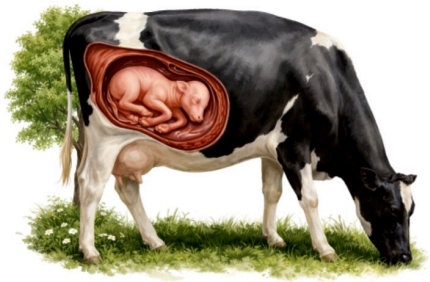
SINA SAKHAEI FAR

Genomic and quantitative genetic analyses of female fertility and calving traits in German Holstein cattle using alternative random regression modelling approaches



Dissertation
to obtain the doctoral degree (Dr. agr.)
at the Faculty of Agricultural Sciences,
Nutritional Sciences and Environmental Management
Justus Liebig University Giessen, Germany

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SINA SAKHAEI FAR

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"This work is dedicated to my spouse, Mrs. Fatemeh Gheitanchi, whose patience, encouragement, and unwavering support helped me overcome the many challenges of my PhD. I am deeply grateful for her sacrifices and her constant presence by my side. I also dedicate this to the loving memory of my mother, Mrs. Zahra Khoramtash, and to my father, Mr. Abdolah Sakhaeifar."

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LIST OF ABBREVIATIONS

CE	Calving Ease
CTFS	Calving To First Service
DO	Days Open
EBV	Estimated Breeding Value
GBLUP	Genomic Best Linear Unbiased Method
GO	Gene Ontology
GWAS	Genome Wide Association Study
KEGG	Kyoto Encyclopedia of Genes and Genome
MTM	Multiple Trait Model
NRR56	Non-Return Rate on Day 56
PCA	Principle Component Analysis
RRM	Random Regression Model
SB	Still Birth
SD	Standard Deviation
SE	Standard Error
SNP	Single Nucleotide Polymorphism

SUMMARY

This thesis presents a comprehensive investigation of the genetic architecture of female fertility and calving traits in German Holstein cattle by integrating advanced quantitative genetic modeling and longitudinal genomic analyses. Due to their low heritability, strong environmental influence, and complex biological regulation, reproductive traits pose a long-standing challenge for dairy breeding programs. The work aims to improve the accuracy and biological relevance of genetic evaluations for traits such as non-return rate at 56 days (NRR56), calving-to-first-service interval (CTFS), days open (DO), calving ease (CE), and stillbirth (SB). To achieve this, the thesis employs classical genetic models, random regression approaches, and longitudinal genome-wide association studies (GWAS), thereby combining statistical, genomic, and biological perspectives.

Chapter 1 provides an extensive introduction to reproductive biology in dairy cattle and reviews key fertility and calving traits, along with their economic and welfare relevance. It discusses factors influencing reproductive performance, including management, behavior, health, and genetics, and outlines the limitations of traditional statistical methods that assume constant genetic effects across time. The chapter emphasizes the importance of longitudinal models and introduces the conceptual foundation of random regression models (RRM) and longitudinal GWAS. These approaches enable the modeling of heterogeneous variances across parities and capture time-dependent genetic effects. The chapter concludes by presenting the main objectives of the thesis: to estimate genetic parameters across reproductive traits using advanced modeling, to integrate genomic data into dynamic analyses, and to evaluate the biological function of identified genomic regions.

Chapter 2 focuses on fertility traits and applies Multiple-trait models (MTM) and random regression models (RRM) to a large dataset comprising more than 592,000 fertility records. Genotypes from approximately 21,300 animals were integrated using a genomic relationship matrix. This chapter demonstrates that genetic variances and heritabilities for NRR56, CTFS, and DO generally increase with parity, particularly distinguishing heifers from cows. The RRM framework proved more biologically realistic, revealing parity-specific genetic patterns and declining genetic correlations as parity distance increased. Notably, correlations between heifer NRR56 and cow NRR56 were low (0.25-0.50), indicating distinct genetic expressions in early versus later reproductive cycles. The chapter shows that RRMs enable dynamic estimated breeding values (EBVs), which support more precise selection across the reproductive lifespan.

Chapter 3 presents a longitudinal genome-wide association study (GWAS) for fertility traits, incorporating time-dependent single-nucleotide polymorphism (SNP) effects. Using repeated fertility measurements across six lactations, the study identifies significant genomic regions whose effects vary across reproductive stages. Circular Manhattan plots and quantile-quantile (QQ) plots illustrate both stage-specific SNP associations and overall model accuracy. Gene annotation and enrichment analysis reveal biological pathways relevant to reproduction, including hormonal regulation (for example, involving the gene *CSMD1*), cell adhesion (genes TMEM132C and *DCHS2*), and cell proliferation and oocyte (egg cell) development (gene CSNK1A1). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis further highlight the contribution of signaling mechanisms such as Hippo, Wnt, and gonadotropin-releasing hormone (GnRH) to fertility regulation. These findings underscore the importance of incorporating temporal genetic variation in genomic evaluations and demonstrate the power of longitudinal GWAS for identifying biologically meaningful candidate genes.

Chapter 4 investigates calving traits, specifically calving ease (CE) and stillbirth (SB), using three modelling approaches: a maternal model with direct and maternal genetic effects, a multiple-trait model (MTM) treating each parity (calving event per cow) as a distinct trait, and a random regression model (RRM), which describes calving performance across multiple parities. Using nearly half a million calving records, the chapter shows that incorporating maternal genetic effects substantially improves model fit and biological interpretation. The RRM approach again provides smoother variances across parities and realistic covariance structures. Moderate heritabilities and strong genetic correlations across parities highlight the potential for genetic improvement of calving traits. This chapter further demonstrates that modeling CE and SB at the dam (mother cow) level, rather than attributing them solely to the calf, better reflects underlying physiology and leads to more accurate estimated breeding values (EBVs).

Chapter 5 synthesizes the results of all studies and discusses their implications for dairy breeding. Building on these findings, the thesis concludes that reproductive traits exhibit dynamic genetic architecture and cannot be fully captured by static models. Notably, random regression models consistently outperform conventional approaches in describing time-varying variances and correlations. Furthermore, longitudinal GWAS complements quantitative models by identifying functional genes and pathways involved at different reproductive stages. Collectively, this research provides a foundation for implementing longitudinal modeling in national breeding programs, enabling more accurate selection for fertility, calving performance, animal welfare, and overall herd sustainability.

ZUSAMMENFASSUNG

Diese Dissertation untersucht umfassend die genetische Architektur weiblicher Fruchtbarkeits- und Kalbeeigenschaften bei Deutschen Holsteinrindern. Aufgrund ihrer geringen Heritabilität, starken Umweltabhängigkeit und komplexen biologischen Regulation zählen Reproduktionsmerkmale zu den Merkmalen, die am schwierigsten mittels züchterischer Methoden verbessert werden können. Ziel der Arbeit ist es daher, die genetische Bewertung für Merkmale wie Non-Return -Rate nach 56 Tagen (NRR56), Kalbe-Erstbesamungs-Intervall (CTFS), Güstzeit (DO), Kalbeverlauf (CE) und Totgeburtenrate (SB) mittels weiterentwickelter statistischer Modelle für longitudinale Datenstrukturen, tiefgreifender genomischer Analysen und der Aufklärung physiologischer und biologischer Mechanismen zu verbessern.

Kapitel 1 bietet eine umfassende Einführung in die Reproduktionsbiologie des Milchviehs und beschreibt die wichtigsten Fruchtbarkeits- und Kalbeeigenschaften sowie ihre wirtschaftliche und tierwohlbezogene Bedeutung. Das Kapitel erläutert Management-, Verhaltens-, Gesundheits- und genetische Einflüsse auf die Reproduktionsleistung und zeigt die Grenzen klassischer genetisch-statistischer Modelle auf, welche konstante genetische Effekte über die Zeit annehmen. Darauf aufbauend werden Random-Regression-Modelle (RRM) und longitudinale genomweite Assoziationsstudien (GWAS) vorgestellt, die zeitabhängigen genetischen Veränderungen adäquat berücksichtigen können. Das Kapitel schließt mit den Zielen der Arbeit: Die Schätzung laktationsspezifischer genetischer Parameter, die Integration genomischer Informationen in statistischer Vorhersagemodelle und die funktionelle und physiologische Interpretation relevanter Genregionen.

Kapitel 2 analysiert die weiblichen Fruchtbarkeitsmerkmale NRR56, CTFS und DO im quantitativ-genetischen Kontext. Auf Basis von mehr als 592.000 Fruchtbarkeitsdaten und

21.300 Geno typisierten Kühen werden verschiedene Modellierungsansätze für longitudinale Datenstrukturen verglichen: Mehrmerkmals-Tiermodell, Wiederholbarkeitsmodell und RRM. Die Ergebnisse zeigen, dass genetische Varianzen und Heritabilitäten mit zunehmender Laktationsnummer ansteigen, wobei besonders deutliche Unterschiede zwischen Färsen und Kühen beim Merkmal NRR56auftreten. Die genetischen Korrelationen im gleichen Merkmal über die Laktationen hinweg nehmen mit zunehmender Paritätsdistanz ab, was auf dynamische genetische Kontrollmechanismen hinweist – verschiedene Gene sind für das gleiche Fruchtbarkeitsmerkmal in verschiedenen Laktationsnummern von Bedeutung. Das Kapitel 2 zeigt, dass RRM zeitpunkt-spezifische Zuchtwertschätzungen ermöglichen, was eine präzisere Selektion von Besamungsbullen gemäß den Altersstrukturen der Milchviehherden impliziert.

Kapitel 3 widmet sich longitudinalen GWAS-Analysen. Durch die Modellierung zeitabhängiger SNP-Effekte über sechs Laktationen hinweg werden Genregionen identifiziert, deren Einfluss sich über die Reproduktionszyklen verändert. Die Analyse detektiert potenzielle Kandidaten Gene wie *CSMD1*, *TMEM132C*, *DCHS2* und *CSNK1A1*, die an Zelladhäsion, hormoneller Regulation, Eizellreifung und Zellproliferation beteiligt sind. GO- und KEGG-Analysen der physiologischen Mechanismen und biologischen Kausalitäten unterstreichen die zentrale Rolle der Hippo-, Wnt- und GnRH-Signalwege. Diese Ergebnisse verdeutlichen, wie wichtig passende Modelle für longitudinale Datenstrukturen sind, um polygen gesteuerte Fruchtbarkeitsmerkmale korrekt abzubilden und die diesbezüglichen ursächlichen genetischen Mechanismen zu verstehen.

Kapitel 4 untersucht Kalbeeigenschaften (CE und SB) anhand dreier Modellierungsansätze: Ein klassisches Tiermodells mit direkten und maternalen Effekten aus der Perspektive des Kalbens sowie ein Mehrmerkmalsmodell und ein RRM mit einer Zuordnung von CE und SB zu den Muttertieren mit anschließender laktationsspezifischer Merkmalsanalyse. Anhand von über

450.000 Kalbedaten zeigt sich, dass maternale genetische Effekte entscheidend zur Modellgüte beitragen und dass die mittels RRM geschätzten Varianzkomponenten sehr gut in einen biologischen Kontext eingeordnet werden können. Im Vergleich zu eher traditionellen genetischen Analysen von CE und SB konnten tendenziell höhere direkte und maternale Heritabilitäten geschätzt werden, Dennoch waren die genetischen Korrelationen im gleichen Merkmal aus verschiedene Laktationen mit > 0,80 recht hoch, was mittels Ranganalysen auf Basis der Bullenzuchtwerte bestätigt wurde.

Kapitel 5 fasst die Erkenntnisse der einzelnen Kapitel zusammen und vertieft die dynamischen genetische Strukturen von reproduktiven Merkmalen der weiblichen Fruchtbarkeit von Milchrindern. Final bleibt festzuhalten, dass RRM sich besonders gut zur Abbildung dieser Dynamik eignen. Longitudinale GWAS sollten angewendet werden, um funktionelle Erkenntnisse zeitabhängiger Genregulation zu generieren, generieren. Insgesamt legt diese Arbeit eine Grundlage für innovative Zuchtwertschätzungssysteme auf Basis wiederholter Messungen für Reproduktionsmerkmale für Milchkühe, welche auch Modellcharakter für andere funktionale Merkmale in der Rinderzucht haben können.

CHAPTER 1

General Introduction

1.1 Background and importance of reproductive traits

Reproductive performance is a cornerstone of dairy cattle production systems, directly influencing herd replacement rates, profitability, and long-term sustainability (Rodriguez-Martinez H 2008, Lenka krpalkova, 2016). Fertility and calving traits are among the most critical functional traits in dairy cattle, not only due to their direct economic impact but also their strong implications for animal health and welfare (Egger-Danner et al., 2015). Declining fertility in modern dairy cows over recent decades has been widely documented (Walsh et al., 2011), while persistent challenges with dystocia and stillbirth continue to compromise productivity (Mee, 2008; Cole et al., 2007). These trends highlight the need for advanced strategies to improve reproductive performance through both genetic and genomic approaches.

The dairy industry has undergone tremendous transformation over the past decades, with increasing emphasis placed on production traits such as milk yield (Liu et al., 2008; Liu et al., 2017a). However, the unfavorable genetic correlation between production and fertility has created challenges for breeders and producers (Wall et al., 2003). While milk production per cow has increased significantly, reproductive performance has often declined, leading to increased inseminations per conception, longer calving intervals, and higher replacement costs (Dobson et al., 2007). The economic consequences of reduced reproductive efficiency include not only increased veterinary and management costs but also losses in lifetime productivity (Inchaisri et al., 2010). Beyond economics, reproductive problems such as dystocia are among the most painful conditions experienced by cattle raising concerns about animal welfare and ethical breeding practices (Abriham Kebede, 2017). Against this background, understanding and improving reproductive traits is central to modern dairy breeding programs.

1.2 Definition of reproductive traits

Reproductive performance is one of the most important determinants of efficiency and profitability in dairy and beef cattle production systems. Fertility and calving traits directly affect the number of calves produced, the length of calving intervals, and the overall lifetime productivity of cows (Cielava et al., 2017). In addition, they are closely linked to animal health, welfare, and farm economics, making their improvement a key target in breeding and management programs (Lucy, 2019).

1.2.1 Fertility traits

As mentioned by Gernand and König (2017), female fertility in dairy cattle can be defined as the ability to show heat or maturity, to conceive, and to recycle. Fertility has decreased during the past decades due to enhanced concentration on production traits (Walsh et al., 2011; Veerkamp et al., 2015). Fertility is a highly complex trait; therefore, new breeding indexes have been developed to encompass its two main components: the initiation of a new cycle after calving and the success of insemination. (González-Recio and Alenda, 2005; Jorjani, 2006). Furthermore there are many studies indicate the negative genetic correlation between production traits and fertility traits in Holstein (VanRaden et al., 2004; Zavadilová and Zink, 2013; Cassandro, 2014) or other breeds (Roy et al., 2024). Also improving fertility will also allow to avoid a reducing on longevity as reported by Oltenacu and Broom (2010).

Besides the strong environmental influence, the intricate genetic basis of female fertility presents a substantial obstacle for reliable genomic prediction (Gajbhiye et al., 2018). The underlying physiological pathways involve a dynamic contribution of multiple genes to reproductive functions, varying over time and across conditions, as noted by Beerda et al. (2008). Furthermore, temporal and environmental shifts can modify gene expression, leading to the activation or suppression of different sets of genes throughout and between lactations (König and May, 2019; Cai et al., 2019).

Among the fertility traits, the non-return rate at 56 days (**NRR56**) measures the proportion of inseminated females that do not return to estrus within 56 days after service and is therefore considered a proxy for pregnancy rate. However, it is influenced by multiple biological and management factors, including fertilization success, early embryo survival, estrus detection accuracy, and record keeping (González-Recio and Alenda, 2005).

Another commonly studied fertility trait is the interval from calving to first service (**CTFS**). This trait reflects both the biological recovery of the reproductive system after parturition and the efficiency of herd management in detecting estrus and performing insemination. A trait that received consideration is the commencement of luteal activity, which is the number of days from calving until the first postpartum luteal activity (Nyman, 2018). A shorter CTFS indicates that cows have resumed normal cycle and are detected in heat promptly, whereas a prolonged interval is often associated with poor postpartum recovery, negative energy balance, or deficiencies in estrus detection (Walsh et al., 2011). Closely related is the trait known as days open (DO), which is defined as the interval between calving and the successful conception that results in pregnancy. This measure integrates several aspects of fertility, such as time to first service, conception rate, and embryo survival, and is therefore considered one of the most informative indicators of reproductive efficiency (Lucy, 2019). Prolonged DO reduces lifetime productivity, increase replacement costs, and are among the major reasons for involuntary culling in dairy herds.

