Yin, and S. König. 2020. Survival analyses in Holstein cows considering direct disease diagnoses and specific SNP marker effects. J Dairy Sci 103:8257–8273. doi:10.3168/jds.2020-18174.

Stephen, M.A., H. Cheng, J.E. Pryce, C.R. Burke, N.M. Steele, C.V.C. Phyn, and D.J. Garrick. 2022a. Estimating heritabilities and breeding values from censored phenotypes using a data augmentation approach. Front Genet 13. doi:10.3389/fgene.2022.867152.

Stephen, M.A., S. Meier, M.D. Price, J.E. Pryce, C.R. Burke, Phyn. C.V.C., and D.J. Garrick. 2022b. Variance parameter estimation for age at puberty phenotypes under two levels of phenotype censorship. Journal of Dairy Science Communications. doi:10.3168/jdsc.2022-0218.

Sun, C., P. Madsen, M.S. Lund, Y. Zhang, U.S. Nielsen, and G. Su. 2010. Improvement in genetic evaluation of female fertility in dairy cattle using multiple-trait models including milk production traits. J Anim Sci 88:871–878. doi:https://doi.org/10.2527/jas.2009-1912.

Thankamony, A., K.K. Ong, D.B. Dunger, C.L. Acerini, and I.A. Hughes. 2009. Anogenital distance from birth to 2 years: A population study. Environ Health Perspect 117:1786–1790. doi:10.1289/ehp.0900881.

Toosi, A., R.L. Fernando, and J.C.M. Dekkers. 2018. Genome-wide mapping of quantitative trait loci in admixed populations using mixed linear model and Bayesian multiple regression analysis. Genetics Selection Evolution 50. doi:10.1186/s12711-018-0402-1.

Verkerk, G. 2003. Pasture-based dairying: Challenges and rewards for New Zealand producers. Theriogenology 59:553–561. doi:10.1016/S0093-691X(02)01239-6.

Welsh, M., P.T.K. Saunders, M. Fisken, H.M. Scott, G.R. Hutchison, L.B. Smith, and R.M. Sharpe. 2008. Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. Journal of Clinical Investigation 118:1479–1490. doi:10.1172/JCI34241.

Williams, G.L., and R.C. Cardoso. 2021. Neuroendocrine Control of Estrus and Ovulation. wiley.

Wolcott, M.L., R.G. Banks, M. Tweedle, and G. Alder. 2018. Genetics of heifer age at puberty in Australian Hereford cattle. Pages 91–94 in Proceedings of the Association for the Advancement of Animal Breeding and Genetics Assoc. Advmt. Anim. Breed. Genet.

Xiang, R., I.M. MacLeod, H.D. Daetwyler, G. de Jong, E. O'Connor, C. Schrooten, A.J. Chamberlain, and M.E. Goddard. 2021. Genome-wide fine-mapping identifies pleiotropic and functional variants that predict many traits across global cattle populations. Nat Commun 12:1–13. doi:10.1038/s41467-021-21001-0.

Zehr, J.L., S.E. Gans, and M.K. McClintock. 2001. Variation in reproductive traits is associated with short anogenital distance in female rats. Dev Psychobiol 38:229–238. doi:10.1002/dev.1017.

Zhang, Z., Y. Chen, J.J. Tang, and X. Xie. 2014. Frequent loss expression of dab2 and promotor hypermethylation in human cancers: A meta-analysis and systematic review. Pak J Med Sci 30:432–437. doi:10.12669/pjms.302.4486.

CHAPTER 7. General Discussion

Unpublished, June 2023

Introduction

In this thesis, I have demonstrated that age at puberty (AGEP) and anogenital distance (AGD) are promising predictors of an animal's genetic merit for fertility. Both traits are moderately heritable, can be measured early-in-life and exhibit moderate genetic correlations with fertility traits measured during lactation. Genetic improvement provides a long-term approach for improving the fertility performance of dairy cattle. Fertility is under direct selection in many countries around the world (Pryce et al., 2014), but progress is limited due to the low heritability (Harris et al., 2005; Bowley et al., 2015; Stachowicz et al., 2021) and delayed expression of breeding-, calving- and pregnancy-based fertility traits. Predictor traits like AGEP and AGD, which are more heritable than common fertility traits and/or can be measured earlier in an animal's life, provide an avenue for improving the early-in-life accuracy of fertility estimated breeding values (EBVs).

Furthermore, early-in-life predictor traits like AGEP and AGD can be complementary to the use of genomic prediction as a strategy for improving the selection accuracy of challenging traits such as fertility. Genomic prediction leverages phenotypes collected on older genotyped animals to estimate EBVs for young animals. This approach provides an important avenue for improving the accuracy of EBVs early in life, particularly for low heritability traits. The accuracy of genomic prediction depends on a wide range of factors, including the size of the reference population (that is, the number of animals with both genotypes and phenotypes), the degree of genomic relatedness and/or similarities between the reference population and the selection candidates (Daetwyler et al., 2012). Recombination (Johnsson et al., 2022) and, to a lesser extent, *de novo* genetic mutations that mean that the genomes of animals change structurally with each new generation. Therefore, the ongoing accuracy and relevance of genomic EBVs can only be maintained by regularly incorporating new phenotype data into an evaluation. Correlated early predictor traits can contribute timely information on new generations of animals, which is valuable for both pedigree and genomic evaluations especially for target traits that are expressed relatively later in life such as calving date or (re)calving interval traits. Furthermore, predictor traits can help improve our understanding of the genetic architecture of a low heritability target trait.

If the number of phenotypes in the training population is a constant, the accuracy of SNP effects depends upon the heritability of a trait (Gonzalez-Recio et al., 2014). Therefore, a genome-wide association study (GWAS) on a higher heritability predictor trait has more power to detect QTL, and some of those QTL may also be relevant to a genetically correlated target trait. However, measuring predictor traits incurs additional cost and effort, and this can present a barrier for implementation. Therefore, the cost and effort associated with measuring a trait is an important consideration when assessing its suitability as a useful predictor trait. In this thesis, I

provide evidence that supports the merit of including large-scale, practical, and cost-effective phenotyping strategies for AGEP and AGD as early predictor traits of genetic merit for fertility.

In the first chapter of this thesis, I reviewed the literature related to the importance of fertility to the NZ dairy industry, the merit of AGEP and AGD as predictors of target fertility traits, and the genetic architectures of AGEP and AGD. My literature review led me to hypothesize that both AGEP and AGD would exhibit moderate genetic correlations with target fertility traits like breeding, calving and pregnancy rates, and that we were likely to detect genomic regions associated with variation in either AGEP or AGD using GWAS. Chapters 2 to 6 of the thesis address key factors that influence the value of AGEP and AGD as predictor traits, including the development of routine data collection and subsequent statistical approaches for analysing AGEP phenotypes, the heritabilities of AGEP and AGD, and the genetic correlations that AGEP and AGD exhibit with fertility traits under selection, as well as a range of body stature traits. In Chapters 5 and 6, I undertook GWAS analysis of AGEP and AGD, respectively, with the objective of improving our understanding of the genetic architecture of these traits. A puberty-at-scale (PS) population of 5,010 animals, farmed across 54 seasonal calving, pasture-based herds around NZ were used for this study. Following various data quality edits, some 4,688 of these animals were included in our analysis, comprising Holstein-Friesian (2,340), Holstein-Friesian cross Jersey (2,276), Jersey (24) and Other (48). These animals were phenotyped for traits of interest from approximately 10 mo of age as peripubertal heifers and during their first and second lactations after calving at approximately 24 mo and 36 mo of age, respectively.

Age at puberty

I explored the potential of AGEP as an early-in-life predictor trait for improving fertility EBVs using data from the PS study , which utilized a novel phenotyping approach to measure puberty across large numbers of animals managed in seasonal, pasture-based dairy herds. The animals in the PS population attained puberty at around 12 mo old, as is typical of breeds with *Bos taurus* origins (Hickson et al., 2011; Meier et al., 2021). Promisingly, the AGEP trait had a moderate heritability of 0.34 (Chapter 5), which aligns well with existing literature (Morris et al., 2000; Fortes et al., 2012; Lefebvre et al., 2021), and it exhibited moderate genetic correlations (0.11 to 0.60) with subsequent breeding, calving and pregnancy phenotypes expressed during first and second lactation (Chapter 5). Overall, the results support the use of AGEP as a predictor of genetic merit for fertility whereby selection for earlier puberty onset is associated with better reproductive performance measured during lactation.

Phenotyping strategy

The AGEP trait has long been established as a promising candidate predictor trait for both reproductive success during lactation (Morris and Amyes, 2005) and subsequent lifetime profitability. This is particularly so in Continental and *Bos indicus* breeds in which AGEP often limits age at first calving to 3 yrs or later (Nogueira, 2004). However, despite this potential to add value to dairy farm systems, large-scale phenotyping initiatives involving AGEP in *Bos taurus* breeds are conspicuously absent from the literature. This is due, in part, to the cost and difficulties associated with measuring precise AGEP phenotypes across large numbers of animals.

My work has contributed towards the development of a cost-efficient approach for genetic evaluation of the AGEP trait. First, the definition for an animal to be categorized as post-pubertal was simplified. Second, phenotype censoring (where phenotypes are not known precisely, but rather only known to fall within a lower and upper bound) was used to reduce the number of observations per animal using a herd-based strategy appropriate for seasonal dairy systems. Finally, a data augmentation approach was implemented to analyze the censored AGEP phenotypes. In Chapters 2 to 4, I demonstrated that AGEP EBVs and variance parameters are relatively unperturbed by increasing the extent of phenotype censoring, especially when using a data augmentation approach.

In this work, an animal was categorized as post-pubertal once it had been observed with elevated blood plasma progesterone (BP4), as this is indicative of a functioning corpus luteum and, therefore, ovulation. In the PS study, blood testing was carried out on 3 occasions by visiting each herd at intervals of approximately 30 d starting when the average age of the heifers in a herd was about 10 mo old.

The results I have presented in Chapters 2, 3, and 4 demonstrate that the simplified phenotyping strategy implemented in the PS study is suitable for the purpose of genetic evaluation. In Chapter 2, I explored the effect of phenotype censorship using a dataset from a prior study of ~500 animals that had weekly BP4 concentrations measured between about 240 to 440 d old and were determined to have reached puberty when 2 of 3 consecutive blood tests showed BP4 elevation (>1 ng/mL). To simulate a simplified phenotyping strategy aligned with the PS study design, data were selectively disregarded from all but 3 blood test events when animals were around 300, 330 and 360 d old. I determined that variance parameters and EBVs were robust to the increased phenotype censoring. In Chapter 3, the data in the PS study were used to explore the implications of even fewer herd visits, by disregarding either 1 or 2 of the possible 3 BP4 test results available for each animal. I found that variance parameters and EBVs were generally robust to reducing the frequency of blood test visits, especially when the BP4 results from the earlier of the 3 visits were included in the analysis. In Chapter 4, the results of Chapters 2 and 3 were extended using simulated data, so that a comparison could be made

between variance parameters and EBVs produced using precise (daily) measurements for AGEP and those produced using the phenotyping strategy implemented in the PS study. In this simulated dataset, the results of our genetic analysis were very stable across blood testing regimes. Together, these 3 chapters provide comprehensive evidence that censored phenotypes derived from relatively infrequent observation data are suitable for the purpose of genetic analysis. Furthermore, in each of these chapters, I reported a moderate heritability for AGEP, indicating that the simplified definition for the on-set of puberty (that is, BP4 elevation >1 ng/mL) did not compromise the genetic parametrization of the AGEP trait.