1.2.2 Calving traits

Calving traits represent synergistic traits, as they depend on the combined genetic contributions of calf, dam, and sire, and they strongly influence both calf survival and the future fertility of the dam (Sakhaei-far et al., 2025). Calving ease (**CE**) describes the amount of assistance required during parturition, ranging from unassisted deliveries to cases requiring veterinary intervention. Dystocia, or difficult calving, is associated with increased risk of injury, uterine

infections, and reduced milk yield, and has strong implications for animal welfare (Probo et al., 2022). The other calving trait is stillbirth (**SB**), which is usually defined as the birth of a dead calf or death within the first 24 to 48 hours after birth (Cole et al., 2007). CE and SB are often referred to as "synergistic traits," since they depend on the interplay of multiple genetic components, including the direct genetic effect of the calf, the maternal genetic effect of the dam, and in some cases the paternal effect of the service sire (König et al., 2007). From a calf perspective, large birth weight or suboptimal body conformation increases the likelihood of dystocia or stillbirth (Simões and Stilwell, 2021). From the maternal perspective, the pelvic size, uterine capacity, gestation length, and metabolic or health status of the dam strongly influence calving outcomes (Cole et al., 2007). Accounting for both direct and maternal components is therefore essential in genetic evaluations of CE and SB, because ignoring these components can bias variance estimates and limit the accuracy of breeding values. SB not only represent a direct economic and welfare loss but also negatively affect the dam, as they are often linked to dystocia, retained placenta, and delayed uterine recovery (Johanson and Berger, 2003; Cole et al., 2007).

1.3 Critical factors shaping reproductive traits

1.3.1 Management factors

Management practices also strongly affect these traits. Efficient heat detection, timely insemination, and careful monitoring during calving can markedly improve reproductive performance (Roche, 2006). Environmental factors further complicate fertility, with nutrition (Butler, 2003), management practices (Evans and Walsh, 2011), and herd hygiene exerting strong influences that can mask genetic effects (Wrzecińska et al., 2021; Jayawardana et al., 2023). These gene × environment interactions can shadow the accuracy of genetic evaluations and complicate selective breeding strategies (Zhang et al., 2019). Inadequate estrus detection may lead to longer CTFS intervals and increased DO, while delayed assistance during

parturition can increase the risk of dystocia and stillbirth (Mee, 2008). Housing conditions and cow comfort are additional management factors; overcrowding, poor flooring, or inadequate lying space can increase stress, lameness, and indirectly impair fertility by reducing estrus expression and mounting behavior (Barkema et al., 2015).

1.3.2 Behavioral factors

Behavioral and physiological stress also influence reproduction. Stressful conditions, such as rough handling, excessive regrouping, or heat stress during summer (Halli et al., 2021), can suppress estrus signs, reduce conception rates, and increase early embryonic loss (Roth and Wolfenson, 2016). Seasonal effects are also well documented, with fertility and calving performance often declining under high temperature-humidity conditions, mainly due to heat stress and reduced feed intake (Vinet et al., 2024).

1.3.3 Disease control

Infectious diseases are another important factor influencing both fertility and calving traits. Uterine infections such as metritis and endometritis delay uterine involution and reduce conception rates, leading to longer intervals to first insemination and increased DO (Sheldon et al., 2008). Systemic diseases such as mastitis or lameness have also been associated with reduced fertility, likely due to metabolic and hormonal disruptions (Tsousis et al., 2022; Wolfenson et al., 2015). In addition, infections during pregnancy can compromise fetal development and increase the risk of stillbirths (Antanaitis et al., 2022).

1.3.4 Economic and welfare implications

Calving traits have profound economic and welfare implications (Abriham Kebede, 2017). Dystocia and stillbirth increase veterinary costs, prolong recovery periods, and reduce subsequent fertility and milk yield (Ghiasi et al., 2015; Sdiri et al., 2023). For instance, González-Recio and Alenda (2005) estimated that even mild assistance at calving incurs an economic cost of nearly €32 per cow, while severe dystocia can exceed €150. Stillbirth in the

U.S. dairy industry has been estimated to cause losses exceeding $125 million annually (Bicalho et al., 2008, Meyer, 2004).

From a welfare perspective, dystocia is among the most painful conditions experienced by cattle, with long-term consequences for both cow and calf health (Hudson et al., 2008). Calves born from difficult calvings have higher risks of respiratory and digestive diseases, while dams are predisposed to retained placenta, uterine infections, and metabolic disorders (Lombard et al., 2007). These issues highlight the importance of including calving traits in breeding programs, not only to enhance economic efficiency but also to promote animal health and ethical breeding practices.

1.3.5 Genetic factors and breed

Fertility is a complex polygenic trait, influenced by numerous genes, each with small additive effects (Gajbhiye et al., 2018). Its dynamic nature arises from fluctuating physiological processes across the reproductive cycle and across different lactations (Sakhaei-far et al., 2025). The expression of fertility-related genes can vary with time, with some genes being switched on or off during the life span, depending on the reproductive stage (McGrath et al., 2021). The combination of polygenicity, dynamic gene expression, and environmental modulation necessitates sophisticated statistical and genomic approaches to accurately assess and improve these traits (Forutan et al., 2024). However in case of fertility Attempts to improve conception rates through genomic herd management, using early genomic breeding values to forecast later phenotypes, have proven largely ineffective (Strabel, 2025).

Calving traits such as CE, SB, and gestation length represent another class of complex reproductive traits. CE reflects both direct genetic effects of the calf, such as size and conformation, and maternal effects related to the dam's pelvic structure and uterine function (Cue and Hayes, 1985). SB, defined as calf death at or within 24 hours of birth, is influenced by multiple environmental and genetic risk factors (Gardosi et al., 2005). These traits are

genetically correlated, with antagonistic relationships between direct and maternal CE and SB (Sakhaeifar & König., 2025). Genetic variation in both SB rate and calving difficulty at first calving is substantial in the Holstein breed whether measured as direct or as maternal traits (Steinbock et al., 2003).

Altogether, fertility and calving traits such as NRR56, CTFS, DO, CE, and SB represent complex traits influenced by both genetic and non-genetic factors. While genetic selection can contribute to gradual improvement, management practices, nutrition, health control, and welfare standards have an immediate and often larger impact on their expression (Rodney et al., 2018). Moreover, both fertility and calving traits indicate a lower heritability which makes difficult to apply breeding programs for genetic selection (Sakhaei-far et al., 2025). Understanding these interactions is therefore essential in designing both breeding strategies and herd management programs aimed at improving reproductive efficiency, animal welfare, and economic sustainability in cattle production (Bach, 2018).

1.4 Statistical models and genetic parameters in reproductive performance analysis

Over the years, numerous statistical models have been developed to evaluate reproductive and calving traits in cattle. For fertility traits, studies use animal models, repeatability models, and multiple-trait models (MTMs) to investigate genetic variation from different perspectives (Kadarmideen et al., 2000; Yin et al., 2014a). The animal model, a widely used linear mixed model, allows estimation of breeding values by incorporating both phenotypic, genotypic and pedigree data. For traits with repeated measures across lactations, repeatability models provide an extension by modeling permanent environmental effects across records (Silva et al., 2020a). In addition, many studies have implemented multiple-trait models (**MTM**)s for the genetic evaluation of female fertility together or in different lactations, allowing distinct assessment of fertility traits in heifers and cows (Muuttoranta et al., 2019; Zhu et al., 2023). For example Liu et al. (2008) utilized the MTM approach for NRR56, partitioning the analysis by parity (heifers

vs. cows). They reported a marginal difference in NRR56 heritability between cows and heifers. Most of the studies indicate similar results for the same traits and slight variations in heritabilities between studies are likely due to statistical modeling choices, such as the designation of the service sire as a fixed effect (Liu et al., 2008) or a random effect, or the method of genetic relationship assessment (pedigree vs. genomic data) (Shabalina et al., 2020). However, both approaches are limited in their ability to capture time-dependent dynamics (Sakhaei-far et al., 2025).

With respect to calving traits, statistical models often include maternal models and sire-maternal grandsire (**MGS**) models to account for both direct (effect of calf genetics) and maternal effects (effects via the mother or dam line) (Eaglen and Bijma, 2009). Different studies have examined calving traits from various perspectives. While most treated these traits as characteristics of the calf (Carnier et al., 2000; Eaglen and Bijma, 2009; Alamer and Nasiruzzaman, 2024), some research has alternatively modeled them as traits of the cow (Axford et al., 2024). This perspective allows for a more comprehensive understanding of calving performance, as it reflects the biological reality that maternal factors such as pelvic size, uterine environment, gestation length, and overall health status strongly influence calving outcomes and neonatal survival (Sakhaeifar & König., 2025).

Random regression models (**RRM**) represent a major advance by modeling genetic (co)variances across continuous trajectories, such as days in milk or parities (Schaeffer, 2004; Yin and König, 2018) or feed efficiency (Khanal et al., 2022). RRMs are especially useful for longitudinal traits and can account for dynamic changes in genetic expression over time (Averill et al., 2006). RRMs offer greater flexibility in modeling suitable covariance functions while accounting for the biological characteristics of the trait. (Kirkpatrick et al., 1990; Veerkamp et al., 1999). However Schaeffer (2004) introduced this method for low-frequency traits, such as NRR56 in CTFS, where each interval corresponds to a lactation number.

Another major advantage compared to MTMs is that RRMs allow genetic predictions and parameter estimation at any point along continuous time or environmental scales, a feature that proves valuable for dense longitudinal data and even remains effective with relatively sparse records (Yin et al., 2014b). They have been applied to fertility traits to explore fluctuations in heritability across lactations and to better estimate genetic parameters. Enhanced accuracy of breeding values has been reported when RRMs are used compared with simpler models (Oliveira et al., 2020).

For calving traits, both linear and threshold models have been used. Threshold models are particularly appropriate for categorical traits such as CE and SB, as they assume an underlying continuous liability scale (López de Maturana et al., 2007). Covariance functions and multivariate models have also been introduced to account for correlations among traits and across parities (Kirkpatrick et al., 1994; Vanderick et al., 2014). Typically, trait observations are attributed to the calf while accounting for both the calf's direct genetic effect and the dam's maternal genetic effect (Wiggans et al., 2003). These methodological developments provide more realistic evaluations and enhance selection accuracy. Moreover, for calving traits using maternal models are necessary as the maternal genetic could be effective in this regard.

In summary, while animal models, repeatability models, and MTM have provided valuable insights into reproductive traits, their assumption of constant genetic effects across time or parity limits their ability to capture biological dynamics (Paneru et al., 2024). RRM represents a major step forward by enabling the modeling of trajectories across parities or lactation stages. More recently, these approaches have been extended into the genomic era by combining dense SNP data with functional modeling, thereby allowing the study of dynamic SNP effects across time. This transition from static to longitudinal and functional models reflects a paradigm shift in cattle breeding, where time-dependent patterns of genetic expression can now be integrated into selection programs.

1.5 The genomic era: GWAS and longitudinal GWAS

1.5.1 Single step GWAS

The advent of dense single nucleotide polymorphism (**SNP**) marker panels has revolutionized genetic analysis of complex traits. Genome-wide association studies (**GWAS**) have become a cornerstone of modern quantitative genetics, offering a robust framework to detect associations between SNPs and traits of interest without requiring prior biological assumptions. This hypothesis-free approach has been particularly valuable in elucidating the genetic basis of complex and polygenic traits that are influenced by numerous loci, each contributing a small fraction of the total phenotypic variance. In livestock species, including dairy cattle, GWAS have been instrumental in advancing our understanding of traits related to production, health, reproduction, and adaptation.

In the context of fertility, which is a multifactorial and economically crucial trait, GWAS have been extensively applied to identify genomic loci influencing reproductive performance. Studies such as those by Ma et al. (2019); Galliou et al. (2020); Wolf et al. (2023) have detected multiple genomic regions and candidate genes associated with key fertility measures, including fertilization success, conception rate, pregnancy rate, and non-return rate. These studies have contributed to the growing evidence supporting the infinitesimal model of inheritance, where a large number of loci each exert small additive effects on the overall phenotype. Such findings align with the theoretical foundations of quantitative genetics that underscore the complexity of genetic architecture underlying fertility.

However, despite their success, traditional GWAS approaches have several limitations when applied to dynamic or time-dependent traits. Classical GWAS models typically assume that the effect of each SNP is constant across time and across varying physiological conditions (Das et al., 2011). This assumption, while simplifying the analytical process, may not reflect biological reality for many traits, particularly those that are influenced by changing physiological states,

environmental exposures, or developmental stages. Fertility in dairy cattle is a prime example of such a dynamic trait, as reproductive performance is intricately linked to metabolic status, lactation stage, and age.

During the postpartum period, for example, dairy cows experience dramatic physiological changes as they transition from gestation to lactation. The energy demands associated with milk production led to negative energy balance, metabolic stress, and hormonal fluctuations, all of which can impact reproductive function. Consequently, the genetic factors that influence fertility may exert different effects depending on the stage of lactation or the parity number. SNPs that are relevant to early postpartum fertility may have little or no effect later in lactation, and vice versa. Traditional GWAS approaches that ignore these temporal patterns may fail to detect such stage-specific genetic effects, thereby underestimating the dynamic nature of genetic regulation.

1.5.2 Longitudinal GWAS

Recognizing this limitation, researchers have developed longitudinal GWAS methodologies that incorporate time-dependent models to better capture the dynamic interplay between genetic effects and physiological processes. Longitudinal GWAS explicitly account for SNP × time interactions, allowing the estimation of how genetic effects evolve across time or across repeated measurements of a trait (Sikorska et al., 2015). By integrating temporal information, these models extend beyond the static framework of traditional GWAS and offer a more nuanced understanding of the genetic architecture of complex traits.

One of the principal advantages of longitudinal GWAS is the ability to identify loci that have stage-specific effects. For instance, certain genomic regions may influence fertility only during the first lactation, when the animal is undergoing its first major reproductive cycle, while others may act consistently across multiple lactations (Ning et al., 2018). This approach also enhances the power to detect genetic associations by leveraging repeated measures within individuals,

thereby reducing environmental noise and increasing statistical precision. Moreover, longitudinal models can disentangle persistent genetic effects from those that are transient or context-dependent, providing a clearer picture of how genes interact with physiological changes over time.

Methodologically, longitudinal GWAS can be implemented using RRM that incorporates time as either a continuous or categorical variable. RRMs, in particular, have been widely applied in animal breeding studies to model genetic effects across time or age, capturing the covariance structure among repeated observations. When combined with dense genomic information, these models can estimate SNP-specific trajectories, revealing how allelic effects vary over time. Such approaches not only enhance our understanding of the biological processes governing fertility but also have practical implications for breeding programs.

In summary, the transition from traditional to longitudinal GWAS marks a significant advancement in the study of complex, dynamic traits. While conventional GWAS has provided invaluable insights into the static associations between genetic markers and phenotypes, longitudinal approaches extend this framework to capture the fluid nature of biological processes. In the context of fertility in dairy cattle, this shift enables researchers to uncover loci that are active at specific physiological stages, thereby improving our understanding of reproductive biology and enhancing the efficiency of genetic selection programs. As genotyping technologies continue to advance and phenotypic recording becomes increasingly automated and precise, longitudinal GWAS will play an even more central role in decoding the temporal dimension of genetic architecture in livestock and beyond.

1.6 Objectives of this study

According to this background, the present thesis integrates statistical and genomic methodologies to improve the understanding and evaluation of reproductive traits in German Holstein cattle. Specifically, it focuses on fertility and calving traits, which are both

economically and biologically important but remain challenging to evaluate due to their complexity.

Chapter 2 evaluates fertility traits using classical and advanced statistical models, including animal, repeatability, and random regression models. The aim was to investigate how different modeling approaches influence the estimation of genetic parameters, estimated breeding value (**EBV**) to explore dynamic changes of different three traits including NRR56, CTFS, and DO across six lactations.