A more comprehensive definition of a post-pubertal heifer generally includes all elements of reproduction (Moran et al., 1989). That is, to be capable of reproducing, an animal must be able to ovulate, exhibit behavioral estrus, and have a normal luteal phase. This is a detailed set of criteria to monitor, and the biological window within which a heifer can attain puberty is around 200 d wide (Dennis et al., 2018). With these factors in mind, obtaining very precise measures of AGEP would require extremely detailed measures carried out daily. Obviously, this is not practical or economically feasible across large numbers of animals, and so like us, other researchers have tended to accept some degree of phenotype censoring and/or simplification of the criterion for AGEP (Johnston et al., 2009; Hickson et al., 2011; Meier et al., 2021). For example, it is common in the literature for animals to have their puberty status measured on a weekly rather than daily basis (Johnston et al., 2009; Hickson et al., 2011; Meier et al., 2021). Simplification of the criterion for AGEP can involve choosing to monitor any subset of the three criteria of puberty (behavioral estrus, ovulation, and normal luteal function). For example, categorizing animals as pubertal once there is evidence of ovulation, even though behavioral estrus and the length of the luteal phase may not have been monitored (Johnston et al., 2009; Meier et al., 2021). That said, even with these concessions, AGEP remains a costly and logistically challenging phenotype to measure across large numbers of animals.

In this thesis, I provide evidence that supports “pushing the boundary” on simplification and censoring of puberty status measures when assessing AGEP in the context of genomic prediction rather than in physiology type studies. Heavily censored observation data may not yield a precise AGEP phenotype for individual animals, but this is not necessarily important in genetic analyses, whereby many phenotypes are analysed simultaneously to make inference on the genetic merit of individuals. Moreover, if a low precision approach enables phenotypes to be measured on a larger number of animals, these additional genotypes and phenotypes can help to reduce the collinearity among genotypes, and thereby improve our ability to separate the effects of individual markers within genomic regions. These results should provide researchers with confidence to strategically use phenotype simplification and censoring to control costs when measuring AGEP phenotypes for the purpose of genetic evaluation.

Going forward, new technologies will likely provide an alternative avenue for reducing the costs associated with measuring AGEP phenotypes. Wearable devices that monitor activity are becoming an increasingly popular tool for monitoring many cow behaviors including estrus detection. Although they are currently mostly fitted to lactating cows and can, therefore, be used to detect estrus during the breeding period, they could also be fitted to yearling replacement cattle to measure age at first estrus as a proxy for AGEP. The main drawback of activity monitoring devices are the upfront costs associated with purchasing the hardware, which are substantial. It is currently unlikely that farmers would invest in this technology solely for the purpose of measuring AGEP, especially given that the devices would need to be fitted to animals as yearlings to capture age at first estrus. There are several financial, logistical, and animal welfare considerations that pose barriers to using activity monitoring devices on calves and yearlings. First, farmers would have to purchase additional hardware to have enough wearables to cover their young replacement heifers, as well as their dairy herd. Second, NZ dairy heifers are often grazed on extensive grazing properties, which may be some kilometers away from the main dairy farm. Many wearable devices require animals to walk near a stationary receiver on a regular basis to facilitate the transfer of data from their device to a database. This receiver is usually placed at the dairy shed, as animals are milked regularly. However, in the context of heifer grazing, a dedicated set up may be expensive or simply impossible to implement. That said, product development across the industry of wearable activity loggers is progressing rapidly, and it is likely that devices that are better suited to an extensive grazing system will be available in the future. Finally, heifers in extensive grazing farm systems are not observed on a daily basis, and therefore any injury that occurs due to a wearable device may not be detected within a reasonable timeframe, causing avoidable suffering for the animal. The risk of this is heightened due to growth of the animals, as fit of the devices must be adjusted to compensate for this. However, if the devices were fitted to yearling heifers for some other purpose, perhaps one with a more direct economic or compliance benefit to farmers (for example, to limit access to waterways, manage grazing, or monitor health indicators in growing heifers) then AGEP data could be obtained marginally from that existing data.

Under a model of genomic selection, it is common for a nucleus of ‘data collection’ herds to be established. In those herds, the herd owner is often compensated in some way for making their animals available for phenotype measurement and, in turn, they are accepting of repeated phenotyping initiatives. A good international example of this concept is the Ginfo initiative in Australia (Pryce et al., 2017), which involves more than 30,000 cows, from over 100 herds. A concept like this could improve the cost-effectiveness of measuring the AGEP trait. At minimum, the relationships with the herd owners are established, and the animals would already be genotyped. It is also feasible that the animals in these herds would already be

subject to other phenotyping activities around the time that puberty status is of interest (for example, weighing), and so there are potential efficiencies regarding the costs of visiting a herd and yarding animals. In the future, it may also make sense to fit animals in these herds with activity monitors, which would dramatically reduce the marginal cost of AGEP phenotypes.

AGEP is genetically correlated with fertility measured during lactation.

In the PS population, the AGEP trait exhibited moderate genetic correlations with subsequent fertility phenotypes, ranging from 0.53 and 0.60 for breeding date in first and second lactation, respectively, to 0.45 and 0.58 for calving date, and 0.34 and 0.11 for pregnancy date (Chapter 5). Moreover, the genetic correlation between AGEP and fertility traits measured during lactation was generally robust to censoring within the AGEP phenotypes. Lefebvre et al. (2021) recently a reported genetic correlation of 0.45 between AGEP and postpartum anoestrus interval (PPAI), which is a trait that shares some similarities with our breeding date trait (that is, success or failure to present for breeding in the first 21 d of the seasonal breeding period). That said, breeding date does not fully align with PPAI. The herds in the PS study were subject to a fixed breeding start date, as is commonplace in a seasonal farm system. A fixed breeding start date creates some left censoring, because some animals would have been cycling but not bred prior to the start of the breeding period. Our results are most comparable to those of Morris et al. (2000), who also reported moderate genetic correlations between AGEP and calving date and pregnancy rate traits in seasonal, pasture-based beef cattle. The fertility EBV used in the NZ dairy industry represents calving rate in second, third and fourth lactation (Stachowicz et al., 2021). Therefore, the results presented in chapter 5 support the use of AGEP as an early predictor trait for dairy cattle fertility EBVs in NZ.

GWAS of age at puberty

A GWAS analysis of AGEP using the PS population as a reference population was undertaken in Chapter 5. A genomic region, located on chromosome 5, was identified as being associated with variation in AGEP phenotypes in the PS population. A further 4 regions, located on chromosomes 14, 6, 1 and 11 (in order of decreasing importance) were suggestively associated with AGEP. Moreover, the associations between 3 of the 5 initial regions (chromosome 5, 6 and 1) and variation in AGEP phenotypes were successfully validated in an independent validation population from the prior fertility research herd (FRH) study (Meier et al., 2021). There was compelling evidence that a genomic region around 105 Mb on chromosome 5 harbors a QTL associated with variation in AGEP, as well as biological rationale for a QTL around 23 Mb on chromosome 6 potentially related to the neurokinin-kisspeptin signaling pathway. My results indicate that further investigations into the identified genomic

regions on chromosomes 5, 6, and 1 are warranted to determine if enrichment of SNP chips in these regions can improve the accuracy of fertility EBVs.

Few authors have reported GWAS for AGEP directly, but GWAS for related traits (such as age at first calving, age at first service, heifer pregnancy etc.) are more common. The lack of literature relating to genomic analysis of AGEP may be due to the costs associated with measuring the phenotype, as GWAS analysis tend to utilize large numbers of animals. One dataset of AGEP phenotypes measured in a population of around 2,000 Australian tropical composite beef cattle (Johnston et al., 2009) has been used for GWAS analysis by Hawken et al. (2012), Fortes et al. (2012) and Fortes et al. (2016). In that beef cattle dataset, AGEP was defined as the age at first detected corpus luteum, using ultrasound scanning carried out at intervals of 4 to 6 weeks. Authors reported numerous significant SNP. The concordance with the GWAS results from the PS population was limited, except for a genomic region on chromosome 14, at around 25 Mb, which was identified in Chapter 5 and had been reported by both Hawken et al. (2012) and Fortes et al. (2012). That region is known to harbor the *PLAG1* gene, which is well documented to affect stature and body weight in cattle (Karim et al., 2011; Littlejohn et al., 2012; Fink et al., 2017), and has been reported to share an association with variation in the fertility traits 'age of first calving' and 'age of first corpus luteum' (Fortes et al., 2016). However, that region on chromosome 14 was not successfully validated in the FRH. This was possibly because the FRH population comprised of 100% Holstein-Friesian animals, and the *PLAG1* allele associated with larger weight and stature is predominant (~86%) in the Holstein-Friesian breed (Karim et al., 2011), whereas the alternative allele is almost fixed in the smaller Jersey breed (Karim et al., 2011). The PS population, comprising ~50% Holstein-Friesian x Jersey animals likely had increased statistical power to detect an effect in the genomic region harboring *PLAG1*, relative to the 100% Holstein-Friesian FRH population. Alternatively, it could be because the FRH was designed to consist of two groups of animals with extreme divergence in fertility genetic merit but minimal variance in other traits such as body weight and milk production (Meier et al., 2021), which is likely to have results in artificially minimized segregation of alleles associated with body weight in the FRH population. In practice, this may reduce the utility of the FRH to validate genomic regions associated with both AGEP and body weight or milk production. Lefebvre et al. (2021) also reported GWAS analysis for AGEP, using a population of 1,163 crossbred Holstein-Normande females. Once again, Lefebvre et al. (2021) detected numerous QTL (on chromosomes 1, 3, 11, 13, 14, 21, and 29), but the cross over with the PS study GWAS results was minimal. Most notably, they detected a region on chromosome 11 at 45 Mb, which is around 4 Mb from the detected region on this chromosome in the PS population. Interestingly, this region validated poorly in the FRH analysis, and with a distance of 4 Mb between the genomic location relative to the Lefebvre et al. (2021) study, these two findings may be unrelated. However, as with chromosome 14, the

FRH population may not have been suitable to validate the GWAS results from the PS population for chromosome 11, as comparing the minor allele frequencies of highest effect SNP within these windows indicates that they were not segregating uniformly across PS and FRH populations. Although the two genomic regions identified on chromosomes 14 and 11 did not validate well in the FRH, the results from the PS population are corroborated by existing literature, especially for the region on chromosome 14. Further research is warranted to determine the association that these two regions may exhibit with variance in AGEP in NZ dairy cattle.

Anogenital distance

In Chapter 6, AGD was measured at two ages using the PS population, first when the animals were peripubertal yearling heifers (AGD1), and second, when they were around 29 mo old at the time of pregnancy diagnosis following seasonal breeding during their first lactation (AGD2). The AGD trait is a highly novel predictor of fertility. This is especially true in the context of dairy cattle, where the earliest paper to investigate a (phenotypic) association between AGD and fertility in dairy cattle was published only a few years ago (Gobikrushanth et al., 2017). To the best of my knowledge, this thesis contains the first published genetic associations between AGD and fertility expressed during lactation, which support the utility of AGD as an early-in-life predictor trait whereby selection for a shorter AGD would be associated with earlier calving, breeding, and pregnancy dates in a seasonal calving system.

Measuring anogenital distance phenotypes at scale

The AGD trait is particularly interesting as a predictor of fertility as it can be measured cheaply and non-invasively. Moreover, measurements can be taken shortly after birth. The AGD phenotypes analysed in this thesis (Chapter 6) were measured when the PS animals were around 11 and 29 mo old, using digital calipers. This is a simple measure to obtain, and it would be feasible for this trait to be recorded by farmers using a standard operating protocol. However, one drawback of the measurement procedure is that the animal must be stationary, as the calipers must be placed carefully to protect the animal from injury and obtain a precise measure. As with AGEP, new technologies could provide alternative approaches that make AGD faster and easier to measure. For example, the AGD measures could be obtained from a video or photo using automated analysis rather than being determined cow-side. However, it is unlikely that AGD could be measured using an entirely hands-off approach, as the tail of the cow often obscures the genital area and must be manually lifted out of the way. Another consideration when measuring this trait is that precision may not be critical for the purposes of genetic evaluation. There are a range of strategies that could be used to expedite the measurement

process, albeit with reduced precision. For example, a flexible ruler could be used in the place of calipers, for faster measurements without concern for the safety of the animals. Grouping continuous measures into ordinal categories could also accelerate measurements, and this approach would make it easier to measure animals using a ruler (instead of digital calipers). The use of ordinal categories in place of more precise measures for a continuous phenotype has been shown to reduce the accuracy of EBVs (Kizilkaya et al., 2014), but the implications of such an approach regarding the utility of predictor traits are not well understood. Even if measuring AGD in ordinal categories reduced the utility of AGD as a predictor of fertility, it may still be justified if it enabled famers to measure phenotypes on a larger number of animals. Furthermore, Kizilkaya et al. (2014) estimated that a 2.25-fold increase in phenotype data would compensate for the loss of EBV accuracy incurred from using ordinal categorical scores. I propose that additional work is warranted to explore alternative measuring procedures that would make it easier for farmers to measure AGD across large groups of animals.