In **chapter 3**, the second study applies a longitudinal GWAS framework to fertility traits. By considering SNP × time interactions, the study provides insights into dynamic genetic mechanisms and identifies candidate genes relevant at different stages of reproduction. These results are further contextualized through gene annotation and functional analyses, including Gene Ontology (GO) terms and KEGG pathways, to highlight biological processes and molecular mechanisms underlying fertility as a polygenic trait.

In **chapter 4** the study extends the scope to calving traits, focusing on calving ease (CE) and Stillbirth (SB). Using different statistical methods including animal, maternal, and random regression models to estimate genetic parameters and estimated breeding value (EBV) across the first three parities, reflecting the biological and practical importance of early calving records. The analysis provides a comprehensive evaluation of genetic parameters and correlations. The findings contribute to improved understanding of the genetic architecture of calving traits in German Holsteins, offering valuable guidance for breeding strategies that aim to reduce dystocia and SB rates.

Generally, these studies advance knowledge of the dynamic genetic basis of fertility and calving traits, highlight the strengths and limitations of different methodological approaches, and support the development of more precise genetic evaluations. The results contribute to

sustainable dairy breeding programs that balance productivity, reproductive performance, animal welfare, and long-term industry viability.

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CHAPTER 2

APPLICATION OF GENOMIC RANDOM REGRESSION MODELS FOR GENETIC PARAMETER ESTIMATIONS OF FEMALE FERTILITY TRAITS IN DIFFERENT PARITIES IN GERMAN HOLSTEINS

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Application of Genomic Random Regression Models for Genetic Parameter Estimations of Female Fertility Traits in Different Parities in German Holsteins

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Keywords: female fertility | genetic parameters | genomic random regressions

ABSTRACT

The aim of the present study was to infer genetic (co) variance components and to estimate parity-specific breeding values for the female fertility traits non-return rate after 56 days, the interval from calving to first service and days open by applying random regression models on a time-dependent parity scale. In this regard, we considered a female fertility dataset comprising 592,829 records on 190,269 German Holstein cows and heifers kept in 45 large-scale dairy contract herds. From a subset of 21,316 cattle with phenotypic records, (imputed) 50 K genotypes were available. The applied genomic random regression model considered Legendre polynomials of order 2 for the additive-genetic effects along the parity scale, and combined pedigree and genomic relationships through the **H**-matrix. Results were compared with genetic parameter estimates from a multiple-trait model, considering the same fertility trait in different parities as different traits. From both modelling approaches, we observed the trend of increasing genetic variances and heritabilities with increasing parity. Especially for the non-return rate, the genetic variance in heifers was substantially smaller than in all parities of cows. With regard to the random regression model, genetic correlations between the same fertility traits from adjacent parities were close to 1, but gradually declined with increasing parity distances. Small genetic correlations were also estimated between non-return rates in heifers with non-return rates in all cow parities, i.e., 0.50 with parity 1, 0.44 with parity 2, 0.41 with parity 3, 0.35 with parity 4, 0.33 with parity 5, and 0.25 with parity 6. A similar pattern for genetic correlations in the same traits across parities was confirmed from the multiple-trait model application. Estimated breeding values for all fertility traits in different parities of sires with at least 10 phenotyped daughters per trait (estimates from the random regression model) were correlated with their official breeding indexes from the national genetic evaluation. In this regard, moderate differences were observed when comparing breeding value correlations for non-return rates in heifers with respective correlations in all cow parities. From a practical breeding perspective, the most important results were the rather small genetic correlations for the same traits in different parities (e.g., 0.24 between calving to first service in parities 1 and 6), and differing breeding value correlations with other breeding indexes in different parities. These findings suggest the implementation of specific genetic evaluations for specific cow parities, as an extension to the existing separation between heifer and cow fertility traits. Parity-specific breeding value correlations from the random regression and the multiple-trait model considering the sires with at least 10 daughters were larger than 0.85, suggesting only minor re-rankings of sires from the two different modeling approaches.

1 | Introduction

As outlined by, e.g., Gernand and König (2017), female fertility in dairy cattle can be defined as the ability to show heat or maturity, to conceive, and to recycle. In consequence, female fertility is a very complex trait, and accordingly, new breeding indexes have been developed to cover both major fertility components, i.e., the start of a new cycle after calving and the success of an insemination (e.g., Gonzalez-Recio and Alenda 2005; Jorjani 2006). Furthermore, in most countries, multiple-trait models (MTM) have been developed for female fertility genetic evaluations, aiming at a separation of heifer and cow fertility traits (e.g., Muuttoranta et al. 2019). Nevertheless, in spite of all these efforts, the fertility status of Holstein Friesian cows gradually declined or stagnated in many countries during the past decades, even in the genomics era (Veerkamp et al. 2015). Efficient breeding approaches for improved female fertility are hampered due to the strong environmental component. In consequence, also the application of the so-called 'genomic herd management', i.e., utilisation of early genomic breeding values to predict later phenotypes, yielded limited success for low heritability conception rates with a correlation coefficient between breeding values and phenotypes of −0.05 (Strabel 2024).

In addition to the strong environmental effect, the complexity of female fertility genetic mechanisms (Gajbive et al. 2018) might be a major challenge to develop accurate genomic predictions. The complexity of physiological mechanisms implies the activity of different genes in reproductive processes with progressive time and in different environments, as outlined by Beerda et al. (2008). Alterations of genetic mechanisms with progressing time or environmental alterations indicate that different genes are 'switched on or off' in the course of lactation as well as across lactations (König and May 2018). From a quantitative-genetic perspective, random regression models (RRM) have the greatest potential for depicting such underlying genomic particularities. For test-day traits, RRM are implemented in national genetic evaluations for more than two decades for the estimation of breeding values and genetic parameters by days in milk (e.g., Swalve 2000). RRM have the potential for a better correction of environmental effects than multiple-trait (MTM) or repeatability models in case of dense longitudinal observations (Swalve 1995). Khanal et al. (2022) outlined the advantages of RRM for feed efficiency traits, due to the pronounced environmental alterations within short periods and respective effects on variance component estimates. The superiority of RRM over, example, MTM, is unclear for traits with repeated measurements at greater distances. In this regard, as an extension for most of the female fertility traits with only one single observation per lactation or for survival analysis, Schaeffer (2004) suggested modeling random regressions by parity. An RRM approach considering genetic parameter alterations in different parities was presented by Veerkamp et al. (1999) for cow longevity. Accordingly, Oliveira et al. (2020) outlined the advantages of RRM in case of a censored data structure and a substantial trait record reduction with increasing aging. In consequence, they applied RRM for genetic analyses of survival traits in beef cattle. In analogy, also for female fertility traits observed at great distances, effects of selection and cullings imply a quite large number of records in heifers and in first parity cows, but an obvious data decline in later lactations.

The above-mentioned RRM applications for survival or longevity during the animals' lifespan considered pedigree relationship matrices. For within-animal observations being far apart, strong fluctuations of genetic covariances in the same traits along the time trajectory are expected, due to evolutionary effects including selection, migration and drift (Do and Whitlock 2023). RRM has greater flexibility in modeling appropriate covariance functions, considering the particularities of the trait biology (Kirkpatrick et al. 1990). Additional value in the genomic era in this regard might be due to the availability of dense SNP markers, capturing genetic covariances more accurately than pedigree-based approaches (Beaulieu et al. 2014).

Consequently, the aim of the present study was to utilise comprehensive phenotype datasets from a large number of genotyped Holstein cows kept in large-scale German contract herds to evaluate genomic RRM for the female fertility traits non-return rate after 56 days (NRR56), interval from calving to first service (CTFS) and days open (DO). Variance components, heritabilities and genetic correlations in the same traits from different parities were compared with respective results from MTM. The estimated breeding values for NRR56, CTFS and DO in different parities from sires with daughter records were correlated with their official breeding values for all indexes included in the German overall net merit index.

2 | Materials and Methods

2.1 | Cow Traits, Pedigree and Genotypes

The female fertility dataset comprised 592,829 records of 190,269 Holstein cows and heifers kept in 45 large-scale dairy contract herds from the German federal states of Hesse, Mecklenburg-West Pomerania and Berlin-Brandenburg. These herds represent the original nucleus used for implementing genomic selection in Germany based on cow training sets (e.g., Klein et al. 2021). Female fertility traits for CTFS, DO and NNR56 were from the years 2010 to 2022. The trait data structure in parities 0 (heifer records for NNR56) to 6 is given in Table 1.

The pedigree of the 190,269 female cattle with phenotypes considered at least three previous generations (sire and dam, respective grandparents and respective great-grandparents). Oldest ancestors were born in 1920. The 190,269 cattle with phenotypes had 5787 different sires, 7116 different maternal grand-sires and 2850 different paternal grand-sires.

In a subset, 21,316 cattle with phenotypic records were genotyped. Of these, 5403 animals were genotyped with the *Illumina Bovine SNP50 v2 Bead Chip*, and 15,913 animals were genotyped with the *Illumina Bovine Eurogenomics 10 K low-density chip*. Among the pool of sires and grand-sires, a further 948 males were genotyped with the *Illumina Bovine SNP50 v2 Bead Chip*. Cattle with low-density 10 K genotypes were imputed to the 50 k panel by the project partner vit Verden using their algorithm as applied for routine national genetic evaluations (Segelke et al. 2012). Genotype quality control was carried out using the software package PLINK (Purcell et al. 2007). The applied filters encompassed the following criteria: a minor allele frequency of 0.05 (exclusion of 3.677 SNP), a minimum call rate of 0.9 (exclusion

TABLE 1 | Number of observations and descriptive statistics for the female fertility traits non-return rate after 56 days (NRR56), interval from calving to first service (CTFS) and days open (DO) by parity (parity 0 = heifers).

Trait	Parity	No. of observations	Mean	SD
NRR56	0	166,736	0.71	0.45
CTFS	0	0	—	—
DO	0	0	—	—
NRR56	1	154,352	0.53	0.49
CTFS	1	158,132	77.09	27.40
DO	1	128,808	117.43	58.75
NRR56	2	114,446	0.49	0.50
CTFS	2	117,442	77.57	26.86
DO	2	89,513	123.00	60.15
NRR56	3	146,790	0.47	0.49
CTFS	3	150,519	79.97	27.15
DO	3	102,042	127.13	60.21
NRR56	4	41,938	0.46	0.49
CTFS	4	43,076	80.45	27.33
DO	4	28,808	128.67	60.73
NRR56	5	20,958	0.47	0.49
CTFS	5	21,512	81.23	27.28
DO	5	13,346	128.94	59.76
NRR56	6	9127	0.46	0.49
CTFS	6	9380	81.22	26.93
DO	6	5503	128.76	59.37

of 866 SNP) and significant deviation (p-value $<1\times10^{-6}$) from Hardy–Weinberg equilibrium (exclusion of 4 SNP). Genomic relationships among all genotyped animals were smaller than 0.95. For one genotyped heifer, we identified Mendelian conflicts, i.e., inconsistencies when comparing the individual genotype with the parental genotypes. The genotype for this animal was ignored. Finally, after filtering, 41,129 SNP from 22,633 genotyped animals were available for the ongoing genomic studies.

2.2 | Statistical Models

The applied MTM simultaneously considered the same trait from different parities as different traits. Hence, the MTM for NRR56 included 7 traits (heifers and 6 cow parities), and for CTFS and DO 6 traits (the 6 cow parities).

The general statistical model defined in matrix notation for the multiple-trait analyses was:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + (\mathbf{SSs}) + \mathbf{e}$$

where **y** was the vector for the same fertility trait in different lactations; **b** was the vector for fixed effects including the combined effect of herd-year-season of insemination and age of cow at insemination (in months), **u** was the vector for additive-genetic effects with $\mathbf{u} \sim N(\mathbf{0}, \mathbf{H}\,\sigma^2_a)$, and σ^2_a denoting the additive genetic variance and **H** denoting the combined (pedigree and genomics) relationship matrix constructed according to Legarra et al. (2009); **s** was the vector for random service sire effects on DO and NRR56 with $\mathbf{s} \sim N(\mathbf{0}, \mathbf{I}\,\sigma^2_s)$, and σ^2_s denoting the service sire variance and **I** denoting an identity matrix for the 6580 service sires, and **e** was the vector for the residual effects with $\mathbf{e} \sim N(\mathbf{0}, \mathbf{I}\,\sigma^2_e)$, and σ^2_e denoting the residual variance and **I** denoting an identity matrix for the cows and heifers with observations. **X**, **Z** and **SS** were incidence matrices for **b**, **u** and **s**, respectively.

The variance–covariance structure for random effects between the same fertility traits in different parities i and j with random service sire effects (NRR56, DO; CTFS without random service sire effects) was:

$$var\begin{bmatrix} u_i \\ u_j \\ s_i \\ s_j \\ e_i \\ e_j \end{bmatrix} = \begin{bmatrix} g_{ii}\mathbf{H} & g_{ij}\mathbf{H} & 0 & 0 & 0 & 0 \\ g_{ji}\mathbf{H} & g_{jj}\mathbf{H} & 0 & 0 & 0 & 0 \\ 0 & 0 & \sigma^2_{s_i}\mathbf{I}_{s_i} & 0 & 0 & 0 \\ 0 & 0 & 0 & \sigma^2_{s_j}\mathbf{I}_{s_j} & 0 & 0 \\ 0 & 0 & 0 & 0 & r_{ii} & 0 \\ 0 & 0 & 0 & 0 & 0 & r_{jj} \end{bmatrix}$$

where $\mathbf{g_{ii}}$, and $\mathbf{g_{ij}}$ were the additive-genetic effects for the same trait in different parities i and j; $\mathbf{g_{ij}}$ and $\mathbf{g_{ji}}$ were additive genetic covariances between the same trait in different parities i and j; **H** was the combined (pedigree and genomics) relationship matrix as outlined above; $\sigma^2_{s_i}$ and $\sigma^2_{s_j}$ were the variances for the service sire effects on traits i and j, respectively, with the respective identity matrices $\mathbf{I}_{s_i}$ and $\mathbf{I}_{s_j}$; r_{ii} and r_{jj} were residual variances for the traits i and j, respectively.

The RRM in matrix notation was defined as follows:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{Wp} + (\mathbf{SSs}) + \mathbf{e}$$

where **y** was a vector for the same fertility trait in different lactations; **b** was a vector for fixed effects including herd-year-season of insemination and age of cow at insemination (in months), and fixed regressions on lactation number modelled with Legendre polynomials of order 2 representing the 3 coefficients intercept, first Legendre polynomial, second Legendre polynomial considering a standardisation between the minimal (parity 0 for heifers) and maximal parity 6; **u** was a vector for random regression coefficients for additive-genetic effects modelled with Legendre polynomials of order 2 representing the 3 coefficients intercept, first Legendre polynomial, second Legendre polynomial considering a standardisation between the minimal (parity 0 for heifers) and maximal parity 6; **p** was a vector for random permanent environmental effects of the cow, **s** was a vector for random service sire effects for NRR56 DO; and **e** was a vector for random residual effects allowing heterogeneous residual variances in different parities. **X**, **Z**, **W**, and **SS** were incidence matrices for **b**, **u**, and **s**, respectively. Random effects were assumed to follow a normal distribution with zero means. The variance–covariance structure for random effects (CTFS without random service sire effects) was:

$$var\begin{bmatrix} u \\ p \\ s \\ e \end{bmatrix} = \begin{bmatrix} \mathbf{G}\otimes\mathbf{H} & 0 & 0 & 0 \\ 0 & \sigma_p^2\mathbf{I_p} & 0 & 0 \\ 0 & 0 & \sigma_s^2\mathbf{I_s} & 0 \\ 0 & 0 & 0 & \sigma_e^2\mathbf{I_n} \end{bmatrix}$$

where **G** was a 3×3 (co)variance matrix of random regression coefficients (intercept and the 2 coefficients for the Legendre polynomials) for the additive genetic effect; **H** was the combined relationship matrix as explained above; σ_p^2, σ_s^2 and σ_e^2 were variances for the permanent environmental, the service sire and the residual effect, respectively; $\mathbf{I_p}$, $\mathbf{I_s}$ and $\mathbf{I_n}$ were identity matrices for *p* cows, *s* service sires and *n* observations, respectively; and ⊗ denotes the Kronecker product.

For the estimation of genetic parameters and breeding values, GBLUP methodology as implemented in the BLUPF90 software packages (Aguilar et al. 2014), was applied.

With regard to model comparisons (i.e., RRM versus MTM), we correlated the respective parity-specific estimated breeding values (EBV) considering the sires with at least 10 daughters (1875 sires for NRR56, 1849 sires for CTFS and 1801 sires for DO). From both modelling approaches, we created multiparity indexes for all three traits by combining the parity-specific EBV with equal weights. Also the sire multiparity indexes were considered in the sire correlation analyses.

2.3 | Correlations With Breeding Indexes From Official National Genetic Evaluations

Parity specific EBV (results from the RRM) for NRR56, CTFS and DO were standardised to relative breeding values (R_NRR56, R_CTFS and R_DO, respectively) using the mean and SD of the original breeding values from animals born in the year 2020. Relative breeding values for all three traits larger than 100 are favourable from a breeding perspective, i.e., increased non-return rates, shorter days from calving to first service, and a shorter period for days open. The R_NRR56 of the 1875 sires, the R_CTFS of the 1849 sires and the R_DO of the 1801 sires with at least 10 daughters were correlated with their respective breeding values of the indexes included in the German total net merit index (RZG) from the official national genetic evaluation from 04/2022. The indexes (also standardised to a mean of 100 and a SD of 12 points) included: production (RZM), health (RZhealth), longevity (RZN), conformation (RZE), daughter fertility (RZR), calf fitness (RZcalfhealth) and calving traits for the paternal (RZKp) and for the maternal component (RZKm).

3 | Results

3.1 | Heritabilities and Variance Components for the Same Female Fertility Traits in Different Parities

Genetic variances by parity with respective SE from the RRM application are displayed in Figure 1 for NRR56, in Figure 2 for CTFS and in Figure 3 for DO. In this regard, there was a general trend of increasing genetic variances with increasing lactation number, i.e., largest estimates in parities 5 and 6. Only for DO, the genetic variance was slightly larger in parity 1 than in the ongoing parities 2 and 3. Consequently, for NRR56 with additional consideration of heifer data, the genetic variance in heifers was substantially smaller than in all other parities of cows.

Increasing genetic female fertility variances with increasing lactation number explained the respective increases of heritabilities. The heritabilities for NRR56, CTFS and DO from the RRM are shown in Figure 4. Nevertheless, the heritabilities were quite small and in a narrow range from 0.01 (NRR56 in heifers) up to 0.08 (CTFS in parity 6). Smallest fluctuations in heritabilities by parity were observed for DO (0.027 in parity 1 up to 0.043 in parity 6).

The MTM estimates confirmed the general pattern for variance component estimates and heritabilities from the RRM, i.e., the trend of increasing genetic variances and heritabilities for NRR56 (Table 2), for CTFS (Table 3) and for DO (Table 4) with increasing lactation number. We also observed increasing service sire and residual variances for all 3 female fertility traits with increasing parity, but due to the stronger fluctuations of the additive-genetic component, heritabilities were

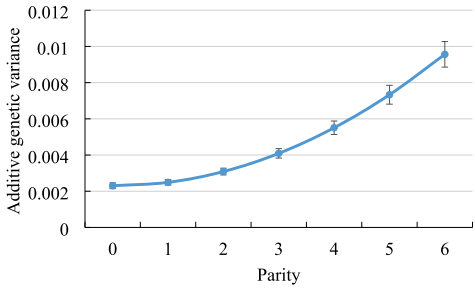

FIGURE 1 | Additive-genetic variances with respective SE for non-return rate after 56 days (NRR56) in different parities (estimates from the random regression model). SE ranged from 0.00015 (parity 1) to 0.00071 (parity 6). [Colour figure can be viewed at wileyonlinelibrary.com]

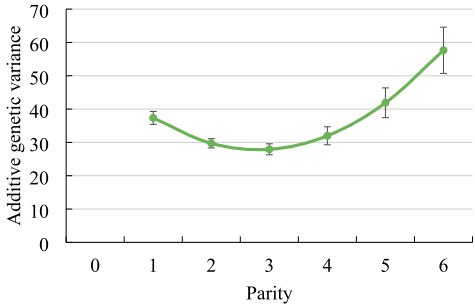

FIGURE 2 | Additive-genetic variances with respective SE for the interval from calving to first service (CTFS) in different parities (estimates from the random regression model). SE ranged from 1.38 (parity 2) to 6.93 (parity 6). [Colour figure can be viewed at wileyonlinelibrary.com]

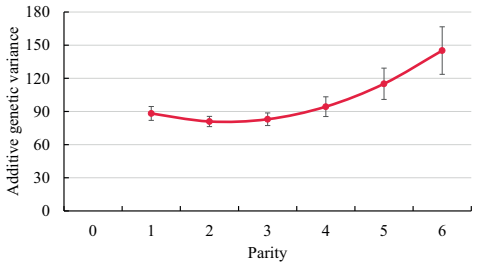

FIGURE 3 | Additive-genetic variances with respective SE for days open (DO) in different parities (estimates from the random regression model). SE ranged from 4.63 (parity 2) to 14.15 (parity 6). [Colour figure can be viewed at wileyonlinelibrary.com]

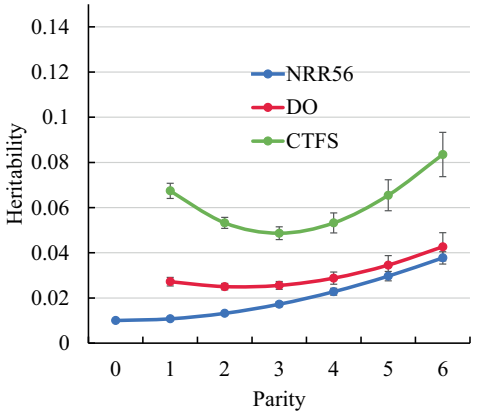

FIGURE 4 | Heritabilities with respective SE for non-return rate after 56 days (NRR56) (SE ranged from 0.001 to 0.003), for the interval from calving to first service (CTFS) (SE ranged from 0.002 to 0.006) and for days open (DO) (SE ranged from 0.001 to 0.006) in different parities (estimates from the random regression model). [Colour figure can be viewed at wileyonlinelibrary.com]

largest in late lactations. Nevertheless, the heritabilities were quite small and only differed marginally in different parities, i.e., in the range from 0.02 to 0.04 for NRR56, in the range from 0.04 to 0.06 for CTFS and in the range from 0.02 to 0.03 for DO.

3.2 | Genetic Correlations Between the Same Fertility Traits From Different Parities

The RRM application enabled the estimation of genetic correlations between the same fertility traits from different parities, e.g., the genetic correlation between NRR56 in heifers with NRR56 in parity 1, between NRR56 in parity 1 with NRR56 in parity 2, etc. From the broad grid of genetic correlation combinations, we depicted the genetic correlations between the lowest cow parity number (parity 1) and all other parities for the same trait (see Figure 5). In this regard, the genetic correlations between the same traits from adjacent parities were close to 1 (0.89 between parity 1 NRR56 and parity 2 NRR56, 0.96 between parity 1 CTFS and parity 2 CTFS, and 0.97 between parity 1 DO and parity 2 DO), but gradually declined with increasing parity distances. Consequently, the smallest genetic correlations were estimated between the same trait from the earliest cow parity 1 and the latest parity 6. In this regard, the smallest genetic correlations were 0.48 (between parity 1 NRR56 and parity 6 NRR56), 0.24 (between parity 1 CTFS and parity 6 CTFS) and 0.52 (between parity 1 DO and parity 6 DO). Small genetic correlations were also identified between NRR56 in heifers and NRR56 in all cow parities, i.e., 0.40 with parity 1, 0.34 with parity 2, 0.31 with parity 3, 0.25 with parity 4, 0.23 with parity 5, and 0.20 with parity 6. The general genetic correlation pattern, i.e., quite large estimates for neighbouring parities, but a substantial decline for parities at greater distances, was also observed for other parity combinations and the same fertility traits.

The genetic correlations between same traits in different parities from the MTM are given in Table 5 for NRR56, in Table 6 for CTFS, and in Table 7 for DO. The genetic correlations generally support the estimate pattern from the RRM, i.e., a decline with increasing parity distances (apart from a few exceptions). However, the genetic correlations differences were smaller

TABLE 2 | Variance components (service sire, additive-genetic, residual) and heritabilities with corresponding SE for the non-return rate after 56 days (NRR56) in different parities from the multiple-trait model application.

Parity	Service sire	SE	Additive-genetic	SE	Residual	SE	Heritability	SE
Heifers	0.001	0.001	0.005	0.001	0.210	0.004	0.023	0.001
1	0.002	0.001	0.007	0.001	0.230	0.003	0.028	0.001
2	0.001	0.001	0.007	0.001	0.236	0.005	0.030	0.001
3	0.002	0.001	0.008	0.001	0.234	0.005	0.033	0.001
4	0.001	0.000	0.009	0.002	0.234	0.005	0.037	0.001
5	0.003	0.001	0.010	0.001	0.234	0.005	0.039	0.001
6	0.006	0.002	0.010	0.001	0.234	0.005	0.041	0.001

TABLE 3 | Variance components (service sire, additive-genetic, residual) and heritabilities with corresponding SE for the interval from calving to first service (CTFS) in different parities from the multiple-trait model application.

Parity	Additive-genetic	SE	Residual	SE	Heritability	SE
1	18.57	5.87	390.74	9.25	0.045	0.015
2	14.54	2.66	367.79	4.98	0.038	0.013
3	18.93	2.61	407.69	5.64	0.044	0.014
4	22.24	4.18	410.27	5.47	0.051	0.017
5	26.89	3.57	420.64	9.43	0.060	0.018
6	27.36	5.01	422.28	11.03	0.061	0.018

TABLE 4 | Variance components (service sire, additive-genetic, residual) and heritabilities with corresponding SE for days open (DO) in different parities from the multiple-trait model application.

Parity	Service sire	SE	Additive-genetic	SE	Residual	SE	Heritability	SE
1	9.30	6.35	52.31	11.05	2310.8	45.17	0.022	0.016
2	16.39	8.63	55.85	9.95	2548.5	31.17	0.021	0.015
3	22.50	8.93	57.26	10.16	2598.1	39.58	0.021	0.016
4	19.27	7.60	62.09	6.99	2642.4	56.10	0.023	0.018
5	29.31	13.47	70.14	6.97	2744.2	56.68	0.025	0.018
6	30.60	21.43	92.37	20.71	2861.5	87.07	0.031	0.017

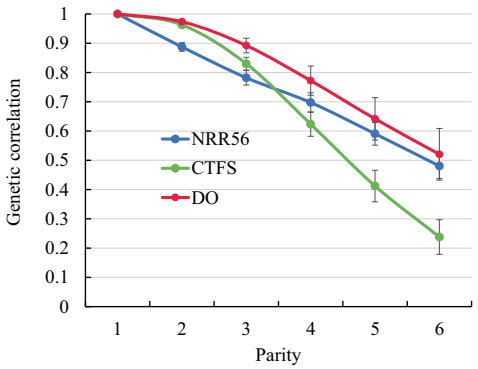

FIGURE 5 | Genetic correlations (estimates from the random regression model) with respective SE between non-return rate after 56 days (NRR56) in parity 1 with NRR56 in all other parities (SE ranged from 0.01 to 0.04), between the interval from calving to first service (CTFS) in parity 1 with CTFS in all other parities (SE ranged from 0.01 to 0.05), and between days open (DO) in parity 1 with DO in all other parities (SE ranged from 0.01 to 0.07). [Colour figure can be viewed at wileyonlinelibrary.com]

compared to the respective estimates from the RRM. The genetic correlations between NRR56 in heifers and NRR56 in all cow parities were in a narrow range from 0.26 (heifers and parity 3 cows) to 0.42 (heifers and parity 1 cows) (Table 5). Regarding NRR56, the genetic correlations among different cow parities were generally larger than 0.57, with a maximal value of 0.79 (parity 1 with parity 2).

Regarding the female fertility interval traits, the genetic correlations between the same trait in different parities were in a narrow range from 0.57 (parity 1 with parity 6) to 0.78 (parity 2 with parity 3) for CTFS (Table 6), and from 0.51 (parity 4 with parity 5) to 0.75 (parity 5 with parity 6) for DO (Table 7).

3.3 | Correlations Between Breeding Values and Indexes From the Multiple Trait and the Random Regression Model

Table 8 displays the correlations between EBV for the same traits and parities from the RRM and MTM, considering the sires with at least 10 daughters. The agreement in parity-specific EBV from both modelling approaches was quite large for the traits from the cow parities with correlation coefficients in the range from 0.93 (parity 1) to 0.87 (parity 6) for NRR56, in the range from 0.96 (parity 1) to 0.91 (parity 6) for CTFS, and in the range from 0.94 (parity 1) to 0.90 (parity 6) for DO. The smallest EBV correlation was found for NRR56 in heifers with a coefficient of 0.85. Accordingly, the correlations between the multiparity indexes for all three traits were large with 0.91 for NRR56 and with 0.92 for CTFS and DO (Table 8).

3.4 | Correlations Between Breeding Values for Female Fertility Traits From Different Parities With Breeding Indexes

Sire breeding value correlations between R_NRR56 with the official breeding indexes (Table 9), R_CTFS with the official breeding indexes (Table 10) and R_DO with the official

TABLE 5 | Genetic correlations with respective SE (below in brackets) between non-return rate after 56 days (NRR56) from different parities from the multiple-trait model application.

	Parity						
Parity	**Heifers**	**1**	**2**	**3**	**4**	**5**	**6**
Heifers		0.42 (0.056)	0.33 (0.057)	0.26 (0.066)	0.29 (0.052)	0.33 (0.044)	0.32 (0.047)
1			0.77 (0.053)	0.70 (0.064)	0.64 (0.062)	0.59 (0.059)	0.57 (0.064)
2				0.78 (0.054)	0.69 (0.063)	0.77 (0.065)	0.70 (0.070)
3					0.74 (0.064)	0.71 (0.067)	0.69 (0.053)
4						0.78 (0.063)	0.64 (0.067)
5							0.66 (0.057)

TABLE 6 | Genetic correlations with respective SE (below in brackets) between the interval from calving to first service (CTFS) from different parities from the multiple-trait model application.

	Parity					
Parity	**1**	**2**	**3**	**4**	**5**	**6**
1		0.77 (0.071)	0.70 (0.064)	0.64 (0.068)	0.59 (0.076)	0.57 (0.062)
2			0.78 (0.075)	0.69 (0.062)	0.77 (0.075)	0.70 (0.068)
3				0.75 (0.072)	0.74 (0.070)	0.69 (0.068)
4					0.78 (0.073)	0.64 (0.058)
5						0.66 (0.069)

TABLE 7 | Genetic correlations with respective SE (in brackets below) between days open (DO) from different parities from the multiple-trait model application.

	Parity					
Parity	**1**	**2**	**3**	**4**	**5**	**6**
1		0.63 (0.080)	0.62 (0.083)	0.64 (0.077)	0.55 (0.076)	0.63 (0.089)
2			0.73 (0.079)	0.71 (0.087)	0.65 (0.085)	0.59 (0.086)
3				0.60 (0.080)	0.61 (0.081)	0.67 (0.089)
4					0.51 (0.074)	0.50 (0.070)
5						0.75 (0.080)

TABLE 8 | Breeding value correlations between the parity-specific breeding values and correlations between multiparity indexes for the non-return rate after 56 days (NRR56), for the interval from calving to first service (CTFS) and for days open from the multiple-trait model (MTM) and the random regression model (RRM) considering the sires with at least 10 daughters.

		Parity						
Trait	**Heifer**	**1**	**2**	**3**	**4**	**5**	**6**	**Multiparity index**
NRR56	0.85	0.93	0.93	0.90	0.89	0.90	0.87	0.91
CTFS		0.96	0.94	0.92	0.93	0.92	0.91	0.92
DO		0.94	0.93	0.91	0.90	0.91	0.89	0.92

breeding indexes (Table 11), were in a narrow range for the same female fertility traits in different cow parities. Largest differences were found for NRR56 when comparing the breeding value correlations in heifers with breeding correlations from the cow parities (Table 9). For example, the correlation between R_NRR56 in heifers with RZG was 0.05, but in the range from 0.18

TABLE 9 | Breeding values correlations between the relative breeding value for non-return rate after 56 days (NRR56) in different parities (estimates from the random regression model) and breeding indexes of 1875 sires with at least 10 daughters.