Anogenital distance is genetically correlated with fertility measured during lactation.

In Chapter 6 I have reported that both AGD1 and AGD2 are moderately heritable traits, exhibiting low to moderate genetic correlations with breeding, calving and pregnancy phenotypes. The heritabilities of AGD1 and AGD2 were of 0.23 and 0.29, respectively. The genetic correlations between AGD and fertility traits measured during lactation ranged from 0.19 – 0.52 for AGD1 and 0.46 – 0.63 for AGD2. The heritabilities that I have reported are slightly lower than the heritability of 0.37 reported by Gobikrushanth et al. (2019) in some 900 mixed-parity cows. To my knowledge, this is the first study where genetic correlations between AGD and fertility trait are reported, and so unfortunately, it is not possible to compare my results to those reported for other populations or measurement strategies.

The effect of age on AGD phenotypes

We reported a moderate to low phenotypic correlation between AGD1 and AGD2 of 0.33, which aligns with the results of Rajesh et al. (2022) who measured AGD at a range of ages between birth and 16 mo old, reporting phenotypic correlations that were at best moderate between all measures. We have reported the first genetic correlations between AGD measured at two ages, and interestingly these were very high, at 0.89, indicating that the underlying genetic drivers of AGD may not change as the animal matures.

Interaction of age on the genetic covariance between AGD and fertility

This thesis provides support for AGD as a useful predictor trait of fertility during lactation, but I was not able to comprehensively investigate the optimal timing of AGD

measurement with respect to the impact of age on the genetic relationship with adult fertility. Although AGD1 and AGD2 exhibited a high genetic correlation, analyses in Chapter 6 suggest that the heritability of AGD and the genetic correlation between AGD and fertility might be higher if animals are older when AGD is measured. There were weak genetic and phenotypic associations among AGD (at either age) and the body stature traits of height, length, and body weight. These results are consistent with existing literature, whereby phenotypic correlations between AGD and stature are reported to be low (Gobikrushanth et al., 2017, 2019; Rajesh et al., 2022). Interestingly, an effect of age on AGD1 was detected and, as a result, age in days was fitted as a fixed effect in those analyses. Conversely, the age effect on AGD2 was near zero, indicating that as animals approach maturity the effect of age on AGD reduces. The effect of age on AGD1 may explain the lower correlation between AGD1 and fertility traits compared with AGD2, as it is possible that fitting age as a covariate does an imperfect job of capturing age effects, and therefore, there may still be some residual effects being incorrectly partitioned into EBVs.

It is important to understand this potential interaction as AGD has greater value as a predictor trait for fertility if it can be measured early in an animal's life. The AGD trait can be measured non-invasively from birth, and so it would be interesting to know whether AGD measured in young calves is still a useful genetic predictor of fertility during lactation. Furthermore, if there is an age at which the genetic association between AGD and fertility traits strengthens, then it is important to understand this, as there still may be a useful timing advantage over breeding, pregnancy, and calving traits. For example, AGD measured at around 18 mo old would have a 6-mo advantage on CR42 in first lactation. Measurement of AGD at a time when other on-farm handling activities are being conducted with replacement heifers, such as weighing or pregnancy testing, would also facilitate the cost-effectiveness of measuring this predictor trait.

Potential for sexual specific genetic covariances between AGD and fertility

The AGD trait has been well established to exhibit a sexually dimorphic phenotypic association with fertility traits in several species, including rats (Hotchkiss et al., 2007), sheep (Lamm et al., 2012), and humans (Thankamony et al., 2009). In these species, longer AGD in males is associated with superior fertility outcomes, while longer AGD in females is associated with poorer outcomes. In this thesis, I present one of the few genetic analyses of AGD and, as far as I am aware, the first reported genetic covariances between AGD and fertility traits in any species. However, the analysis in Chapter 6 was limited to females only, and so it is unclear if the genetic covariance between AGD and fertility traits is sexually dimorphic in cattle. Moreover, the response in male AGD if we were to begin selecting for shorter AGD in females is currently unknown. Given that AGD is a marker of androgen dysfunction (Lamm et al., 2012;

Thankamony et al., 2016), it is plausible that selection for shorter AGD in females would result in longer AGD in males, as both phenotypes associated with normal androgen function in the respective genders (Hotchkiss et al., 2007; van den Driesche et al., 2011; Lamm et al., 2012). However, it is also possible that selection for shorter AGD in females would result in shorter AGD in males, which could be problematic given that shorter AGD is associated with poorer fertility in males (van den Driesche et al., 2011; Dean and Sharpe, 2013). If the latter scenario were true, then selection for ADG (measured in either gender) would not be advisable. Therefore, it is critical that the genetic association between AGD in males and AGD in females is understood before selection is applied to this trait. Furthermore, understanding the genetic association between male AGD and fertility would be useful insofar as a bull's own AGD may provide insight into his genetic merit for both his own fertility as well as the fertility of his daughters.

GWAS of anogenital distance

In Chapter 6, I report on a GWAS analysis of AGD1 and AGD2 using the PS population as a reference population. The GWAS of AGD1 identified genomic region, located at around 35 Mb on chromosome 20, as well as another region on chromosome 13 with a suggestive association with variation in AGD1. Gobikrushanth et al. (2019) have previously reported a genomic region on chromosome 20 as being suggestively associated with AGD in some 900 mixed-parity, Irish Holstein Friesian cows managed in seasonal, pasture-based herds; however, this region was about 10 Mb from the region that we identified, and so the association they detected may have been driven by an unrelated QTL. Grala et al. (2021) also reported GWAS analysis for AGD measured during first lactation in the FRH; however, there was no cross over between the PS population results and the 1 region on chromosome 26 that they identified in their study, which may be due to the different population structure of these two studies as discussed above for the GWAS results for the AGEP trait. Moreover, the GWAS of AGD2 did not detect any associated genomic regions, which may have been due to the reduced number of animals with this phenotype (n = 1,956), relative to the AGD1 phenotype (n = 4,688). Although Chapter 6 describes the largest published study with a GWAS of AGD to date, it appears that more animals would be required to fully explore a GWAS for AGD measured at different ages, and to determine if any identified genomic regions harbor QTL for AGD that provide benefits for genetic improvement in fertility.

Age at puberty and anogenital distance as early in life predictors of fertility

My research provides support for AGEP and AGD as useful predictors of fertility in NZ Holstein-Friesian cows. First, both traits were expressed early in life. Second, their heritabilities were moderate. Third AGEP and AGD exhibited moderate genetic correlations with breeding, calving and pregnancy date traits. Finally, AGEP and AGD exhibit a weak genetic correlation with each other, indicating that they can separately be used to predict fertility, likely via different biological pathways.

The timing of phenotype collection for a trait under selection is important under both pedigree and genomic evaluation systems, but for different reasons. In a pedigree evaluation system, parent average EBVs are available at birth, but have relatively low reliability (only 25% of the sum of the two parents' reliabilities [Mrode, 2005]). Reliabilities do not change markedly until the animals have phenotype data measured on them individually or on their progeny. This dependence on direct (own or daughter) phenotypes for EBV reliability means that accurate selection requires delaying culling and selection decisions until after such phenotypes have been measured. Therefore, the timing of phenotype measurements will antagonistically influence either the generation interval or the selection accuracy for a trait. A predictor trait that can be measured earlier in life than a target trait can improve the rate of genetic progress in a pedigree-based evaluation system by shortening the generation interval or improving the accuracy (and therefore reliability) of EBVs at the time of selection. Conversely, the accuracy of EBVs in a genomic evaluation system does not have the same dependency on the timing of phenotype measurement, as phenotypes and genotypes measured in older animals can be used to infer EBVs for young, genotyped animals. That said, the effects of recombination and *de novo* mutations are not captured by genomic EBVs until the phenotypes that represent these changes have been incorporated into reference populations. Early-in-life predictor phenotypes have value within a genomic evaluation system as they can facilitate a more rapid 'refresh' of the genomic and phenotypic associations that genomic prediction relies on.

The accuracies of EBVs are a function of the number of phenotypes measured on cohorts that include the individual, cohorts that include its close relatives, and the heritability of a trait (Mrode, 2005). The results presented in this thesis establish AGEP and AGD as moderately heritable traits, with much higher (10- to 20-fold) heritabilities then those estimated for the breeding, calving and pregnancy date traits that were measured in the same population. The higher heritabilities of AGEP and AGD mean that for the same number of phenotyped animals, EBVs for AGEP or AGD will be more accurate than the EBVs for breeding, calving and pregnancy date traits such as the current fertility BV based on CR42 (that is, success or failure to calve within the first 42 d of the seasonal calving period). While neither AGEP nor

AGD are targeted for selection, higher accuracies will likely improve their utility as predictors of correlated traits. Further, the higher heritabilities of both AGEP and AGD relative to fertility traits will improve the accuracy of SNP effects (Gonzalez-Recio et al., 2014) and, therefore, improve the likelihood of identifying QTL associated with variance in the respective phenotypes. Although the GWAS results presented in this thesis do not directly improve our understanding of the genetic architecture of fertility traits, some of the genomic regions identified as being associated with either AGEP or AGD may also be relevant to genetically correlated fertility traits. Estimation of SNP effects does not depend on knowledge of the genetic architecture of a trait, but there is some evidence that enrichment of SNP chips across regions known to harbor QTL can improve accuracy of genomic predictions (Xiang et al., 2021). Therefore, GWAS of AGEP or AGD may contribute improved accuracy of fertility EBVs.

The three characteristics of AGEP and AGD that make them appealing candidate traits to predict fertility EBVs should be viewed in concert when assessing their utility, and this can be done using selection index theory (Hazel et al., 1992). Figures 7.1 and 7.2 illustrate the potential gain in reliability for a CR42 EBV that could be achieved by using either AGEP or AGD (Figure 7.1: AGD1, Figure 7.2: AGD2), or both, as predictor traits, based on the timing of phenotype measurement (represented as the age of a bull when daughter phenotypes are collected), the heritability of each trait, and the genetic correlation exhibited between these traits and CR42 in first and second lactation in the PS population, as reported in this thesis. These plots were produced with the aid of the MTIndex software (Van der Werf, 2023; see supplementary material 2 for full details). The reliabilities shown in Figure 7.1 demonstrate that both AGEP and AGD1 add value as predictors of CR42 EBVs, even when a bull has daughter phenotypes for the CR42 trait. Further, the reliability gains from these predictor traits are realized when a bull is between 3 and 4 yrs old (and his daughters are 1 yr old). This is a year before daughter phenotypes for CR42 can be measured and, importantly, also a year before a bull's daughters' express phenotypes for production traits, which are under strong selection and in competition with fertility traits due to an antagonistic genetic association (Berry et al., 2014). It is worth noting that Figure 7.1 indicates a greater reliability gain from using AGEP as a predictor, relative to using AGD1. This is because the Chapter 5 results suggest that AGEP exhibits a stronger genetic correlation with CR42 in both first and second lactation than AGD1. Further, in Chapter 6, it was determined that the genetic correlations between AGD2 (measured around 29 mo old) and fertility traits tended to be higher than those reported using AGD1 (measured around 11 mo old). These higher genetic correlations manifest as a greater increase in reliability in CR42 EBVs when AGD2 is used as a predictor in the place of AGD1 (Figure 7.2). Although AGD2 is measured later-in-life than AGD1, the trait does still have both a timing and a heritability advantage over CR42. Figure 7.2 illustrates the value of using AGD2

as a predictor of fertility EBVs, and notably the reliability gains expected from using AGD2 as a predictor of CR42 EBVs is comparable to those expected from using AGEP, albeit with a 1 yr delay.

Furthermore, an important finding of this thesis is that AGEP and AGD exhibit minimal genetic covariance. The genetic correlation exhibited between either AGD1 or AGD2 and AGEP did not exceed 0.30. This independence between the two candidate predictor traits means that they may make an additive contribution to the prediction of fertility EBVs. Therefore, considering the genetic correlation exhibited between each trait and fertility measured during lactation in isolation will not represent the potential value that the two traits could have if used together. Figure 7.1 and Figure 7.2 illustrate this potential for additive value, whereby using both AGEP and AGD as predictors of fertility EBVs is expected to yield greater reliabilities than either predictor used in isolation. Based on these results, it may be useful to incorporate AGD1, AGD2 and AGEP as predictors of fertility EBVs, such that the timing advantage of AGD1 over AGD2 can be realized, without compromising the reliabilities of EBVs that can be achieved by incorporating AGD2 in the following year. In the simplified context of single trait selection, we would expect the use of AGD and AGEP as predictor traits to illicit a selection response in CR42 of up to 125% (relative to the selection response expected from using CR42 phenotypes alone [see supplementary material 2]). Future work is required to quantify the expected selection response in the context of multi-trait selection, as is common practice in the NZ dairy industry.

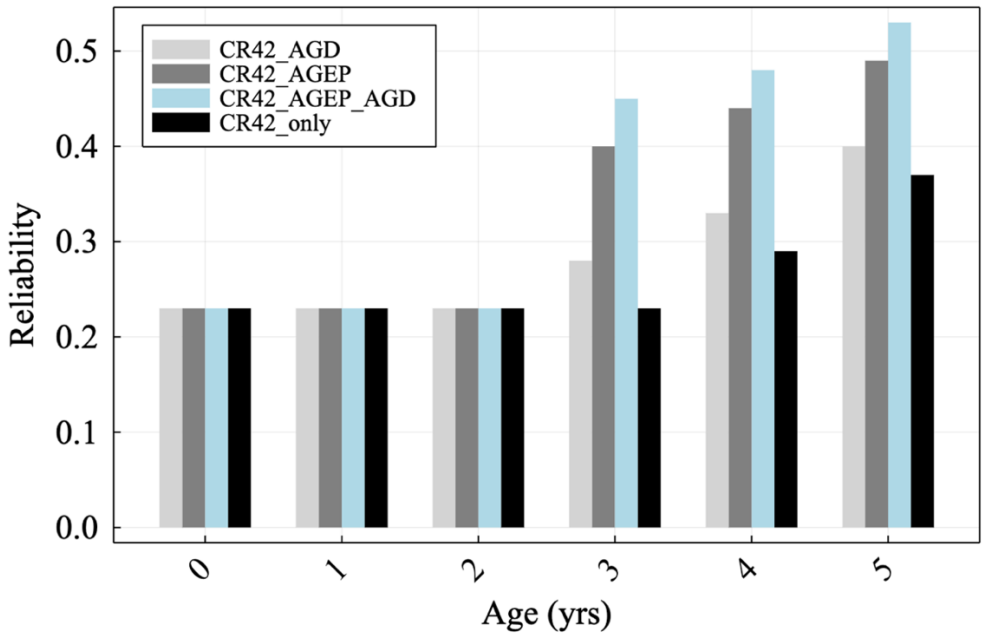


Figure 7.1 Reliability of fertility EBVs for a typical AI bull in New Zealand, where the (pedigree-based) evaluation approach is varied to included either age at puberty (AGEP; dark

grey), anogenital distance measured when daughters are 11 mo old (AGD1; light grey) or both (blue) as novel predictors of an animals EBV for CR42 (success or failure to calve within the first 42 d of the seasonal calving period). The black bar approximately represents the current evaluation for fertility, where daughter CR42 phenotypes from first and second lactation contribute to a bull's fertility EBVs when it is 5 yr old. EBVs accuracies were estimated using selection index theory, with the aid of the MTIndex software (Van der Werf, 2023).

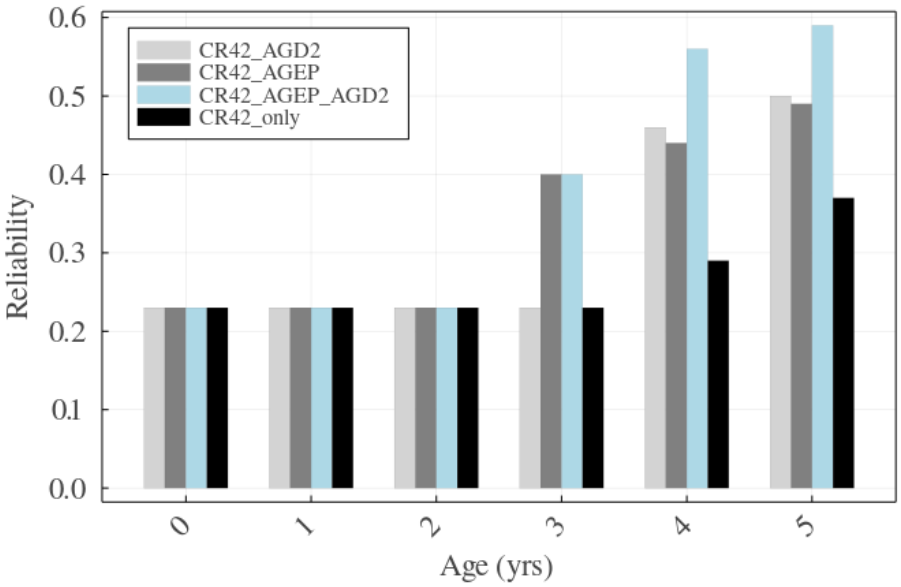


Figure 7.2 Reliability of fertility EBVs for a typical AI bull in New Zealand, where the (pedigree-based) evaluation approach is varied to included either age at puberty (AGEP; dark grey), anogenital distance measured when daughters are 29 mo old (AGD2; light grey) or both (blue) as novel predictors of an animals EBV for CR42 (success or failure to calve within the first 42 d of the seasonal calving period). The black bar approximately represents the current evaluation for fertility, where daughter CR42 phenotypes from first and second lactation contribute to a bull's fertility EBVs when it is 5 yr old. EBVs accuracies were estimated using selection index theory, with the aid of the MTIndex software (Van der Werf, 2023).

General limitations

Animal numbers

The PS study population analysed in this thesis comprised of some 5,000 heifers with AGEP and AGD1 phenotypes, and after natural attrition and various data quality exclusions there were around 3,500 animals with fertility phenotypes measured in second lactation. To my knowledge, this is the largest dataset of puberty and AGD phenotypes ever measured in dairy cattle. Even so, it is relatively small in the context of national genetic evaluation, and this is

especially true given the low heritability of fertility traits. The size of the dataset, and the low heritabilities of breeding, calving and pregnancy traits has resulted in some uncertainty in our results, and this is reflected in our credibility intervals. Collection of additional phenotypes in greater numbers of animals will help to validate the results of this thesis and improve the precision of estimated genetic covariances between the novel predictor traits, AGEP and AGD, and subsequent fertility traits based upon calving, breeding and pregnancy dates measured during lactation .

Breed composition

In this thesis, I analysed phenotypes measured in a population of animals that were predominantly Holstein-Friesian or Holstein-Friesian cross Jersey. There were very few (n=24) predominantly Jersey animals represented in the analysis of the PS population. The lack of Jersey animals in this study was by design, as the research presented here is a continuation of earlier work undertaken in the FRH, which consisted of Holstein-Friesian dairy cattle divergent in the NZ fertility BV (Meier et al., 2021). In that population, AGEP and AGD were highlighted as candidate predictors of subsequent fertility due to the earlier onset of puberty, shorter AGD, earlier resumption of cyclicity postpartum, and markedly superior reproductive success of high fertility BV cows relative to low fertility BV cows (Grala et al., 2021; Meier et al., 2021; 2022). Because that prior research was undertaken in the Holstein-Friesian breed, it was decided that to validate those initial findings, the herds recruited for the PS study should be predominantly Holstein-Friesian. However, selecting animals based on breed introduced a limitation to the research, as the results of an analysis involving Holstein-Friesians cannot be assumed to directly apply to other breeds. The NZ dairy cattle population is a highly ad-mixed combination of Holstein-Friesians and Jersey breeds, and so there is risk that the results of the research presented in this thesis may not be applicable to a substantial proportion of dairy cattle in NZ. Having said that, although the intent during herd enrolment was to maximize the proportion of animals that were Holstein-Friesian, the breed composition of the NZ dairy herd meant that it was somewhat inevitable that a proportion of animals involved in the study would be Holstein-Friesian cross Jersey (approximately 45%). On one hand, the crossbreed animals may be beneficial to my study, as they provide some indication that the results would apply to crossbreed animals as well as Holstein-Friesians. However, on the other hand, the presence of these crossbreed animals may have compromised the ability to validate the results presented by Meier et al. (2021) and Grala et al. (2021), especially if there is an interaction of breed on the association between the two candidate predictor traits, AGEP and AGD, and fertility traits during lactation. Furthermore, the inclusion of these crossbreed animals may have reduced the statistical power of our GWAS analysis to quantify the effects of alleles that only segregate in the Holstein-Frisian breed, especially if the MAF frequencies were already low in the Holstein-

Friesian breed. It would have been more ideal if the current dataset included a greater proportion of Holstein-Friesian animals. Further work is required to confirm our findings in a Jersey population and, therefore, it would be beneficial to repeat the current study in one or more cohorts of predominantly Jersey animals.

Preselection on fertility in later parity phenotypes

We were limited in our ability to estimate the genetic covariance that both predictor traits exhibited with fertility phenotypes in second lactation, because the animals' contributing phenotypes to those second lactation traits were subject to preselection based on their fertility performance. That is, the cows that failed to conceive in first lactation had been removed from the herd, and some of those animals represented the poorest fertility animals in each cohort. Preselection based on fertility performance would be present in both lactations; however, it is much more apparent in the second lactation cohort, where only around 70% of the initial 5,000 animals with AGEP phenotypes had a CR42 phenotype in second lactation (compared with around 87% in first lactation). Although some of these missing animals will be animals that were sold to a herd outside of this study, this level of attrition is in line with national averages, and largely representative of widespread adherence to a strict protocol of culling cows that are not-in-calf or will be late calving in the following season in pasture-based, seasonal farm systems. The occurrence of pre-selection is difficult to control in a commercial setting, as retaining those animals in the herd to give them an opportunity to become pregnancy in subsequent lactations would incur cost for the farmers and disrupt their normal farm routines. Therefore, this is not a limitation that can be readily mitigated.

Concluding statements

The research presented in this thesis provides support for both AGEP and AGD as promising candidate early predictors of genetic merit for fertility measured during lactation. I would expect that used of these traits in multi-trait selection for fertility would result in improved reliabilities of fertility EBVs in NZ Holstein-Friesian and Holstein-Friesian cross Jersey cattle.

References

Berry, D.P., E. Wall, and J.E. Pryce. 2014. Genetics and genomics of reproductive performance in dairy and beef cattle. Animal 8:105–121. doi:10.1017/S1751731114000743.

Bowley, F.E., R.E. Green, P.R. Amer, and S. Meier. 2015. Novel approaches to genetic analysis of fertility traits in New Zealand dairy cattle. J Dairy Sci 98:2005–2012. doi:10.3168/jds.2014-8266.

Daetwyler, H.D., A.A. Swan, J.H. Van Der Werf, and B.J. Hayes. 2012. Accuracy of pedigree and genomic predictions of carcass and novel meat quality traits in multi-breed sheep data assessed by cross-validation. Genetics Selection Evolution 44:1–11. doi:10.1186/1297-9686-44-33.