		Relative breeding values for NNRR56 in different parities					
Index[a]	**Heifer**	**1**	**2**	**3**	**4**	**5**	**6**
RZG	0.05	0.18	0.28	0.25	0.38	0.36	0.46
RZM	−0.08	−0.12	−0.09	−0.15	−0.12	−0.25	−0.22
RZN	0.09	0.31	0.24	0.25	0.38	0.43	0.46
RZE	−0.01	−0.11	−0.08	−0.14	−0.16	−0.22	−0.17
RZR	0.35	0.59	0.62	0.65	0.61	0.75	0.70
RZHealth	0.10	0.23	0.20	0.31	0.33	0.37	0.39
RZcalfhealth	−0.01	0.06	0.03	0.10	0.11	0.13	0.15
RZKp	0.01	0.12	0.17	0.13	0.19	0.24	0.22
RZKm	0.04	0.16	0.15	0.23	0.24	0.26	0.24

[a]RZcalfhealth = calf fitness index, RZE = conformation index, RZG = overall net merit index, RZhealth = health index, RZKm = index for calving ease maternal, RZKp = index for calving ease paternal, RZM = production index, RZN = longevity index, RZR = daughter fertility index.

TABLE 10 | Breeding values correlations between the relative breeding value for the interval from calving to first service (CTFS) in different parities (estimates from the random regression model) and breeding indexes of 1849 sires with at least 10 daughters.

	Relative breeding values for CTFS in different parities					
Index[a]	**1**	**2**	**3**	**4**	**5**	**6**
RZG	0.16	0.11	0.11	0.09	0.06	0.07
RZM	−0.40	−0.34	−0.30	−0.31	−0.26	−0.22
RZN	0.28	0.33	0.37	0.40	0.41	0.47
RZE	0.00	−0.03	−0.05	−0.07	−0.04	−0.03
RZR	0.45	0.46	0.49	0.48	0.52	0.59
RZHealth	0.25	0.23	0.38	0.34	0.40	0.47
RZcalfhealth	0.03	−0.01	−0.05	0.00	−0.02	0.00
RZKp	0.07	0.04	0.08	0.03	0.01	0.03
RZKm	0.11	0.10	0.05	0.07	0.03	0.01

[a]RZcalfhealth = calf fitness index, RZE = conformation index, RZG = overall net merit index, RZhealth = health index, RZKm = index for calving ease maternal, RZKp = index for calving ease paternal, RZM = production index, RZN = longevity index, RZR = daughter fertility index.

to 0.46 between R_NRR56 from the different cow parities with RZG. A general antagonistic relationship was found between RZM with R_NRR56 in heifers (−0.08) and with R_NRR56 in all parities (−0.09 to −0.25). The largest favourable breeding value correlations were identified between R_NRR56 from all parities with RZR in the range from 0.35 (heifers) to 0.75 (parity 3). Moderate and favourable were the breeding value correlations between R_NRR56 with longevity (RZN) and with the overall health index (RZHealth), again with largest differences between associations in heifers and in cow parities.

With regard to R_CTFS breeding value correlations with all other indexes (Table 10), only minor to moderate differences were observed across parities. The antagonistic associations between R_CTFS and RZM declined from parity 1 (−0.40) to parity 6 (−0.22). In analogy with non-return rates, the strongest favourable associations were found between R_CTFS and RZN in the range from 0.25 to 0.47, with RZHealth in the range from 0.23 to 0.47, and with RZR in the range from 0.45 to 0.59. Due to the favourable effect of R_CTFS on other functional traits or indexes in all parities, but the consistently unfavourable associations with RZM, the breeding value correlations with RZG were close to zero.

Breeding value correlations between R_DO with all other indexes (Table 11) reflect the pattern as presented for R_CTFS, displaying only minor to moderate deviations in correlation coefficients for the same indexes with R_DO in different cow parities. Quite strong and favourable were the correlations between R_DO in different parities with RZR in the range from 0.61 to 0.87. In analog with CTFS, moderate and favourable correlations were found between R_DO with RZN in the range from 0.39 (in parity 1) to 0.56 (in parity 3), and between R_DO with RZHealth in the range from 0.26 (in parity 2) to 0.46 (in parity

TABLE 11 | Breeding values correlations between the relative breeding value for days open (DO) in different parities (estimates from the random regression model) and breeding indexes of 1801 sires with at least 10 daughters.

	Relative breeding values DO in different parities					
Index[a]	**1**	**2**	**3**	**4**	**5**	**6**
RZG	0.18	0.23	0.20	0.17	0.17	0.15
RZM	−0.27	−0.22	−0.16	−0.15	−0.15	−0.14
RZN	0.39	0.41	0.56	0.54	0.50	0.50
RZE	−0.16	−0.10	−0.11	−0.09	−0.13	−0.09
RZR	0.61	0.74	0.68	0.77	0.87	0.80
RZHealth	0.32	0.26	0.40	0.38	0.37	0.46
RZcalfhealth	0.07	0.03	0.01	0.03	0.00	0.01
RZKp	0.17	0.20	0.26	0.23	0.28	0.27
RZKm	0.26	0.25	0.20	0.20	0.17	0.18

[a]RZcalfhealth = calf fitness index, RZE = conformation index, RZG = overall net merit index, RZhealth = health index, RZKm = index for calving ease maternal, RZKp = index for calving ease paternal, RZM = production index, RZN = longevity index, RZR = daughter fertility index.

6). Antagonistic in all parities were the breeding value correlations between R_DO with RZM in the range from −0.27 (in parity 1) to −0.14 (in parity 6).

4 | Discussion

4.1 | Heritabilities and Variance Components for the Same Female Fertility Traits in Different Parities

From both modelling approaches (RRM and MTM applications), we found slight increases in additive-genetic variances and heritabilities in all three female fertility traits with increasing parities, with the smallest estimates for NRR56 in heifers. Liu et al. (2008) applied MTM for NRR56 and distinguished between heifers and cows. In their study, the NRR56 heritability was only slightly larger in cows (0.015) than in heifers (0.012). For the cow female fertility interval traits, the heritabilities reported by Liu et al. (2008) reflect the estimates from our present study. Slight deviations in heritabilities from different studies might be due to the statistical modelling approach, e.g., treating the service sire as fixed (Liu et al. 2008) or as a random effect as done in our study, or due to the modelling of genetic relationships (pedigree based versus genomics) (Shabalina et al. 2020). Recently, also Zhu et al. (2023) indicated smaller variance components and heritabilities for female fertility traits in heifers than in cows, but on a generally very low level, e.g., 0.0014 for NRR56 in heifers and 0.002 for NRR56 in cows. Muuttoranta et al. (2019) applied a multiple-trait multiple-lactation model for female fertility traits in Nordic Holstein cows, and reported the smallest heritabilities in heifers and increasing heritabilities with increasing lactation number, also for the female fertility interval traits as considered in our present study. Interestingly, such effects were negligible in Nordic Red Dairy cattle, indicating breed-specific effects on female fertility variance components in different parities. Albeit the only small differences between NRR56 heritabilities observed in heifers and cows, the slight increase in genetic variations with increasing parity seems to be a bit surprising when addressing aspects of natural and artificial selection. Usually, as indicated by König et al. (2005) for claw disorders, diseased cows have a greater risk for disposals before reaching the subsequent parity, narrowing genetic trait variation. The same might be the case for female fertility, being a major reason for involuntary cow cullings (Shabalina et al. 2019). Doublet et al. (2019) outlined the effects of artificial selection on declining genetic diversity measurements including genetic variations. Furthermore, with regard to female fertility variations, natural selection is a major driving component, especially in harsh environments, supporting Darwin's concept of 'survival of the fittest', with effects on reproduction rates with progressing time in specific genetic lines (Paul 1988). However, for studying the effects of selection, genetic variations in heifer fertility over years, or in cow fertility in specific parities over years, might be a better indicator than comparing the same traits in cows and in heifers. Differing genetic parameters in the same trait in heifers and cows might indicate a differing genetic trait architecture and differing gene activities for fertility with aging, as outlined via gene expressions in humans (Zhang et al. 2020).

A main focus was the comparison of MTM estimates with RRM estimates along the parity or aging trajectory. In this regard, Paneru et al. (2024) recommended RRM applications for weight traits in sheep, justified by a more accurate capturing of the genetic (co)variance structure and more accurate genetic evaluations over time than MTM. However, for weight traits in sheep, the repeated measurements were in closer intervals compared to the different parities in the present female fertility study, and the number of repeated weight measurements per sheep (minimum: 4 records per animal) was generally larger than the repeated female fertility structure in cows (maximum: 6 records per animal). Interestingly, also in the sheep study by Paneru et al. (2024) and in analogy with NRR56, CTFS and DO in the present study, additive-genetic variances and heritabilities from the RRM increased with increasing age (apart from the very early beginning). However, increased genetic parameters at the 'extreme ends' of the age scale could be due to limited data to model the covariance function (Meyer 2004). In the present

study, the number of observations and animals was quite large in all parities. Furthermore, in the present study, the genetic parameter pattern by parity as obtained from the RRM is confirmed through the estimates from the MTM. Both applications RRM and MTM enabled the estimation of breeding values of animals without records in distinct parities or age classes. In this regard, with a focus on an environmental climate scale, Bohlouli et al. (2018) indicated more accurate predictions in case of missing records when applying genomic RRM compared to other modelling approaches. Paneru et al. (2024) indicated very large breeding value correlations for weight traits from the RRM and the MTM at the same age classes. Furthermore, Paneru et al. (2024) showed very similar genetic parameter estimates for the same ages based on RRM and MTM applications, supporting the similarities in RRM–MTM comparisons for variance components and heritabilities for the female fertility traits in our study. Nevertheless, the great advantage when applying an RRM compared to an MTM is the ability of genetic predictions and genetic parameter estimations at any point of the continuous time scale or along environmental scales. This is especially the well-known case for dense longitudinal data structures. Such concepts were carefully evaluated by Yin, Pimentel, et al. (2014), displaying the advantages of RRM also for only a few (maximum 5) records per cow for consecutive climatic levels.

4.2 | Genetic Correlations Between the Same Fertility Traits From Different Parities

The decline of genetic correlations in the same fertility traits with increasing parity distance is well-known for other cow traits and data structures, e.g., for monthly test-day production records (e.g., Swalve 1995, 2000) or for cattle weights along the age scale (Yin and König 2017), but also for production and health indicator traits along a climatic trajectory (Bohlouli et al. 2018). The genetic correlations from the RRM are confirmed through the respective estimates from the MTM as additionally applied in the present study, and through previous MTM publications. For example, Liu et al. (2017) indicated the effect of proximity in lactation numbers on the shared genetic mechanisms among traits. However, in contrast, Liu et al. (2017) estimated negative genetic correlations between non-return rates in heifers and in cows. The only moderate genetic correlations between the same female fertility traits from different cow parities (minimal coefficient: 0.24 for CTFS in parity 1 with CTFS in parity 6 from the RRM) suggest indicating specific breeding values for specific parities, which will contribute to more precise mating and selection strategies.

With regard to NRR56 and RRM applications, our estimates are in agreement with results by Averill et al. (2006), but they based their study on a denser data structure within lactations for the success of an insemination. In contrast, Averill et al. (2006) applied threshold methodology for binary female fertility traits, but for binary NRR56, we used a linear RRM. With regard to heritabilities, differences on the underlying liability scale and on the observed scale are expected (Dempster and Lerner 1950). However, in the case of NRR56 with intermediate frequencies for 'pregnant' or 'non-pregnant', and for such a large dataset, only minor differences from linear and threshold model applications are expected. Especially with regard to genetic correlations, estimates from linear and threshold models are theoretically expected to be the same, as shown by Vinson and Kliewer (1976) for type traits. From a random regression modelling perspective, further differences in (co)variance components might be due to the applied covariance function and the polynomial order for the time dependent variable (e.g., Meyer 2005). In the present study, we ran RRM with Legendre polynomials of order 2, which probably explains increasing variances at the extreme ends of the time scale and smaller genetic correlations between the same traits from different parities. However, such a modelling approach can be justified with the large dataset in the present study, because a strong impact of the polynomial order on genetic parameter estimates was only observed for small datasets and small herd sizes with small contemporary groups (Yin, Bapst, et al. 2014). Furthermore, the chosen covariance function or polynomial structure should be interpreted in the context of computing time. All estimations and related computations were conducted on high-performance computing systems with 192 GB RAM and 24-core CPU nodes. In preliminary runs using first order Legendre polynomials, evaluations took 7–8 days per trait, but 10–12 days per trait for the second order Legendre polynomials. Regarding computation time, the MTM did not perform better than the RRM.

The correlations between the parity-specific sire EBV from the MTM with the respective sire EBV from the RRM were throughout larger than 0.87 for the cow fertility traits in the present study. The EBV correlation coefficient was slightly smaller in heifers with 0.84 for NRR56. In consequence, the large EBV correlations imply only minor re-rankings of sires due to a change from an MTM approach towards an RRM. Interestingly, regarding the cow parities, the smallest correlations were observed in parity 6, but still displayed coefficients larger than 0.87. An explanation of larger EBV deviations in late parities from both modelling approaches might address the data structure due to the effect of selection. Only the high fertile cows are kept in the herds in late lactations. In this regard, the possible effect of pre-selection on biased EBV was intensively discussed in horse breeding for single-trait models (e.g., Bugislaus et al. 2006). In such context, an RRM with greater flexibility for the definition of covariance functions might be superior over MTM.

4.3 | Breeding Value Correlations Between Female Fertility Traits From Different Parities With Breeding Indexes

With regard to EBV correlations between R_NRR56, R_CTFS and R_DO with the indexes from the German official national genetic evaluation, minor to moderate differences were identified across the cow parities. Stronger differences in correlation coefficients were observed when comparing heifers with cows. In consequence, a general separation between female fertility heifer traits and female fertility cow traits as being current practice in most of the national genetic evaluations worldwide, is justified (e.g., Jorjani 2006; Gredler et al. 2007).

From a physiological perspective, it is interesting to note that antagonistic relationships between productivity (in terms of RZM) and female fertility traits do exist over the whole cow lifespan, i.e., in heifers up to parity 6. These unfavourable breeding value correlations based on the RRM approach confirm estimates

from previous studies with a focus on single specific lactations (Jayawardana et al. 2022), on repeatability models (Chafai et al. 2024) or on MTM (Sewalem et al. 2010). In Jersey crosses kept in stressful tropical conditions, the genetic correlations between 305-days milk yield with numbers of services per conception, days open and calving interval were large and positive (Roy et al. 2024), supporting the antagonistic relationships between productivity and female fertility in breeds other than Holstein Friesian and in differing production systems.

Consistent favourable correlations between breeding values for female fertility traits and longevity (RZN) were identified across all parities. Accordingly, moderately favourable genetic correlations between longevity (in terms of the length of productive life) and female fertility traits (DO, CTFS and the interval from first to last insemination) were reported by Zavadilová and Zink (2013) in Czech Holsteins by applying bivariate linear animal models. Hence, the estimates from our applied RRM reflect the correlation pattern between female fertility and longevity traits as outlined before on the basis of more simple modelling approaches. Also, breeding value correlations were consistent and favourable with the overall health index (RZHealth) across all parities, but a weak correlation (0.10) was found between NRR56 in heifers and RZHealth. The overall health index (RZHealth) includes cow health traits that are directly related to female fertility, i.e., endometritis or retained placenta, explaining the favourable breeding value correlations in all cow parities. In consequence, Gernand and König (2017) estimated quite strong genetic correlations between fertility diseases and the female fertility traits CTFS, the interval from first service to pregnancy and the interval from calving to pregnancy. Respective genomic associations between fertility and uterine diseases were inferred by May et al. (2021).

The quite strong EBV correlations between female fertility traits from different parities with the overall fertility index (RZR) are the logical consequence, because RZR is strongly determined through the single female fertility composites (Pasman et al. 2006).

4.4 | Further Explanations for Differing Genetic Parameters in Different Parities

In quantitative-genetic studies, the most common argument for differing genetic parameters with progressing time is that different genes might be 'switched on' or 'switched off'. For fertility traits in cattle, such explanations were confirmed via differing gene expression patterns (e.g., Cai et al. 2019). Recently, alterations of genetic parameters and variance components with progressing time were discussed in the context of epigenomic mechanisms, induced by environmental stressors during early pregnancy (Kipp, Brügemann, Yin, et al. 2021). Such postulations are mostly based on observations made in humans, e.g., the effect of prenatal famine in the embryonic stage on epigenetic modifications, and in causality, on chronic diseases related to infertility (Heijmans et al. 2008). Kipp, Brügemann, Yin, et al. (2021) studied heat stressors during pregnancy, and identified time-lagged genotype × heat stress interactions and alterations of (co)variance components especially for NRR56. Interestingly, in another study by Kipp, Brügemann, Zieger, et al. (2021), the long-lasting effects were strongest for traits recorded in late parities (e.g., longevity). In contrast, Halli et al. (2021) reported only minor effects on early available weight traits in beef cattle. Physiological explanations in both studies (Kipp, Brügemann, Yin, et al. 2021; Halli et al. 2021) addressed heat stressors during maturation and late pregnancy and their associations with genome methylation patterns, with the strongest effects on pregnant cows in their first parity. An argument in this regard might be an additional stress component due to an increased energy deficit (Gross and Bruckmeier 2019). First parity cows need energy for milk production and for growth, causing stronger clinical signs of metabolic stress and metabolic disorders compared to adult cows. Accordingly, López-Catalina et al. (2024) hypothesised that 'calves gestated by nonlactating mothers have a different methylation profile than those gestated by lactating cows'. Such epigenomic aspects as outlined above are speculative, but moderate to strong genetic parameter alterations for female fertility traits across parities and related physiological explanations in other species, suggest ongoing genomic research in this regard.

5 | Conclusion

The application of RRM for NRR56, CTFS and DO along the time-dependent parity scale resulted in reliable genetic parameters, which were confirmed via MTM. Especially for NRR56, heritabilities and variance components differed with regard to heifers and cow parities, supporting the approach to distinguish between heifer and cow female fertility in genetic evaluations. For all three female fertility traits (from the RRM as well as from the MTM), heritabilities and additive-genetic variances increased with increasing parity. Genetic correlations between the same fertility traits from different parities declined with increasing parity distance, also across the cow parity scale. The differing genetic background of fertility traits in different parities resulted in differing breeding value correlations between R_NRR56, R_CTFS and R_DO and other breeding indexes along the parity trajectory. However, larger differences in this regard were only found for NRR56 in heifers and in cows. Correlations between cow parity-specific EBV for the same trait from both modelling approaches MTM and RRM were larger than 0.87.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

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CHAPTER 3

LONGITUDINAL GENOME-WIDE ASSOCIATION STUDY FOR FEMALE FERTILITY TRAITS IN GERMAN HOLSTEIN CATTLE

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Longitudinal Genome-Wide Association Study for Female Fertility Traits in German Holstein Cattle

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ABSTRACT

The aim of this genome-wide association study (GWAS) was to detect significant SNP effects influencing the dynamic process of female fertility in dairy cattle over lactations, to identify possible candidate genes and to study their role in pathway analyses. We considered records for the female fertility traits non-return rate after 56 days (NRR56), interval from calving to first service (CTFS) and days open (DO) up to parity six from 190 269 lactating Holstein cows and heifers. The longitudinal GWAS followed a 2-step approach. In step 1, we estimated (co)variance components by combining pedigree and genomic relationships in random regression models with additive-genetic and permanent environmental regressions on the time-dependent gradient 'parity'. The matrix for estimated (co)variance components for random regression coefficients from step 1 was integrated into the longitudinal GWAS in step 2 to estimate SNP effects and significance for (a) the outer 'layer' representing baseline effects (intercept), (b) the middle 'layer' representing the slope and (c) the inner 'layer' indicating significant SNPs in all lactations, but with differing effects. For the 'inner layer', we detected the following five potential candidate genes: *TMEM132C* and *IMPG1* for NRR56, *DCHS2* for CTFS and *CSMD1* and *CSNK1A1* for DO. The identified genes also play a dominant role in biological pathways related to physiological fertility mechanisms. Overall, the longitudinal GWAS illustrated the dynamic genetic mechanisms of gene regulations on female fertility traits with progressing time.

1 | Introduction

Fertility plays an essential role in dairy cow farming systems, with direct impact on the herd reproduction status, indirect effects on production traits and ultimately determining overall profitability (Shalloo et al. 2014). From a breeding perspective, improvements in female fertility traits strongly determine intra-herd replacement rates, with causal effects on selection intensities (VanRaden et al. 2004). In the context of the interplay between production and reproduction, high reproduction rates ensure the onset of new lactations with high milk production after calving (Beerda et al. 2008; Pinedo and de Vries 2010; Kiser et al. 2019). However, genetic antagonistic relationships among production and female fertility traits have been reported in numerous studies (e.g., Gernand and König 2017).

Female fertility traits are typically polygenic, implying that they are controlled by multiple genes, each contributing small individual effects (Gajbhiye et al. 2018). The expression of these fertility-related genes can vary throughout an animal's lifespan, with some genes switching on or off during different life stages, making them more challenging to detect (McGrath et al. 2021). Environmental factors related to, for example, nutrition, management practices and herd hygiene status also play substantial roles in shaping fertility outcomes, partly with genetic interactions. Strong environmental influences can overshadow genetic contributions, complicating the use

of genetic effects in selective breeding programmes (Sakali et al. 2024).

In recent years in the genomic era, based on the availability of dense high-throughput SNP marker data, genome-wide association studies (GWAS) have been widely applied to identify genetic variants for female fertility traits including the fertilisation success at first service (Galliou et al. 2020), pregnancy rates (Wright et al. 2020) and non-return rates (Frischknecht et al. 2018). The detection of a broad pattern of potential candidate genes associated with the above-mentioned female fertility traits in Holstein-Friesian cows supported the infinitesimal model of inheritance.

Inferring genetic mechanisms remains a challenge, because female fertility is a dynamic trait with alterations and fluctuations across life stages and reproductive cycles. The genetic mechanisms influencing fertility may shift over time, with different genes being expressed at varying stages of lactation, as well as across lactations. As outlined by Das et al. (2011), traditional GWAS to capture static genetic effects do not fully account for such dynamic variation. To address this limitation in a quantitative-genetic context, Schaeffer (2004) introduced the applications of random regression models (RRM) for a more accurate estimation of genetic parameters across lactation stages, age classes and parities. As a further extension based on dense genomic marker data, so-called 'longitudinal GWAS' were suggested to infer SNPs with altering effects in progressing time, for example, to capture the dynamic processes of disease processes in humans (Wiegrebe et al. 2024). Applying this approach in an animal genetics context, Ning et al. (2018) studied SNP effects at multiple time points, providing a comprehensive understanding of altering genetic mechanisms for milk production of Chinese Holstein cows. Unlike traditional GWAS, which assume constant effects for each SNP, longitudinal GWAS capture the dynamic nature of genetic influences over time, for example, across lactations. In longitudinal GWAS, only a limited number of SNPs are expected to exhibit significant effects over the whole time trajectory, implying the crucial importance of the SNP×time interaction term (Sikorska et al. 2015). Sikorska et al. (2015) applied linear mixed models to longitudinal phenotypes to estimate effects for the slope, and in a subsequent step, slope estimates were considered as dependent variables in genomic analyses. Wendel et al. (2021) conducted longitudinal GWAS in a single step by defining linear mixed models with a SNP×time interaction term. Ning et al. (2018) initially estimated the variance components from a random regression model, and subsequently, these estimates were considered as phenotypes in the model for whole-genome association analyses. Such approaches are especially relevant for female fertility traits, where gene expression and regulatory mechanisms are known to fluctuate throughout the dairy cow's reproductive cycle (Pimentel et al. 2011), suggesting the importance of different genes in different lactations.

Consequently, the aim of this study was to infer SNP effects for female fertility traits in German Holstein cows considering the physiological dynamics from an across-lactation perspective by applying a longitudinal genome-wide association approach and considering the (co)variance components for additive-genetic and permanent environmental effects from a genomic random regression model. Subsequently, significant SNPs for the longitudinal component influencing female fertility across lactations were used to identify candidate genes and explore the functional and biological roles of these genes and their relevance to regulatory processes of female fertility.

2 | Materials and Methods

2.1 | Data

2.1.1 | Female Fertility Traits

The phenotypic dataset for the female fertility traits included records for the non-return rate after 56 days (NRR56), for the interval from calving to first service (CTFS), and for days open (DO) from 190 269 lactating cows and heifers kept in 45 large-scale contract herds located in the German federal states of Hesse, Berlin-Brandenburg and Mecklenburg-West Pomerania. Female fertility traits were recorded in the calving years 2011–2022. The number of cattle by parity and trait is given in Table 1. Phenotypic averages were 0.71 (SD = 0.45)

TABLE 1 | Number of observations and descriptive statistics for the female fertility traits non-return rate after 56 days (NRR56), interval from calving to first service (CTFS) and days open (DO) by parity (parity 0 = heifers).

Trait	Parity	No. of observations	Mean	SD
NRR56	0	166 736	0.71	0.45
CTFS	0	0	—	—
DO	0	0	—	—
NRR56	1	154 352	0.53	0.49
CTFS	1	158 132	77.09	27.40
DO	1	128 808	117.43	58.75
NRR56	2	114 446	0.49	0.50
CTFS	2	117 442	77.57	26.86
DO	2	89 513	123.00	60.15
NRR56	3	146 790	0.47	0.49
CTFS	3	150 519	79.97	27.15
DO	3	102 042	127.13	60.21
NRR56	4	41 938	0.46	0.49
CTFS	4	43 076	80.45	27.33
DO	4	28 808	128.67	60.73
NRR56	5	20 958	0.47	0.49
CTFS	5	21 512	81.23	27.28
DO	5	13 346	128.94	59.76
NRR56	6	9127	0.46	0.49
CTFS	6	9380	81.22	26.93
DO	6	5503	128.76	59.37

for NRR56 in heifers, 0.49 for NRR56 in cows (SD = 0.49), 78.24 days for CTFS (SD = 27.50) and 122.08 days for DO (SD = 60.91). Descriptive female fertility trait statistics by parity indicating the phenotypic trend across ages are shown in Table 1.

2.1.2 | Genotypes and Pedigree

The genotype dataset included 21 316 cows with phenotypes for all three female fertility traits. In this regard, 5403 cows were genotyped using the *Illumina Bovine SNP50 v2 BeadChip* and 15 913 cows were genotyped using the *Illumina Bovine Eurogenomics 10 K* low-density chip. The genotypes obtained from the 10 K chip were subsequently imputed to the 50 K panel in the routine process of national genetic evaluations for German Holstein (Segelke et al. 2012). Quality control (QC) of the genotype data was performed using the preGSf90 programme, as implemented in the BLUPf90 software package (Misztal et al. 2018). The filters applied included a minimum minor allele frequency (MAF) threshold of 0.05, a minimum call rate of 0.9, detection of deviation from Hardy–Weinberg equilibrium (p-value $< 10^{-6}$), exclusion of animals with genomic relationships exceeding 0.95, resolution of Mendelian conflicts (i.e., inconsistencies between genotypes of parents and offspring), and elimination of monomorphic SNPs. After QC, the dataset consisted of 41 129 SNPs from 21 048 genotyped cows. The genomic relationship matrix for these animals was constructed applying the algorithm of VanRaden et al. (2004). The principal components (PCs) from the genomic relationship matrix considering the female cattle with phenotypes were generated by applying the software package GCTA (Yang et al. 2011). PC1 and PC2 explained 11.6% and 9.7% of the total genetic variation, respectively. Afterwards, k-means clustering was applied by considering the first five PCs to allocate the animals to four groups with similar genomic relationships. The plot for the first two PCs (Figure 1) and the smooth gradient observed between clusters confirm that the genotyped cows belong to a largely homogeneous population, implying that we did not need to account for further population stratification in the genome-wide association analysis.

The pedigree of the phenotyped 190 269 female cattle comprised at least three generations backwards on the maternal as well as on the paternal side, with 5787 different sires, 7116 different maternal grand-sires, and 2850 different paternal grand-sires. Oldest founder animals were from the birth year 1920.

2.2 | Statistical Models

We applied a two-step strategy. In the first step, we combined genomic and pedigree data to estimate (co)variance components via RRM. The estimated (co)variance components for random regression coefficients and further random effects were used as input data in the longitudinal GWAS in step 2.

2.2.1 | Estimation of Variance Components

The single-trait RRM (model 1, consecutive runs for the three traits NRR56, DO and CTFS) in matrix notation for the estimation of (co)variance components was the following:

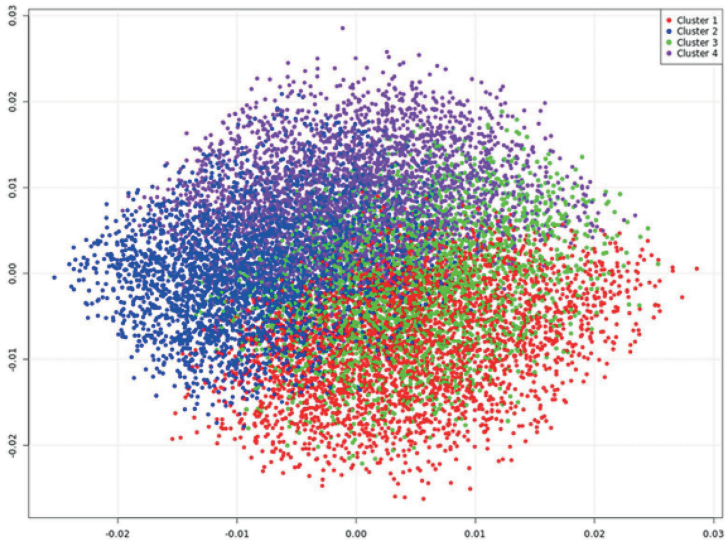

FIGURE 1 | Plot of the first two principal components (PC 1 and PC 2) of the genomic relationship matrix for the genotyped female cattle. The four different clusters represent the allocation to four groups based on k-means clustering considering the first five PCs of the genomic relationship matrix.

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Qu} + \mathbf{Zp} + \mathbf{Ss} + \mathbf{e} \quad (1)$$

where $\mathbf{y}$ = vector for the observations for NRR56, DO or CTFS in different lactations, $\mathbf{b}$ = vector for fixed effects including herd-year-season of insemination, insemination age in months and linear regressions on scaled lactation number (first order Legendre polynomials with intercept and slope); $\mathbf{u}$ = vector of additive genetic effects for random regression coefficients on the scaled lactation number (first order Legendre polynomials with intercept and slope) $\mathbf{u} \sim N(0, \mathbf{H} \otimes \mathbf{G})$, and $\mathbf{H}$ denoting the combined (pedigree and genomics) relationship matrix constructed according to Legarra et al. (2009), $\otimes$ representing the Kronecker product and $\mathbf{G}$ = (co)variance matrix for random regression coefficients of additive genetic effect; $\mathbf{p}$ = vector of permanent environmental effects for random regression coefficients on the scaled lactation number (first order Legendre polynomials with intercept and slope) with $\mathbf{p} \sim N(0, \mathbf{I} \otimes \mathbf{P})$ and $\mathbf{P}$ = (co)variance matrix for random regression coefficients of permanent environmental effects, $\mathbf{s}$ = vector for the random service sire effects (for NRR56 and DO) with $\mathbf{s} \sim N(0, \mathbf{I}\,\sigma_s^2)$ and σ_s^2 denoting the service sire variance; and $\mathbf{e}$ = vector for the random residual effects with $\mathbf{e} \sim N(0, \mathbf{I}\,\sigma_e^2)$ and σ_e^2 denoting the residual variance; and $\mathbf{X}$, $\mathbf{Q}$, $\mathbf{Z}$ and $\mathbf{S}$ = the respective incidence matrices. The concept of random regression modelling with intercept and slope is based on the reaction norm approach to study environmental sensitivity in German Holstein cows by Streit et al. (2012), but adapted to a temporal perspective in the present study.