Dean, A., and R.M. Sharpe. 2013. Anogenital distance or digit length ratio as measures of fetal androgen exposure: Relationship to male reproductive development and its disorders. Journal of Clinical Endocrinology and Metabolism 98:2230–2238. doi:10.1210/jc.2012-4057.

Dennis, N.A., K. Stachowicz, B. Visser, F.S. Hely, D.K. Berg, N.C. Friggens, P.R. Amer, S. Meier, and C.R. Burke. 2018. Combining genetic and physiological data to identify predictors of lifetime reproductive success and the effect of selection on these predictors on underlying fertility traits. J Dairy Sci 101:3176–3192. doi:10.3168/jds.2017-13355.

van den Driesche, S., H.M. Scott, D.J. MacLeod, M. Fisken, M. Walker, and R.M. Sharpe. 2011. Relative importance of prenatal and postnatal androgen action in determining growth of the penis and anogenital distance in the rat before, during and after puberty. Int J Androl 34:e578–e586. doi:10.1111/j.1365-2605.2011.01175.x.

Fink, T., K. Tiplady, T. Lopdell, T. Johnson, R.G. Snell, R.J. Spelman, S.R. Davis, and M.D. Littlejohn. 2017. Functional confirmation of PLAG1 as the candidate causative gene underlying major pleiotropic effects on body weight and milk characteristics. Sci Rep 7:1–8. doi:10.1038/srep44793.

Fortes, M.R.S., S.A. Lehnert, S. Bolormaa, C. Reich, G. Fordyce, N.J. Corbet, V. Whan, R.J. Hawken, and A. Reverter. 2012. Finding genes for economically important traits: Brahman cattle puberty. Anim Prod Sci 52:143–150. doi:10.1071/AN11165.

Fortes, M.R.S., L.T. Nguyen, L.R. Porto Neto, A. Reverter, S.S. Moore, S.A. Lehnert, and M.G. Thomas. 2016. Polymorphisms and genes associated with puberty in heifers. Theriogenology 86:333–339. doi:10.1016/j.theriogenology.2016.04.046.

Gobikrushanth, M., T.C. Bruinjé, M.G. Colazo, S.T. Butler, and D.J. Ambrose. 2017. Characterization of anogenital distance and its relationship to fertility in lactating Holstein cows. J Dairy Sci 100:9815–9823. doi:https://doi.org/10.3168/jds.2017-13033.

Gobikrushanth, M., D.C. Purfield, J. Kenneally, R.C. Doyle, S.A. Holden, P.M. Martinez, E.R. Canadas, T.C. Bruinjé, M.G. Colazo, D.J. Ambrose, and S.T. Butler. 2019. The relationship between anogenital distance and fertility, and genome-wide associations for anogenital distance in Irish Holstein-Friesian cows. J Dairy Sci 102:1702–1711. doi:10.3168/jds.2018-15552.

Gonzalez-Recio, O., M.P. Coffey, and J.E. Pryce. 2014. On the value of the phenotypes in the genomic era. J Dairy Sci 97:7905–7915. doi:10.3168/jds.2014-8125.

Grala, T.M., M.D. Price, B. Kuhn-Sherlock, C.R. Burke, and S. Meier. 2021. Investigating anogenital distance and antral follicle count as novel markers of fertility within a herd of cows with positive or negative genetic merit for fertility traits. J Dairy Sci 104:12939–12952. doi:10.3168/jds.2020-19948.

Harris, B.L., J.E. Pryce, Z.Z. Xu, and W.A. Montgomerie. 2005. Fertility Breeding Values in New Zealand, the Next Generation. Interbull Bulletin 33:47–50.

Hawken, R.J., Y.D. Zhang, M.R.S. Fortes, E. Collis, W.C. Barris, N.J. Corbet, P.J. Williams, G. Fordyce, R.G. Holroyd, J.R.W. Walkley, W. Barendse, D.J. Johnston, K.C. Prayaga, B. Tier, A. Reverter, and S.A. Lehnert. 2012. Genome-wide association studies of female reproduction in tropically adapted beef cattle. J. Anim. Sci 90:1398–1410. doi:10.2527/jas2011-4410.

Hazel, L.N., G.E. Dickerson, and A.E. Freeman. 1992. The Selection Index-Then, Now, and for the Future. J Dairy Sci 77:3236–3251.

Hickson, R.E., C.C. Balcomb, K.R. Fraser, N. Lopez-Villalobos, P.R. Kenyon, and S.T. Morris. 2011. The effect of breed on the onset of puberty in heifers. Pages 51–54 in Proceedings of the Association for the Advancement of Animal Breeding and Genetics, Perth, Australia.

Hotchkiss, A.K., C.S. Lambright, J.S. Ostby, L. Parks-Saldutti, J.G. Vandenbergh, and L.E. Gray. 2007. Prenatal testosterone exposure permanently masculinizes anogenital distance, nipple development, and reproductive tract morphology in female sprague-dawley rats. Toxicological Sciences 96:335–345. doi:10.1093/toxsci/kfm002.

Johnsson, M., A.M. Johansson, T. Klingström, and E. Rius-Vilarrasa. 2022. The effect of recombination rate on genomic selection in simulation. Pages 853–856 in Proceedings of 12th World Congress on Genetics Applied to Livestock Production (WCGALP) . Wageningen Academic Publishers.

Johnston, D.J., S.A. Barwick, N.J. Corbet, G. Fordyce, R.G. Holroyd, P.J. Williams, and H.M. Burrow. 2009. Genetics of heifer puberty in two tropical beef genotypes in northern Australia and associations with heifer- and steer-production traits. Anim Prod Sci 49:399–412. doi:10.1071/EA08276.

Karim, L., H. Takeda, L. Lin, T. Druet, J.A.C. Arias, D. Baurain, N. Cambisano, S.R. Davis, F. Farnir, B. Grisart, B.L. Harris, M.D. Keehan, M.D. Littlejohn, R.J. Spelman, M. Georges,

and W. Coppieters. 2011. Variants modulating the expression of a chromosome domain encompassing PLAG1 influence bovine stature. Nat Genet 43:405–415. doi:10.1038/ng.814.

Kizilkaya, K., R.L. Fernando, and D.J. Garrick. 2014. Reduction in accuracy of genomic prediction for ordered categorical data compared to continuous observations. Genetics Selection Evolution 46:1–13. doi:10.1186/1297-9686-46-37.

Lamm, C.G., P.M. Hastie, N.P. Evans, and J.E. Robinson. 2012. Masculinization of the distal tubular and external genitalia in female sheep with prenatal androgen exposure. Vet Pathol 49:546–551. doi:10.1177/0300985811419533.

Lefebvre, R., H. Larroque, S. Barbey, Y. Gallard, J.J. Colleau, A.L. Laine, D. Boichard, and P. Martin. 2021. Genome-wide association study for age at puberty and resumption of cyclicity in a crossbred dairy cattle population. J Dairy Sci 104:5794–5804. doi:10.3168/jds.2020-18228.

Littlejohn, M., T. Grala, K. Sanders, C. Walker, G. Waghorn, K. MacDonald, W. Coppieters, M. Georges, R. Spelman, E. Hillerton, S. Davis, and R. Snell. 2012. Genetic variation in PLAG1 associates with early life body weight and peripubertal weight and growth in Bos taurus. Anim Genet 43:591–594. doi:10.1111/j.1365-2052.2011.02293.x.

Meier, S., L.R. McNaughton, R.C. Handcock, P.R. Amer, P.R. Beatson, J.R. Bryant, K.G. Dodds, R. Spelman, J.R. Roche, and C.R. Burke. 2021. Heifers with positive genetic merit for fertility traits reach puberty earlier and have a greater pregnancy rate than heifers with negative genetic merit for fertility traits.. J Dairy Sci 104:3707–3721. doi:10.3168/jds.2020-19155.

Moran, C., J.F. Quirke, and J.F. Roche. 1989. Puberty in heifers: a review. Anim Reprod Sci 18:167–182. doi:10.1016/0378-4320(89)90019-5.

Morris, C.A., and N.C. Amyes. 2005. Response to selection for age at puberty in an angus herd. Pages 157–160 in Proceedings of the Association for Advancement of Animal Breeding and Genetics.

Morris, C.A., J.A. Wilson, G.L. Bennett, N.G. Cullen, S.M. Hickey, and J.C. Hunter. 2000. Genetic parameters for growth, puberty, and beef cow reproductive traits in a puberty selection experiment. New Zealand Journal of Agricultural Research 43:83–91. doi:10.1080/00288233.2000.9513411.

Mrode, R.A. 2005. Linear Models for the Prediction of Animal Breeding Values.

Nogueira, G.P. 2004. Puberty in South American Bos indicus (Zebu) cattle. Anim Reprod Sci 82–83:361–372. doi:10.1016/j.anireprosci.2004.04.007.

Pryce, J.E., P. Douglas, C.M. Reich, A.J. Chamberlain, P.J. Bowman, T.T.T. Nguyen, B.A. Mason, C.P. Prowse-Wilkins, G.J. Nieuwhof, T. Hancock, M. Shaffer, and B.J. Hayes. 2017. Reliabilities of Australian dairy genomic breeding values increase through the

addition of genotyped females with excellent phenotypes. Pages 133–136 in Proc. Assoc. Advmt. Anim. Breed. Genet.

Pryce, J.E., R. Woolaston, D.P. Berry, E. Wall, M. Winters, R. Butler, and M. Shaffer. 2014. World trends in dairy cow fertility. Pages 1–10 in 10th World Congress of Genetics Applied to Livestock Production.

Rajesh, I., M. Gobikrushanth, J.E. Carrelli, M. Oba, and D.J. Ambrose. 2022. Repeatability of anogenital distance measurements from birth to maturity and at different physiological states in female Holstein cattle. J Dairy Sci 86. doi:10.3168/jds.2021-21419.

Stachowicz, K., P.R. Amer, B. Falconer, and D.P. Garrick. 2021. New model for genetic evaluation of fertility in New Zealand dairy cattle. Pages 373–376 in Association for the Advancement of Animal Breeding and Genetics.

Thankamony, A., K.K. Ong, D.B. Dunger, C.L. Acerini, and I.A. Hughes. 2009. Anogenital distance from birth to 2 years: A population study. Environ Health Perspect 117:1786–1790. doi:10.1289/ehp.0900881.

Thankamony, A., V. Pasterski, K.K. Ong, C.L. Acerini, and I.A. Hughes. 2016. Anogenital distance as a marker of androgen exposure in humans. Andrology 4:616–625. doi:10.1111/andr.12156.

Van der Werf, J. 2023. Teaching Software Used for Quantitative Genetics at UNE. Accessed June 28, 2023. https://jvanderw.une.edu.au/software.htm.

Xiang, R., I.M. MacLeod, H.D. Daetwyler, G. de Jong, E. O'Connor, C. Schrooten, A.J. Chamberlain, and M.E. Goddard. 2021. Genome-wide fine-mapping identifies pleiotropic and functional variants that predict many traits across global cattle populations. Nat Commun 12:1–13. doi:10.1038/s41467-021-21001-0.

SUPPLEMENTARY MATERIAL 1. Prior Distributions

Supplementary to Chapters 5 and 6

This section contains the prior values used for the statistical analyses described in chapters 5 and 6. Table S1.1 provides the prior values used in the univariate analyses, while Table S1.2 provides the prior values used in the bivariate and trivariate analyses.

Univariate analyses

Prior values for (co)variance parameters were required for the statistical analyses described in chapters 5 and 6. For most of the traits analysed, we were able to obtain an estimate of the phenotypic variance and heritability from scientific literature. We used the heritability to partition the phenotypic variance into an estimate of genetic and residual variance (Table S1.1).

Table S1.1 Prior values for genetic and residual variances used in univariate analyses (anogenital distance measured at 1 yo [AGD1], anogenital distance measured at 2 yo [AGD2], age at puberty [AGEP], calving rate in 42 d in first lactation [CR42L1], calving rate in 42 d in second lactation [CR42L2], presented for breeding in 21 d in first lactation [PM21L1], presented for breeding in 21 d in second lactation [PB21L2], pregnant in 42 d in first lactation [PR42L1], pregnant in 42 d in second lactation [PR42L2], height at 1 yo, length at 1 yo and body weight at 1 yo (BW). Phenotypic variances and heritabilities were sourced from scientific literature. If a parameter was reported in multiple studies, we have selectively included those studies that were most relevant (i.e. studies that involved cow of a similar breed to those in our study were preferred). If more than one source was included, we have used the mean of the values reported for the parameter of interest. *We were not able to find a reported heritability for body length and so we used the heritability of height to partition the phenotypic variance of this trait.

Trait	Genetic Variance	Residual Variance	Phenotypic Variance	Heritability	Notes
AGD1	41	69	110	0.37	(Gobikrushanth et al., 2019; Carrelli et al., 2021)
AGD2	48	81	129	0.37	(Gobikrushanth et al., 2017, 2019)
AGEP	647	1202	1849	0.35	(Martin et al., 1992; Mialon et al., 2000; Morris et al., 2000; Dennis et al., 2018)
CR42L1	0.0015	0.099	0.10	0.015	(Harris et al., 2005; Craig et al., 2018)
CR42L2	0.0028	0.187	0.19	0.015	(Harris et al., 2005; Craig et al., 2018)
PM21L1	0.0054	0.145	0.15	0.037	(Harris et al., 2005; Craig et al., 2018)
PM21L2	0.0054	0.155	0.16	0.035	(Harris et al., 2005; Craig et al., 2018)
PR42L1	0.0059	0.194	0.20	0.03	(Grosshans et al., 1997)
PR42L2	0.0058	0.184	0.19	0.03	(Grosshans et al., 1997)
HEIGHT	18	23	41	0.44	(Ahlborn and Dempfle, 1992; Pryce et al., 2000; Macdonald et al., 2007)
LENGTH	17	22	39	0.44*	(Macdonald et al., 2007)
BW	187	305	492	0.38	(Ahlborn and Dempfle, 1992; Van Der Waaij et al., 1997; Macdonald et al., 2007)

Bi-variate and Tri-variate analyses

As with the univariate analyses, prior values for (co)variance parameters were required for bivariate and trivariate analyses described in chapters 5 and 6. Prior values for genetic and residual variances were obtained from our univariate analysis of each trait. Prior values for the genetic and residual covariances between each of our traits were obtained from existing scientific literature (Table S1.2). We organized our traits into 4 categories and then searched for literature where correlations between traits across categories were reported. The four categories were: 1. Anogenital Distance (AGD; measured at both time points); 2. Body weight and size traits (body; height, length and body weight measured at around 12 mo); 3. Age at puberty (AGEP); and 4. Fertility traits for calving rate (CR42), breeding rate (PB21), and pregnancy rate (PR42) expressed during first (L1) or second (L2) lactation (fert; CR42L1, PB21L1, PR42L1, CR42L2, PB21L2, PR42L2). For 4 of the category pairings (AGEP with fert, AGEP with body, fert with fert and body with body), we were able to find literature where both genetic and residual correlations were reported. For 3 of the category pairings (AGD with fert, AGD with body, and AGD with AGD), we were only able to source phenotypic correlations. For analysis of traits across these 3 category pairings, we used the phenotypic correlation to determine prior values for both the genetic and phenotypic covariances. For the AGD with AGEP category pairing, to our knowledge, there have been no associations previously reported in dairy cattle. For our analyses between AGD traits and AGEP, the prior values for correlations were the same as the prior values used for trait pairings across the AGD and fert categories.

Table S1.2 Priors for covariances used in bivariate and trivariate analyses. We organized our traits into 4 categories and then searched for literature where correlation between traits across categories were reported. The four categories were: 1. Anogenital Distance (AGD; measured at both time points); 2. Body weight and size traits (body; height, length and liveweight measured at around 12 mo); 3. Age at puberty (AGEP); and 4. Fertility traits expressed during lactation (fert; CR42L1, PB21L1, PR42L1, CR42L2, PB21L2, PR42L2).

Trait category 1	Trait category 2	Genetic	Residual	Phenotypic	Notes
AGD	Fert	0.30	0.30	0.30 (Gobikrushanth et al., 2017, 2019; Carrelli et al., 2021)	To our knowledge, genetic correlations have not been previously reported.
AGD	Body	0.00	0.00	0.00 (Gobikrushanth et al., 2019)	To our knowledge, genetic correlations have not been previously reported.
AGD	AGEP	0.30	0.30	0.30	To our knowledge, not previously reported – these priors are based on reported associations between AGD and Fertility.
AGEP	Fert	0.40 (Mialon et al., 2000; Morris et al., 2000; Lefebvre et al., 2021)	0.10 (Mialon et al., 2000)	0.25 (Mialon et al., 2000)	
AGEP	Body	-0.30 (Mialon et al., 2001)	-0.25	-0.25 (Mialon et al., 1999)	
Fert	Body	0.25	0.25	0.25	Weak correlations between weight/stature type traits and fertility (Pryce et al., 2000; Berry et al., 2002)
AGD	AGD	0.60	0.60	0.60 (Rajesh et al., 2022)	To our knowledge, genetic correlations have not been previously reported.
Fert	Fert	0.80 (Bowley et al., 2015)	0.25	0.30 (Bowley et al., 2015)	
Body	Body	0.95 (Cue et al., 1996)	0.80	0.85 (Cue et al., 1996)	Strong genetic and phenotypic correlations between stature and LWT (Cue et al., 1996)

References

Ahlborn, G., and L. Dempfle. 1992. Genetic parameters for milk production and body size in New Zealand Holstein-Friesian and Jersey. Livest Prod Sci 31:205–219. doi:10.1016/0301-6226(92)90018-Y.

Berry, D.P., F. Buckley, P. Dillon, R.D. Evans, M. Rath, and R.F. Veerkamp. 2002. Genetic Parameters for Level and Change of Body Condition Score and Body Weight in Dairy Cows. J Dairy Sci 85:2030–2039. doi:10.3168/jds.S0022-0302(02)74280-X.

Bowley, F.E., R.E. Green, P.R. Amer, and S. Meier. 2015. Novel approaches to genetic analysis of fertility traits in New Zealand dairy cattle. J Dairy Sci 98:2005–2012. doi:10.3168/jds.2014-8266.

Carrelli, J.E., M. Gobikrushanth, M. Corpron, I. Rajesh, W. Sandberg, M.G. Colazo, A. Ahmadzadeh, M. Oba, and D.J. Ambrose. 2021. Relationship of anogenital distance with fertility in nulliparous Holstein heifers. J Dairy Sci 104:8256–8264. doi:10.3168/jds.2020-19940.

Craig, H.J.B., K. Stachowicz, M. Black, M. Parry, C.R. Burke, S. Meier, and P.R. Amer. 2018. Genotype by environment interactions in fertility traits in New Zealand dairy cows. J Dairy Sci 101:10991–11003. doi:10.3168/jds.2017-14195.

Cue, R.I., B.L. Harris, and J.M. Rendel. 1996. Genetic parameters for traits other than production in purebred and crossbred New Zealand dairy cattle. Livest Prod Sci 45:123–135. doi:10.1016/0301-6226(96)00009-7.

Dennis, N.A., K. Stachowicz, B. Visser, F.S. Hely, D.K. Berg, N.C. Friggens, P.R. Amer, S. Meier, and C.R. Burke. 2018. Combining genetic and physiological data to identify predictors of lifetime reproductive success and the effect of selection on these predictors on underlying fertility traits. J Dairy Sci 101:3176–3192. doi:10.3168/jds.2017-13355.

Gobikrushanth, M., T.C. Bruinjé, M.G. Colazo, S.T. Butler, and D.J. Ambrose. 2017. Characterization of anogenital distance and its relationship to fertility in lactating Holstein cows. J Dairy Sci 100:9815–9823. doi:https://doi.org/10.3168/jds.2017-13033.

Gobikrushanth, M., D.C. Purfield, J. Kenneally, R.C. Doyle, S.A. Holden, P.M. Martinez, E.R. Canadas, T.C. Bruinjé, M.G. Colazo, D.J. Ambrose, and S.T. Butler. 2019. The relationship between anogenital distance and fertility, and genome-wide associations for anogenital distance in Irish Holstein-Friesian cows. J Dairy Sci 102:1702–1711. doi:10.3168/jds.2018-15552.

Grosshans, T., Z.Z. Xu, L.J. Burton, D.L. Johnson, and K.L. Macmillan. 1997. Performance and genetic parameters for fertility of seasonal dairy cows in New Zealand. Livest Prod Sci 51:41–51. doi:10.1016/S0301-6226(97)00104-8.

Harris, B.L., J.E. Pryce, Z.Z. Xu, and W.A. Montgomerie. 2005. Fertility Breeding Values in New Zealand, the Next Generation. Interbull Bulletin 33:47–50.

Lefebvre, R., H. Larroque, S. Barbey, Y. Gallard, J.J. Colleau, A.L. Laine, D. Boichard, and P. Martin. 2021. Genome-wide association study for age at puberty and resumption of cyclicity in a crossbred dairy cattle population. J Dairy Sci 104:5794–5804. doi:10.3168/jds.2020-18228.

Macdonald, K.A., L.R. McNaughton, G.A. Verkerk, J.W. Penno, L.J. Burton, D.P. Berry, P.J.S. Gore, J.A.S. Lancaster, and C.W. Holmes. 2007. A comparison of three strains of Holstein-Friesian cows grazed on pasture: Growth, development, and puberty. J Dairy Sci 90:3993–4003. doi:10.3168/jds.2007-0119.

Martin, L.C., J.S. Brinks, R.M. Bourdon, and L. V. Cundiff. 1992. Genetic effects on beef heifer puberty and subsequent reproduction. J Anim Sci 70:4006–4017. doi:10.2527/1992.70124006x.

Mialon, M.M., G. Renand, D. Krauss, and F. Ménissier. 1999. Puberty of Charolais heifers in relation to growth rate. 1. Phenotypic variability. Animal Research 48:413–426. doi:10.1051/animres:19990601.

Mialon, M.M., G. Renand, D. Krauss, and F. Ménissier. 2000. Genetic variability of the length of postpartum anoestrus in Charolais cows and its relationship with age at puberty. Genetics Selection Evolution 32:403–414. doi:10.1051/gse:2001119.

Mialon, M.M., G. Renand, D. Krauss, and F. Ménissier. 2001. Genetic relationship between cyclic ovarian activity in heifers and cows and beef traits in males. Genetics Selection Evolution 33:273–287. doi:10.1186/1297-9686-33-3-273.

Morris, C.A., J.A. Wilson, G.L. Bennett, N.G. Cullen, S.M. Hickey, and J.C. Hunter. 2000. Genetic parameters for growth, puberty, and beef cow reproductive traits in a puberty selection experiment. New Zealand Journal of Agricultural Research 43:83–91. doi:10.1080/00288233.2000.9513411.

Pryce, J.E., M.P. Coffey, and S. Brotherstone. 2000. The genetic relationship between calving interval, body condition score and linear type and management traits in registered Holsteins. J Dairy Sci 83:2664–2671. doi:10.3168/jds.S0022-0302(00)75160-5.

Rajesh, I., M. Gobikrushanth, J.E. Carrelli, M. Oba, and D.J. Ambrose. 2022. Repeatability of anogenital distance measurements from birth to maturity and at different physiological states in female Holstein cattle. J Dairy Sci 86. doi:10.3168/jds.2021-21419.

Van Der Waaij, E.H., P.J.B. Galesloot, and D.J. Garrick. 1997. Some relationships between weights of growing heifers and their subsequent lactation performances. New Zealand Journal of Agricultural Research 40:87–92. doi:10.1080/00288233.1997.9513235.