2.2.2 | Longitudinal Genome-Wide-Associations

Step 2 was the longitudinal GWAS, which was restricted to phenotyped female cattle with genotypes, due to the challenges of the algorithms presented below and computation time limitations. The respective datasets for the longitudinal GWAS included 38 939 records for NRR56, 38 398 records for CTFS and 22 740 records for DO. The model 2 for the longitudinal GWAS in matrix notation was

$$\mathbf{y} = \mathbf{Xb} + \mathbf{X}_{\mathbf{snp}}\mathbf{b}_{\mathbf{snp}} + \mathbf{Qu} + \mathbf{Zp} + \mathbf{Ss} + \mathbf{e} \quad (2)$$

where $\mathbf{y}$ = the vector of observations for NRR5, CTFS and DO; $\mathbf{b}_{\mathbf{snp}}$ = vector for fixed effects for 41 129 SNPs and $\mathbf{X}_{\mathbf{snp}}$ = the related incidence matrix for SNPs considering the three possible SNP-genotypes. The remaining effects were the same as defined for model 1.

For solving the equations of model 2 with regard to SNP effects and significance levels, we followed the 'Eigen decomposition' approach according to Ning et al. (2018), by applying our own R code (R Core Team 2023) (Appendix S1). The technical description of the respective workflow is outlined in Appendix S2.

2.3 | Candidate Gene Identification

Candidate genes were identified based on the significant SNP effects from the inner layer of the circular Manhattan plots. We applied the biomaRt R package from Bioconductor to retrieve the rs accession numbers of SNPs associated with the traits of interest, using the getBM() function (Durinck et al. 2005, 2009). Candidate genes were identified and mapped to these SNPs based on the latest gene annotations from ENSEMBL (McLaren et al. 2016), using the *Bos taurus* ARS-UCD1.2 genome assembly (Yates et al. 2020). A gene was considered as a candidate if at least one SNP exceeding the *Psug* threshold was located either within the gene or within a 200 kb window (100 kb upstream and 100 kb downstream). The identified potential candidate genes were manually submitted to DAVID version 2021 (Huang et al. 2009; Sherman et al. 2022) to retrieve Gene Ontology (GO) terms and associated biological functions. In addition, the Bgee database (Bastian et al. 2021) was queried to obtain gene expression scores, that is, values for these genes quantifying their activity in a specific cattle tissue sample.

2.4 | Pathway Analysis Including the Identified Genes

For the investigations of gene expressions and associated biological pathways, we utilised the SRplot online platform for pathway analysis, visualisation and graphing (Tang et al. 2023), which uses the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa and Goto 2000). This approach enabled us to identify KEGG pathways related to the identified genes in *B. taurus*.

3 | Results

3.1 | Variance Components

The estimates for (co)variance components from the RRM (model 1) are displayed in Table 2. The (co)variance components comprise the intercept and slope for additive-genetic effects and for permanent environmental effects, and the variances for the service sire and residual components. The matrices for these (co)variance components were input data for the algorithm of the subsequent longitudinal GWAS in step 2. Considering the variance components, the heritability for NNR56 was 0.010 in parity 0 for heifers, 0.011 in parity 1, 0.013 in parity 2, 0.017 in parity 3, 0.023 in parity 4, 0.030 in parity 5 and 0.038 in parity 6. The SE of the NRR56 heritability estimates ranged from 0.001 (heifers) to 0.003 (cows from parity 6). For CTFS, the heritability was 0.072 in parity 1, 0.053 in parity 2, 0.047 in parity 3, 0.052 in parity 4, 0.066 in parity 5 and 0.091 in parity 6, with SE in the range from 0.015 (parity 1) to 0.022 (parity 6). The heritability for DO was 0.037 in parity 1, 0.026 in parity 2, 0.022 in parity 3, 0.021 in parity 4, 0.024 in parity 5 and 0.031 in parity 6. The SE for the DO heritabilities ranged from 0.013 (parity 1) to 0.020 (parity 6).

The genetic correlations between the same traits from different parities from the RRM (model 1) were high for adjacent cow parities, but gradually declined with increasing parity distance. The genetic correlations between NRR56 in parity 1 with NRR56 in parities 2, 3, 4, 5 and 6 were 0.89, 0.79, 0.70, 0.69 and 0.67, respectively. Smaller genetic correlations in the range from 0.54 to 0.65 were identified between NRR56 in heifers with NRR56 in all cow parities. Genetic correlations between CTFS from adjacent parities exceeded 0.91, and

TABLE 2 | Variance and covariance components for the traits non-return rate after 56 days (NRR56), calving to first service (CTFS) and days open (DO).

Trait	(Co)variance components							
	var_{a1}	cov_a	var_{a2}	var_{p1}	cov_p	var_{p2}	var_s	var_e
NRR56	0.0033	0.0013	0.0006	0.0048	0.004	0.0034	0.0023	0.2235
CTFS	16.43	2.56	8.15	58.19	29.41	19.09		411.87
DO	128.04	69.83	63.69	67.88	92.91	138.67	19.41	2576.88

Abbreviations: cov_a, additive genetic covariance between the intercept and slope; cov_p, phenotypic covariance between the intercept and slope; var_{a1}, additive genetic variance for the intercept (baseline genetic variance); var_{a2}, additive genetic variance for the slope (change in genetic variance over time); var_e, residual variance; var_{p1}, phenotypic variance for the intercept (baseline phenotypic variance); var_{p2}, phenotypic variance for the slope (change in phenotypic variance over time); var_s, service sire variance.

those between DO from adjacent parities exceeded 0.90. As for NRR56, the genetic correlations for CTFS and DO declined with increasing parity distances.

3.2 | Longitudinal Genome-Wide Associations

The circular Manhattan plots for the SNP effects and the respective *Q*–*Q* plots from the longitudinal GWAS are displayed in Figure 2 for NRR56, in Figure 3 for CTFS and in Figure 4 for DO. With regard to NRR56 and the inner layer for the longitudinal GWAS, no SNP variant surpassed the *Pbonf* threshold. However, three SNPs including ARS-BFGL-NGS-116933 (rs109487947) at BTA 7:55,181,283, BTB-00380633 at BTA 9:15,966,478 and ARS-BFGL-NGS-104247 (rs109066308) at BTA 17:49,578,439 were associated according to *Psug*. The genomic inflation factor (λ) of 1.01 and the *Q*–*Q* plot (included in Figure 2) indicated unbiased estimates. The three significant SNPs for the inner layer explained 3.56% (rs109487947), 0.23% (BTB-00380633) and 0.11% (ARS-BFGL-NGS-104247) of the total genetic variation for NRR56. No single SNP was significant for the outer layer (intercept). Associated SNPs according to *Psug* for the middle layer (slope) included the variants ARS-BFGL-NGS-84234 (rs109703134) at BTA 7:111,655,507, ARS-BFGL-NGS-59605 (rs109476806) at BTA 15:52,762,755, ARS-BFGL-NGS-104247 (rs109066308) at BTA 17:49,578,439 and ARS-BFGL-NGS-62254 (rs42782475) at BTA 22:55,770,123. Notably, ARS-BFGL-NGS-104247 (rs109066308) on BTA 17 was significant for both the middle and inner layer. The significant SNPs for the middle layer explained between 0.10% and 4.03% of the total genetic variation for NRR56. The correlation coefficient between SNP effects for the intercept and slope for NRR56 was 0.87, indicating stability of associated SNPs across all lactations while displaying changing effects over time.

With regard to CTFS, no SNP surpassed the significance threshold for the outer layer (intercept) or the middle layer (slope) (Figure 3). According to *Psug*, the two significant SNPs for the inner layer were BTA-109611 (rs41572882) at BTA 17:3,009,412 and Hapmap51513-BTA-88215 (rs41596684) at BTA 27:8,689,042. The genomic inflation factor (λ) was 0.91, indicating slight but acceptable deflation, as displayed in the corresponding *Q*–*Q* plot (Figure 3). The two significant SNPs for the inner layer explained 0.10% (rs41572882) and 0.08% (rs41596684) of the total genetic variation for CTFS. The correlation coefficient between SNP effects for the intercept and for the slope was 0.72.

The Manhattan plots for the three layers for DO are shown in Figure 4. No single SNP surpassed the suggestive significance threshold for the outer layer (intercept) or the middle layer (slope). For the inner layer combining both effects (intercept and slope), no SNP exceeded the *Pbonf* threshold, but two SNPs surpassed the *Psug* threshold, including UA-IFASA-7691 (rs41655307) at BTA 7:62,860,816 and ARS-BFGL-NGS-1430 (rs109833308) at BTA 27:1,467,500. The genomic inflation factor (λ) was 0.90, indicating slight but acceptable deflation. The *Q*–*Q* plot confirmed the absence of inflation (Figure 4). The two significant SNPs for the inner layer explained 1.13% (rs41655307) and 0.15% (rs109833308) of the total genetic variation for DO. The correlation coefficient between SNP effects for intercept and slope was 0.83.

3.3 | Identification of Potential Candidate Genes and Their Roles in Biological Pathways

Genes located within 100 kb windows of significant or suggestive SNP positions were considered as candidate genes. For example, the SNP ARS-BFGL-NGS-116933 (rs109487947) was excluded due to its location in an intergenic region, outside any known gene boundaries or regulatory elements.

The identified potential candidate genes for the three female fertility traits along with the respective locations of the identified significant SNPs and brief descriptions of the gene functions are listed in Table 3. The GO terms and biological functions of these five genes as retrieved from DAVID, and the respective gene expressions scores are shown in Table 4. With regard to NRR56, one significant variant, ARS-BFGL-NGS-104247 (rs109066308) on BTA 17, is located within the *TMEM132C* gene, which encodes transmembrane protein 132C. *TMEM132C* serves as a neural adhesion molecule due to its structural features, including cohesion and immunoglobulin domains. These domains are characteristic of proteins involved in cell adhesion processes, which are vital for neural development and function. While specific pathways involving *TMEM132C* have not been fully elucidated in cattle, its potential role in cell adhesion with GO:0016020~membrane and the expression scores of 72.20 in the fornix of vagina, 71.90 in the uterine cervix and 62.47 in the isthmus of fallopian tube suggest influence in reproductive processes by affecting cell–cell interactions within the reproductive tract. Another potential candidate gene for NRR56 is *IMPG1* on BTA 9, within the window of SNP BTB-00380633. *IMPG1* is well-known for its role in the visual system, contributing to the structural integrity of the interphotoreceptor

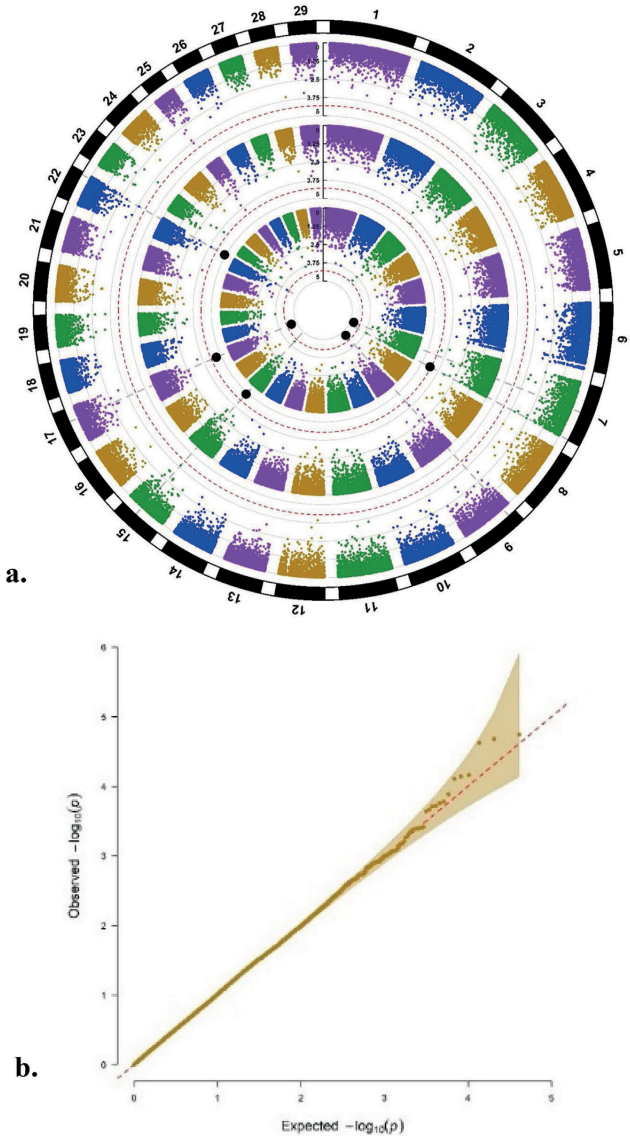

FIGURE 2 | (a) Circular Manhattan plot for the non-return rate after 56 days (NRR56) displaying the *p*-values of SNPs for the three 'layers' intercept (outer layer), slope (middle layer) and the combination of both (inner layer) (the dotted red circular line for each layer represents the respective suggestive threshold and the enlarged black dots the significant SNPs). (b) *Q*–*Q* plot illustrating observed *p*-values plotted versus expected *p*-values, highlighting any deviation from the null hypothesis ($\lambda = 1.01$).

matrix (GO:0007601~visual perception, GO:0030198~extracellular matrix organisation). The expression scores are 97.35 in the retina, 57.74 in the anterior segment of eyeball and 47.38 for the abomasum (Table 4).

With regard to CTFS, the potential candidate gene *DCHS2* on BTA 17 harbours the significant SNP BTA-109611 (rs41572882). *DCHS2* encodes the dachsous cadherin-related protein 2. The expression scores of *DCHS2* in follicular cells of the ovary, in

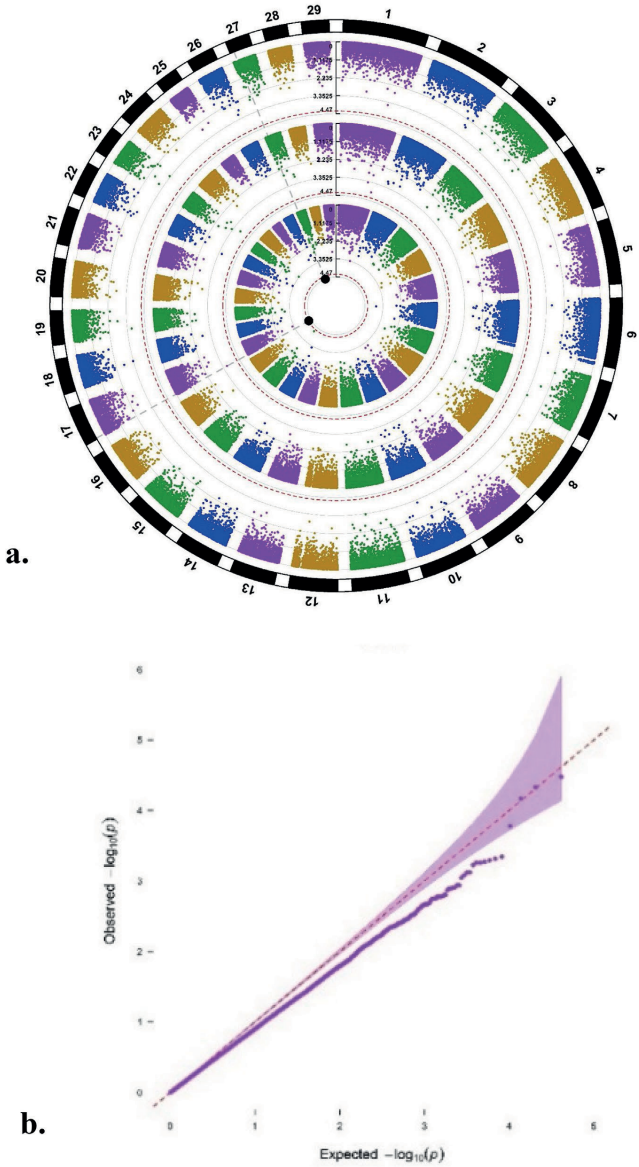

FIGURE 3 | (a) Circular Manhattan plot for the interval from calving to first insemination (CTFS) displaying the *p*-values of SNPs for the three 'layers' intercept (outer layer), slope (middle layer), and the combination of both (inner layer) (the dotted red circular line for each layer represents the respective suggestive threshold and the enlarged black dots the significant SNPs). (b) *Q*–*Q* plot illustrating observed *p*-values plotted versus expected *p*-values, highlighting any deviation from the null hypothesis ($\lambda = 0.91$).

bone marrow, in the hypothalamus, in theca cells and in granulosa cells of female cows are 40.96, 56.06, 47.49, 42.65 and 40.26, respectively (Table 4). These follicular cells are key players in reproductive processes, including androgen secretion. GO-analyses (Table 4) indicate that *DCHS2* is related to the following relevant terms: (a) nephron development (GO:0072006),