SUPPLEMENTARY MATERIAL 2. Accuracy of selection for calving rate using multi-trait analysis

Supplementary to Chapter 7 General Discussion

Background and objectives

In this thesis, I have demonstrated that both age at puberty (AGEP) and anogenital distance (AGD) are moderately heritable and exhibit moderate genetic correlations with subsequent fertility phenotypes measured during lactation. These results suggest that both traits have value as predictors of fertility EBVs for New Zealand, but it would be interesting to quantify this value. The aim of this paper is to quantify the value of AGEP and AGD as predictors of fertility EBVs, in terms of the potential gains in EBV reliability for breeding bulls, the timeliness of those gains and the estimated response to selection. This analysis was carried out in the context of pedigree-based evaluation system, where an animal's EBVs are the average of its parents until it has direct or daughter phenotypes.

Material and methods

We estimated EBV accuracies using selection index theory (Hazel et al., 1992), with the aid of the MTIndex software (Van der Werf, 2023). The EBVs were produced using either bi-variate (calving rate in first and second lactations only), or multi-trait (using either AGEP, AGD or both as predictor traits) analyses.

Our assumptions regarding (co)variance parameters were informed by the analyses reported in chapters 5 and 6 of this thesis (Table S2.1, Table S2.2). We assumed that the dam of a typical bull candidate had 1 phenotype measured for each of CR42_first (success or failure to calve in first 42 d of seasonal calving period [CR42] in first lactation) and CR42_second (CR42 in second lactation), but not AGEP or AGD (Table S2.3). We assumed that the sire of a typical bull candidate was well proven for both CR42 traits, with 5,000 progeny (represented as half-siblings), but none of these half-siblings had AGEP or AGD phenotypes (Table S2.3). The number of daughters that a widely used bull might have for each trait varied depending on the age of the bull, as would be normal in the New Zealand dairy sector (DairyNZ bull search, Table S2.4).

Table S2.1 Variance parameters. The phenotypic standard deviation and heritability of calving rate* in first (CR42_first) and second (CR42_second) lactations, age at puberty (AGEP) and anogenital distance measured at 11 mo (AGD1) and 29 mo (AGD2) old. *Calving rate is a binary trait denoting a cow's success (1) or failure (2) to calve within the first 42 d of the herds seasonal calving period.

Name	Units	Phenotypic Stand. Dev	Heritability
CR42_second	%	0.447	0.01
CR42_first	%	0.283	0.01
AGEP	mm	43.451	0.34
AGD1	days	7.905	0.23
AGD2	days	9.06	0.29

Table S2.2 Correlations. The genetic (below the diagonal) and phenotypic (above the diagonal) correlations between calving rate* in first (CR42_first) and second (CR42_second) lactation, age at puberty (AGEP) and anogenital distance measured at 11 mo (AGD1) and 29 mo (AGD2) old. *Calving rate is a binary trait denoting a cow's success (1) or failure (2) to calve within the first 42 d of the herds seasonal calving period.

	CR42_Second	CR42_first	AGEP	AGD1	AGD2
CR42_second	NA	0.11	0.01	0.00	0.11
CR42_first	0.78	NA	0.02	-0.01	0.07
AGEP	0.58	0.45	NA	-0.02	-0.02
AGD1	0.32	0.24	0.10	NA	NA
AGD2	0.63	0.58	0.3	NA	NA

Table S2.3 Number of phenotype records for sires. The number of phenotypes measured on a bull's dam, sire, siblings, and progeny*. Phenotypes include calving rate** in first (CR42_first) and second (CR42_second) lactation, age at puberty (AGEP) and anogenital distance measured at 11 mo (AGD1) and 29 mo (AGD2) old. *Progeny numbers vary within the provided bounds, depending on the age of the bull (See Table S2.4). **Calving rate is a binary trait denoting a cow's success (1) or failure (2) to calve within the first 42 d of the herds seasonal calving period.

Trait	own	dam	sire	full sibs	half sibs	progeny
CR42_second	0	1	0	0	5,000	0 to 85
CR42_first	0	1	0	0	5,000	0 to 85
AGEP	0	0	0	0	0	0 to 45
AGD1 or AGD2	0	0	0	0	0	0 to 45

Table S2.4 Daughter (Dtr) numbers by age of the bull. Number of phenotyped daughters per trait, by the age of the bull. Phenotypes include calving rate* in first (CR42_first) and second (CR42_second) lactation, age at puberty (AGEP) and anogenital distance measured at 11 mo (AGD1) and 29 mo (AGD2) old. *Calving rate is a binary trait denoting a cow's success (1) or failure (2) to calve within the first 42 d of the herds seasonal calving period.

Age (yrs)	Age of Dtrs (yrs)	CR42_second, CR42_first	CR42_second, CR42_first, AGEP	CR42_second, CR42_first, AGD	CR42_second, CR42_first, AGEP, AGD
0	NA	0,0	0,0,0	0,0,0	0,0,0,0
1	NA	0,0	0,0,0	0,0,0	0,0,0,0
2	0	0,0	0,0,0	0,0,0	0,0,0,0
3	1	0,0	0,0,45	0,0,45	0,0,45,45
4	2	0,85	0,85,45	0,85,45	0,85,45,45
5	3	85,85	85,85,45	85,85,45	85,85,45,45

Results and discussion

The reliability (accuracy squared) of fertility EBVs ranged from 0.23 to 0.59 (Table S2.5), depending on the age of the bull, the model (bivariate, CR42_first and CR42_second; trivariate, CR42_first and CR42_second with either AGEP or AGD as a predictor; multivariate, CR42 first and CR42_second with both AGEP and AGD as predictors) and the AGD trait that was used (AGD1 or AGD2).

In our analysis the reliability of parent average fertility EBVs is the same regardless of the model used, as we assumed that the parents and siblings of a bull would only have CR42

phenotypes. When a bull is > 3 yr old, we estimated that the trivariate model would yield the highest EBV accuracies, ranging from 0.45 to 0.48 and 0.56 to 0.59 when using AGD1 and AGD2 respectively (Figure 7.1, Figure 7.2). Conversely, we estimated the reliability of a bull's fertility EBVs to be lowest using just CR42 phenotypes, ranging from 0.23 to 0.37. Further, the reliability of fertility EBVs estimated using just CR42 phenotypes do not increase from parent average (0.23) until a bull's daughters are at least 2 yr old, when the bull is at least 4 years old.

The dependency of EBV reliability on the age of the bull, the choice of model (bivariate, multivariate with AGEP, multivariate with AGD, multivariate with AGEP and AGD) determined the estimated response to selection (Table S2.6, Table S2.8), which was around 20% higher when using both AGEP and AGD as predictors of fertility EBVs, compared to using CR42 phenotypes alone. As the EBV reliabilities suggest, most of these gains in selection response can be realized in much younger animals, as the benefit of the AGEP and AGD phenotypes is captured when a bull's daughters are 1 yr old, 12 mo prior to their first calving.

Table S2.5 Reliability (accuracy squared) of the fertility EBV for bulls aged up to 4 yr old, using bivariate (calving rate; CR42) or multivariate (CR42 and age at puberty [AGDP], CR42 and anogenital distance at 12 mo old [AGD1] or CR42, AGEP and AGD1) analyses.

Age (years)	Daughters	CR42 only	CR42, AGEP	CR42, AGD1	CR42, AGEP, AGD1
Birth to 1	NA	0.23	0.23	0.23	0.23
1 to 2	NA	0.23	0.23	0.23	0.23
2 to 3	Born	0.23	0.23	0.23	0.23
3 to 4	Puberty	0.23	0.4	0.28	0.45
4 to 5	First lactation	0.29	0.44	0.33	0.48
5 to 6	Second lactation	0.37	0.49	0.4	0.53

Table S2.6 Selection response[+] for the fertility EBV in bulls using bivariate (calving rate; CR42) or multivariate (CR42 and age at puberty [AGEP], CR42 and anogenital distance at 12 mo old [AGD1] or CR42, AGEP and AGD1) analyses. Selection response is expressed as a percentage of the response estimated to occur when selecting bulls at 6 yr old, with 85 daughter phenotypes for CR42 in both first and second lactation. [+](Selection response per unit of selection intensity = Accuracy * genetic standard deviation).

Age (years)	Daughters	CR42 only	CR42, AGEP	CR42, AGD1	CR42, AGEP, AGD1
Birth to 1	NA	79%	79%	79%	79%
1 to 2	NA	79%	79%	79%	79%
2 to 3	Born	79%	79%	79%	79%
3 to 4	Puberty	79%	104%	87%	110%
4 to 5	First lactation	89%	109%	94%	114%
5 to 6	Second lactation	100%	115%	104%	120%

Table S2.7 Reliability (accuracy squared) of the fertility EBV for bulls aged up to 4 yr old, using bivariate (calving rate; CR42) or multivariate (CR42 and age at puberty [AGEP], CR42 and anogenital distance at 29 mo old [AGD2] or CR42, AGEP and AGD2) analyses.

Age (years)	Daughters	CR42 only	CR42, AGEP	CR42, AGD2	CR42, AGEP, AGD2
Birth to 1	NA	0.23	0.23	0.23	0.23
1 to 2	NA	0.23	0.23	0.23	0.23
2 to 3	Born	0.23	0.23	0.23	0.23
3 to 4	Puberty	0.23	0.40	0.23	0.40
4 to 5	First lactation	0.29	0.44	0.46	0.56
5 to 6	Second lactation	0.37	0.49	0.5	0.59

Table S2.8 Selection response[+] for the fertility EBV in bulls using bivariate (calving rate; CR42) or multivariate (CR42 and age at puberty [AGEP], CR42 and anogenital distance at 29 mo old [AGD2] or CR42, AGEP and AGD2) analyses. Selection response is expressed as a percentage of the response estimated to occur when selecting bulls at 6 yr old, with 85 daughter phenotypes for CR42 in both first and second lactation. [+](Selection response per unit of selection intensity = Accuracy * genetic standard deviation).

Age (years)	Daughters	CR42 only	CR42, AGEP	CR42, AGD2	CR42, AGEP, AGD2
Birth to 1	NA	79%	79%	79%	79%
1 to 2	NA	79%	79%	79%	79%
2 to 3	Born	79%	79%	79%	79%
3 to 4	Puberty	79%	104%	79%	104%
4 to 5	First lactation	89%	109%	112%	123%
5 to 6	Second lactation	100%	115%	116%	126%

References

Hazel, L.N., G.E. Dickerson, and A.E. Freeman. 1992. The Selection Index-Then, Now, and for the Future. J Dairy Sci 77:3236–3251.

Van der Werf, J. 2023. Teaching Software Used for Quantitative Genetics at UNE. Accessed June 28, 2023. https://jvanderw.une.edu.au/software.htm.

APPENDIX 1. Related publications

This appendix contains the abstracts for related publications that I have authored or co-authored.

Variance parameter estimation for age at puberty phenotypes under two levels of phenotype censorship

Stephen et al., 2022. **American Dairy Science Association annual meeting**, 2022, Kansas City, Missouri, USA. An oral presentation (virtual) with live Q+A.

Link:
https://www.adsa.org/Portals/0/SiteContent/Docs/Meetings/PastMeetings/Annual/2022/199.pdf

Variance parameter estimation for age at puberty phenotypes under two levels of phenotype censorship.

M. Stephen*[1,4], S. Meier[1], M. Price[1], J.E. Pryce[2,3], C. Burke[1], C. Phyn[1], D.Garrick[4], *[1] DairyNZ Ltd, 605 Ruakura Road, Hamilton 3240, New Zealand, [2] Agriculture Victoria Research, AgriBio, Centre for AgriBioscience, Bundoora, Victoria 3083, Australia, [3] School of Applied Systems Biology, La Trobe University, Bundoora, Victoria 3083, Australia, [4] AL Rae Centre for Genetics and Breeding - Massey University, Ruakura, Hamilton 3214, New Zealand.*

Abstract

Age at puberty (AGEP) is a moderately heritable trait in cattle that may be beneficial as an early-in-life predictor of an animal's genetic merit for lifetime reproductive success. Unfortunately, AGEP is difficult to measure precisely as animals must be observed frequently over several months. However, it is possible that genetic selection for AGEP could be successful using censored phenotypes. That is, when observations are less frequent and/or occur over a shorter time period. Our objectives for this study were two-fold. First, to produce variance components for AGEP. Second, to investigate the implications of a simplified phenotyping strategy on the genetic evaluation of AGEP, where censoring of the phenotype was increased. We measured AGEP in a closely monitored population of approximately 500 Holstein-Friesian heifers, born in 2015 and managed under a seasonal, pasture-based dairy system. Animals were blood tested weekly from approximately 240 to 440 days of age and were deemed to have reached puberty when blood plasma progesterone (BP4) elevation (>1 ng/mL) was detected in two of three consecutive blood tests (AGEP_Weekly). To simulate a simplified phenotyping strategy based upon monthly herd visits (AGEP_Monthly), we selectively disregarded data from all but three blood tests, when animals were approximately 300, 330 and 360 days of age (SD = 14.5 d). The posterior mean of estimated heritabilities for AGEP_Weekly was 0.54, with a 90% credibility interval (CI) of 0.41 to 0.66, whereas it was 0.44 (90% CI 0.32 to 0.57) for AGEP_Monthly. The correlation between EBV for AGEP_Weekly and AGEP_Monthly was 0.87 (90% CI, 0.84 to 0.89). We conclude that, in this population, AGEP is a moderately heritable trait. Further, increasing phenotype censorship from weekly to monthly observations over a shorter period did not alter the main conclusions of this analysis. Our results support the use of censoring to reduce costs and logistical challenges associated with collection of puberty phenotypes.

Key words: Puberty, Fertility, Heritability, Censored

(Co)variances between anogenital distance and fertility in Holstein-Friesian dairy cattle

Stephen et al., 2023. **European Federation of Animal Science (EAAP) annual meeting**, 2023, Lyon, France. Accepted for an oral presentation.

(Co)variances between anogenital distance and fertility in Holstein-Friesian dairy cattle.

M.A. Stephen*[1,4], C.R. Burke[1], N. Steele[1], J.E. Pryce[2,3], S. Meier[1], P.R. Amer[5], C.V.C. Phyn[1], D.J. Garrick[1,4],[1] *DairyNZ Ltd, 605 Ruakura Road, Hamilton 3240, New Zealand,* [2] *Agriculture Victoria Research, AgriBio, Centre for AgriBioscience, Bundoora, Victoria 3083, Australia,* [3] *School of Applied Systems Biology, La Trobe University, Bundoora, Victoria 3083, Australia,* [4] *AL Rae Centre for Genetics and Breeding - Massey University, Ruakura, Hamilton 3214, New Zealand,* [5] *AbacusBio, Dunedin, New Zealand; melissa.stephen@dairynz.co.nz*

Abstract

Reproductive performance is an economically important trait for dairy farmers, particularly in pasture-based, seasonal farm systems that use an annual calving pattern to align pasture growth with the feed demands of the herd. Traditional fertility phenotypes are lowly heritable and expressed late in an animal's life, which contributes to relatively slow progress from genetic selection for those traits. Anogenital distance (AGD) is a moderately heritable candidate trait for predicting fertility estimate breeding values (EBVs) in dairy cattle. The objectives of this study were twofold. First, to estimate the genetic and phenotypic (co)variances between AGD measured at two ages and subsequent fertility traits expressed during lactation. Second, to estimate the genetic (co)variances between AGD and body conformation traits. We measured AGD, shoulder height, body length and body weight at 11 ± 0.5 months of age in a population of 5,010 Holstein-Friesian and Holstein-Friesian x Jersey cows, born in 2018 and farmed across 54 seasonal, pasture-based herds. We also measured AGD at 29 ± 0.7 months of age in a subset of 17 herds (n=1,956 cows). Calving, breeding, and conception dates were recorded for all available animals in first and second lactations, which commenced when animals were approximately 24 and 36 months of age, respectively. We report moderate heritabilities of 0.23 and 0.29 for 11-month and 29-month AGD, respectively. Both AGD measures exhibited moderate genetic correlations between 0.19 and 0.63 with calving, breeding, and conception traits. Genetic correlations between both AGD measures and body stature traits were weak (≤0.16). We conclude that AGD is a promising candidate predictor for fertility EBVs in dairy cattle, and genetic selection for shorter AGD in female cattle should result in improved fertility outcomes during lactation.

Animal- and herd-level factors associated with onset of puberty in grazing dairy heifers

Steele et al., 2023. Full paper, in press with New Zealand Veterinary Journal, June 2023

Animal- and herd-level factors associated with onset of puberty in grazing dairy heifers.

NM Steele[1], MA Stephen[1,2], B Kuhn-Sherlock[1], SJ Hendriks*[,3], S Meier[1], CVC Phyn[1] and CR Burke[1],[1]DairyNZ *Ltd.*, Private Bag 3221, Hamilton, New Zealand, [2]*AL Rae Centre for Genetics and Breeding, Massey University, Hamilton, New Zealand,* [3]School of Agriculture and Environment, Massey University, Palmerston North, New Zealand,[*]Author for correspondence: Email: s.hendriks@massey.ac.nz

Abstract

Aims: To explore animal- and herd-level risk factors influencing age at puberty in predominantly Holstein-Friesian dairy heifers managed in seasonal, pasture-based systems.

Methods: Heifers born in spring 2018 (n = 5,010) from 54 commercial dairy herds in New Zealand were visited on three occasions when the average heifer age, within herd, was 10 (visit 1; V1), 11 (V2) and 12 (V3) months old. Blood samples were collected on each visit and liveweight, stature and anogenital distance (AGD) were measured at V2. Heifers were defined as having reached puberty at the first visit where blood progesterone was elevated (≥ 1 ng/mL). Animal-level response variables included pubertal status by V1, V2 and V3, and age at puberty (or age at V3 plus 31 days for those that had not attained puberty by V3). To explore herd-level management factors, farmers answered a questionnaire relating to animal location, land type, health, feeding, and management between weaning and mating. A partial least squares regression was undertaken to identify herd-level factors associated with the greatest influence on puberty rate within herd.

Results: The mean age at puberty was 352 (SD 34.9) days. Heavier animals at a greater proportion of expected mature liveweight based on their breeding value for liveweight, or animals with a higher breed proportion of Jersey and lower breed proportion of Holstein, were associated with earlier puberty. Herd puberty rates varied widely among enrolled herds, and averaged 20%, 39% and 56% by V1, V2 and V3, respectively. Liveweight, followed by breed and land type, had the greatest influence on the herd puberty rate. Heifer herds with a greater mean liveweight (absolute and proportion of expected mature weight) or greater Jersey proportion had more animals that reached puberty at any visit, whereas herds located on steep land or with greater Holstein breed proportions had lower puberty rates. Management-related factors such as vaccinations, provision of feed supplements, and weighing frequency were also herd-level risk factors of puberty but had less influence.

Conclusions and clinical relevance: This study highlights the importance of having well-grown heifers for increasing the chances of earlier puberty onset and the effect of breed and youngstock management to achieve growth targets. These outcomes have important implications for the optimal management of heifers to achieve puberty before their maiden breeding and for the timing of measurements to potentially incorporate a puberty trait in genetic evaluations.

APPENDIX 1. DRC 16 forms

The ‘Statements of Contribution’ to Doctoral thesis containing publications or prepared for publication.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Melissa Anne Stephen
Name/title of Primary Supervisor:	Dorian Garrick
In which chapter is the manuscript /published work:	Chapter Two

Please select one of the following three options:

(X) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Stephen, M.A., S. Meier, M.D. Price, J.E. Pryce, C.R. Burke, Phyn. C.V.C., and D.J. Garrick. 2022b. Variance parameter estimation for age at puberty phenotypes under two levels of phenotype censorship. J Dairy Sci. Comms. doi:10.3168/jdsc.2022-0218.

( ) The manuscript is currently under review for publication – please indicate:

- The name of the journal:

- The percentage of the manuscript/published work that was contributed by the candidate:

- Describe the contribution that the candidate has made to the manuscript/published work:

( ) It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Candidate's Signature:	Mel Stephen
Date:	30/06/2023
Primary Supervisor's Signature:	Dorian Garrick
Date:	30/06/2023

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis.

GRS Version 5 – 13 December 2019
DRC 19/09/10

DRC 16

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Melissa Anne Stephen
Name/title of Primary Supervisor:	Dorian Garrick
In which chapter is the manuscript /published work:	Chapter Three

Please select one of the following three options:

◯ The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

(X) The manuscript is currently under review for publication – please indicate:

- The name of the journal:

In review with Journal of Animal Science and Biotechnology, Submitted May 2023.

- The percentage of the manuscript/published work that was contributed by the candidate: **90 %**
- Describe the contribution that the candidate has made to the manuscript/published work:

I collated and analysed existing phenotype data collected in the 'Puberty as Scale' trial. I selected the analytical approaches, completed all statistical analysis, interpreted the results and wrote the manuscripts, under the guidance of my supervisors and co-authors.

◯ It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Candidate's Signature:	Mel Stephen
Date:	30/06/2023
Primary Supervisor's Signature:	Dorian Garrick
Date:	30/06/2023

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis.

GRS Version 5 – 13 December 2019
DRC 19/09/10

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Melissa Anne Stephen
Name/title of Primary Supervisor:	Dorian Garrick
In which chapter is the manuscript /published work:	Chapter Four

Please select one of the following three options:

(X) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Stephen, M.A., H. Cheng, J.E. Pryce, C.R. Burke, N.M. Steele, C.V.C. Phyn, and D.J. Garrick. 2022a. Estimating heritabilities and breeding values from censored phenotypes using a data augmentation approach. Front Genet 13. doi:10.3389/fgene.2022.867152.

( ) The manuscript is currently under review for publication – please indicate:

- The name of the journal:
- The percentage of the manuscript/published work that was contributed by the candidate:
- Describe the contribution that the candidate has made to the manuscript/published work:

( ) It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Candidate's Signature:	Mel Stephen
Date:	30/06/2023
Primary Supervisor's Signature:	Dorian Garrick
Date:	30/06/2023

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis.

GRS Version 5 – 13 December 2019
DRC 19/09/10

DRC 16

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Melissa Anne Stephen
Name/title of Primary Supervisor:	Dorian Garrick
In which chapter is the manuscript /published work:	Chapter Five

Please select one of the following three options:

○ The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

○ The manuscript is currently under review for publication – please indicate:

- The name of the journal:
- The percentage of the manuscript/published work that was contributed by the candidate:
- Describe the contribution that the candidate has made to the manuscript/published work:

(X) It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Candidate's Signature:	Mel Stephen
Date:	30/06/2023
Primary Supervisor's Signature:	Dorian Garrick
Date:	30/06/2023

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis.

GRS Version 5 – 13 December 2019
DRC 19/09/10

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Melissa Anne Stephen
Name/title of Primary Supervisor:	Dorian Garrick
In which chapter is the manuscript /published work:	Chapter Six

Please select one of the following three options:

(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Stephen, M.A., C.R. Burke, N. Steele, J.E. Pryce, S. Meier, P.R. Amer, C.V.C. Phyn, and D.J. Garrick. 2023. Genome-Wide Association Study of anogenital distance and its (co)variances with fertility in growing and lactating Holstein-Friesian dairy cattle. J Dairy Sci In Press.

( ) The manuscript is currently under review for publication – please indicate:

- The name of the journal:
- The percentage of the manuscript/published work that was contributed by the candidate:
- Describe the contribution that the candidate has made to the manuscript/published work:

( ) It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Candidate's Signature:	Mel Stephen
Date:	30/06/2023
Primary Supervisor's Signature:	Dorian Garrick
Date:	30/06/2023

This form should appear at the end of each thesis chapter/section/appendix submitted as a manuscript/ publication or collected as an appendix at the end of the thesis.

GRS Version 5 – 13 December 2019
DRC 19/09/10