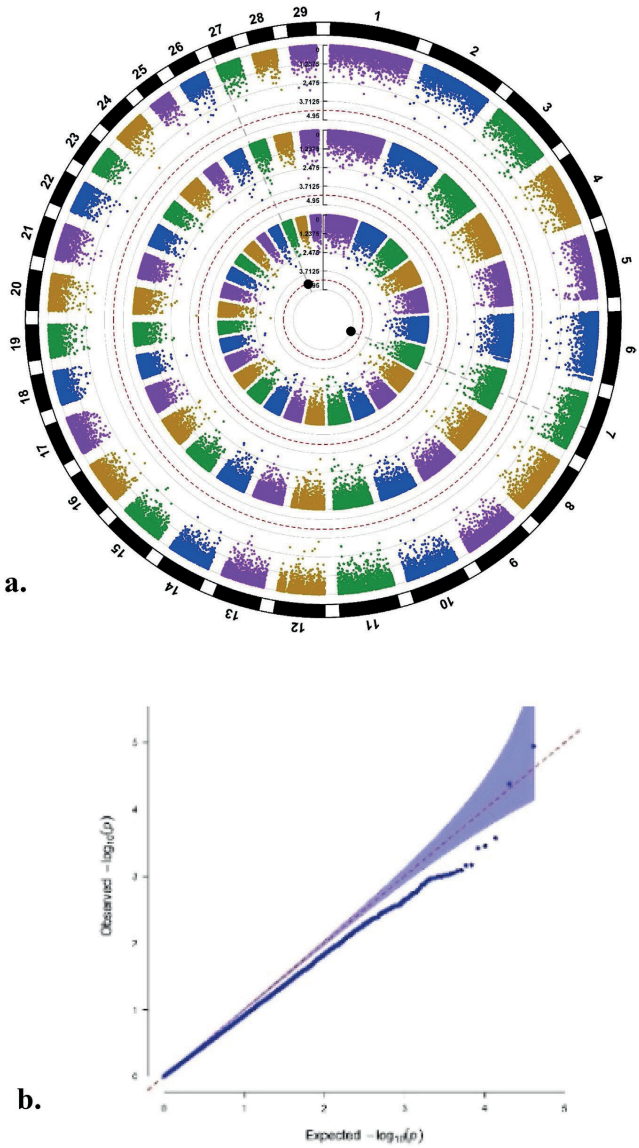

FIGURE 4 | (a) Circular Manhattan plot for days open (DO) displaying the *p*-values of SNPs for the three 'layers' intercept (outer layer), slope (middle layer), and the combination of both (inner layer) (the dotted red circular line for each layer represents the respective suggestive threshold and the enlarged black dots the significant SNPs). (b) *Q*–*Q* plot illustrating observed *p*-values plotted versus expected *p*-values, highlighting any deviation from the null hypothesis ($\lambda = 0.9$).

suggesting its involvement in cellular differentiation and organ development, particularly in kidney formation, which may extend to reproductive health and (b) condensed mesenchymal cell proliferation (GO:0072137), which indicates the role of *DCHS2* in mesenchymal cell behaviour during organogenesis, potentially impacting reproductive tissues.

TABLE 3 | Identified potential candidate genes for non-return rate after 56 days (NRR56), interval from calving to first service (CTFS) and days open (DO) based on the significant SNPs from the longitudinal GWAS.

Trait	SNP	rs-id	Position	BTA	Identified gene	SNP loca-tion[a]	Description
NRR56	ARS-BFGL-NGS-104247	rs109066308	48550804	17	*TMEM132C*	yes	Transmembrane protein 132C
NRR56	BTB-00380633	—	15766008	9	*IMPG1*	no	Interphotoreceptor matrix proteoglycan 1
CTFS	BTA-109611	rs41572882	3027194	17	*DCHS2*	yes	Dachsous cadherin-related 2
DO	ARS-BFGL-NGS-1430	rs109833308	2599527	27	*CSMD1*	yes	CUB and Sushi multiple domains 1
DO	UA-IFASA-7691	rs41655307	60864104	7	*CSNK1A1*	yes	Casein kinase 1 alpha 1

[a]Yes = SNP is located within the intron of the potential candidate gene; no = SNP is located in the 100 kb upstream or 100 kb downstream region of the potential candidate gene.

TABLE 4 | Candidate genes associated with fertility traits, annotated with Gene Ontology (GO) biological processes using the DAVID tool (version 2021), and gene expression scores retrieved from the Bgee database.

Gene name	GO-term and biological process	Gene expression score
TMEM132C	GO:0016020 ~ membrane, GO:0110165 ~ cellular anatomical entity	Fornix of vagina (72.20), uterine cervix (71.90), isthmus of fallopian tube (62.47)
IMPG1	GO:0007601 ~ visual perception, GO:0030198 ~ extracellular matrix organisation	Retina (97.35), anterior segment of eyeball (57.74), abomasum (47.38)
DCHS2	GO:0007156 ~ haemophilic cell adhesion via plasma membrane adhesion molecules, GO:0005509 ~ calcium ion binding, GO:0005911 ~ cell–cell junction, GO:0005886 ~ plasma membrane, GO:0072137 ~ condensed mesenchymal cell proliferation	Follicular cell of ovary (40.96), bone marrow (56.06), hypothalamus (47.49), theca cells (42.65) and granulosa cells (40.26)
CSNK1A1	GO:0006468 ~ protein phosphorylation, GO:0007030 ~ Golgi organisation, GO:0007049 ~ cell cycle, GO:0007165 ~ signal transduction, GO:0016055 ~ Wnt signalling pathway, GO:0016310 ~ phosphorylation, GO:0018105 ~ peptidyl-serine phosphorylation, GO:0031670 ~ cellular response to nutrient, GO:0032436 ~ positive regulation of proteasomal ubiquitin-dependent protein catabolic process, GO:0035025 ~ positive regulation of Rho protein signal transduction, GO:0045104 ~ intermediate filament cytoskeleton organisation, GO:0051301 ~ cell division, GO:0090090 ~ negative regulation of canonical Wnt signalling pathway, GO:1900226 ~ negative regulation of NLRP3 inflammasome complex assembly, GO:1904263 ~ positive regulation of TORC1 signalling	Neutrophils (98.23), oviduct epithelium (98.02) and milk (97.48)
CSMD1	GO:0001964 ~ startle response, GO:0007613 ~ memory, GO:0008584 ~ male gonad development, GO:0008585 ~ female gonad development, GO:0035846 ~ oviduct epithelium development, GO:0042593 ~ glucose homeostasis, GO:0060745 ~ mammary gland branching involved in pregnancy, GO:1990708 ~ conditioned place preference	Cumulus cells (61.43), oocytes (57.27), occipital lobe (53.07), prefrontal cortex (52.75), hypothalamus (52.57 in males, 52.35 in females), temporal cortex (47.45), pituitary gland (46.85) and cerebellum (46.67)

With regard to DO, the significantly associated SNP ARS-BFGL-NGS-1430 (rs109833308) on BTA 27 is located within the *CSMD1* gene, which encodes CUB and Sushi multiple domains 1 (Table 3). *CSMD1* is expressed in several tissues and brain regions relevant to fertility and reproductive health in cows. As shown in Table 4, *CSMD1* shows moderate expression scores in cumulus cells (61.43) and oocytes (57.27), both with relevance for oocyte maturation and linked to GO:0035846 (oviduct epithelium development) and GO:0008585 (female gonad development), highlighting its potential role in oocyte quality and gonadal development. Additionally, *CSMD1* is moderately expressed in brain regions involved in stress and behavioural

regulation of fertility, including the occipital lobe (expression score 53.07), prefrontal cortex (52.75), hypothalamus (52.57 in males, 52.35 in females), temporal cortex (47.45), pituitary gland (46.85) and cerebellum (46.67). These regions are associated with GO terms such as GO:0001964 (startle response), GO:0007613 (memory), GO:0008584 (male gonad development) and GO:0008585 (female gonad development), suggesting involvement in the hypothalamic–pituitary–gonadal axis and neuroendocrine pathways that influence reproductive function.

The second potential candidate gene for DO was *CSNK1A1* on BTA 7 harbouring the significant SNP UA-IFASA-7691 (rs41655307). The C*SNK1A1* gene encodes casein kinase 1 alpha 1 (Table 3). *CSNK1A1* is highly expressed in key reproductive tissues, such as neutrophils, oviduct epithelium and milk, with expression scores of 98.23, 98.02 and 97.48, respectively (Table 4). GO-analysis indicated that *CSNK1A1* is involved in several biological processes, including protein phosphorylation (GO:0006468, GO:0016310), cell cycle regulation (GO:0007049) and signal transduction (GO:0007165). These processes are fundamental for proper cellular functions and are directly linked to reproductive health. *CSNK1A1* is also implicated in the regulation of the TORC1 signalling mechanisms (GO:1904263), which play a role in cell growth and metabolism and in the development of healthy oocytes.

The GO enrichment analysis revealed significant enrichment in all three GO domains: biological processes (BP), cellular components (CC) and molecular functions (MF), which are presented in Figure 5. Terms including the intermediate filament-based process, intermediate filament cytoskeleton organisation and Wnt signalling pathway were enriched in the BP category, indicating a potential role in fertility processes. For the CC category, the terms cilium, keratin filament and photoreceptor segments were enriched, suggesting a role of cellular projections. For MF, enriched terms included heparin binding and protein kinase activity, highlighting potential regulatory and signalling functions. The genes *CSNK1A1* and *IMPG1* may play central roles in signal transduction and structural integrity related to reproductive processes. The convergence of structural and signalling pathways across GO categories supports the hypothesis that fertility-associated genes function at multiple biological levels.

The pathway analysis, considering the identified candidate genes described above, suggests the involvement of several pathways related to regulation of key reproductive organs, female fertility and developmental functions, particularly highlighting the Hippo signalling pathway with an enrichment score of 2.2 (Table 5, with a visual illustration in Figure 6).

4 | Discussion

4.1 | Longitudinal Genome-Wide Associations

The declining genetic correlations with increasing parity distances between the same traits from different parities suggest alterations of SNP effects for the same female fertility traits with progressing time. Specifically, the correlations between SNP

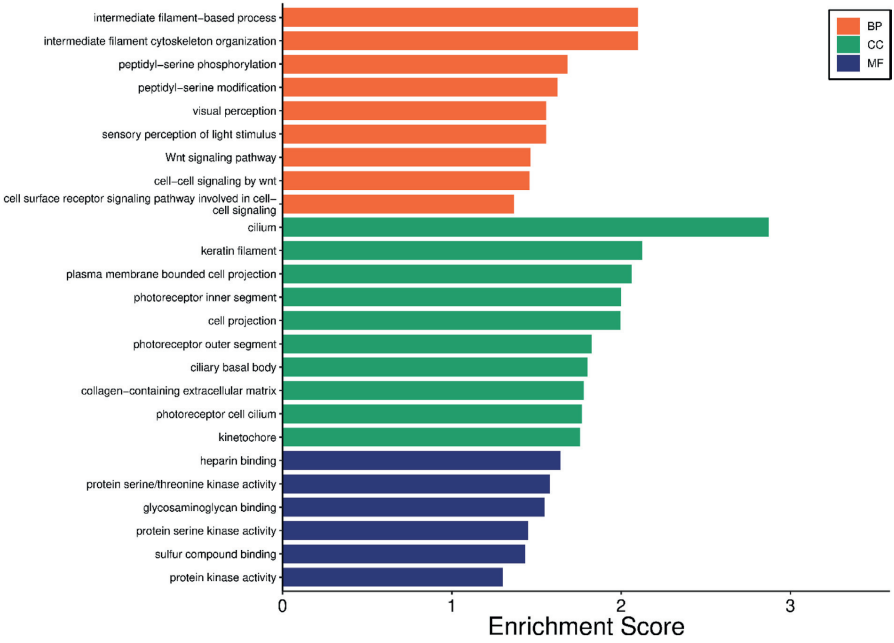

FIGURE 5 | Gene Ontology (GO) enrichment analysis for fertility traits in dairy cows. The bar plot displays significantly enriched GO terms categorised into biological process (BP), cellular component (CC), and molecular function (MF).

TABLE 5 | Pathways related to specific KEGG (Kyoto Encyclopedia of Genes and Genomes) database entries based on identified candidate genes associated with female fertility traits days open (DO) and the interval from calving to first service (CTFS) and supported by previous studies from the literature.

Pathway	KEGG entry	Related trait	Identified candidate gene (BTA)	Same pathways addressed in other studies
Hedgehog signalling pathway	bta04340	DO	*CSNK1A1* (7)	Dilower et al. (2023)
Wnt signalling pathway	bta04310	DO	*CSNK1A1* (7)	Zhang et al. (2022), Hernandez Gifford (2015)
Hippo signalling pathway	bta04392	CTFS	*DCHS2* (17)	Clark et al. (2022), Lv et al. (2019), Plewes et al. (2019)
GnRH pathway	bta04912	DO	*CSNK1A1* (7)	Sun et al. (2022), Dai et al. (2022), Huo et al. (2022)

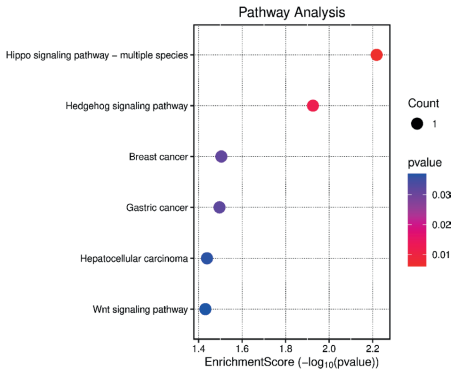

FIGURE 6 | Pathway enrichment analysis of identified genes associated with the female fertility traits non-return rate after 56 days, interval from calving to first insemination and days open. The plot indicates significantly enriched pathways, based on gene set analysis. The *y*-axis represents pathways, the *x*-axis shows the enrichment score ($-\log_{10}$ (*p*-value)), and the dot size reflects the number of genes per pathway.

effects for the outer layer reflecting the baseline effect (intercept) and the middle layer reflecting the slope were 0.87 for NRR56, 0.72 for CTFS and 0.83 for DO, indicating changes in the important SNPs with progressing time. However, the single SNP effects on the female fertility traits were generally small. Even the significant SNPs only explained at most 4.03% of the total genetic variation for the trait of interest (i.e., the effect of the SNP variant ARS-BFGL-NGS-84234 on BTA 7 for NRR56).

In the past, many studies reported significant SNPs linked to fertility traits such as calving interval, days open and conception rate, providing valuable insights into the genetic basis of fertility (e.g., Höglund et al. 2015; Nayeri et al. 2016; Wolf et al. 2021). However, these studies assumed static effects of SNPs and potential candidate genes, overlooking dynamic genetic influences or temporal variations in gene expression across lactations. In our present study, the applied longitudinal GWAS identified seven SNPs associated with five potential candidate genes, influencing changes in fertility traits across parities. We explicitly focussed on the longitudinal genome-wide associations (i.e., the inner layer of the circular Manhattan plot), which is a combination of intercept and slope, and considers the covariance between intercept and slope. In this regard, we identified three significantly associated SNPs for NRR56, two significantly associated SNPs for CTFS and two significantly associated SNPs for DO. A different set of significantly associated SNPs and important genomic segments for CTFS were reported by Wolf et al. (2021), who applied a 'classical static' approach for only one single record per cow. Mohammadi et al. (2020) focussed on same fertility traits, but they identified most of the significant SNPs on BTA 19, indicating the differences between 'static' and longitudinal GWAS for female fertility traits. However, other factors, such as differences between analysed populations, might explain the differences in genome-wide associations for the same traits.

To the best of our knowledge, this is the first study focusing on longitudinal GWAS for female fertility traits in dairy cattle, allowing us to capture time-dependent genetic effects and offering a more comprehensive understanding of fertility as a dynamic trait. Our approach for female fertility traits aligns with previous research by Sikorska et al. (2015) and by Ning et al. (2018), which emphasised the importance in incorporating time-dependent genetic effects for a better and more precise understanding of complex traits. Ning et al. (2018) examined the genomic background of milk production traits and analysed a very dense longitudinal data structure based on daily measurements. In contrast, our female fertility study considered records from very distant periods, that is, from different lactations. As a further challenge for fertility traits compared to milk production is the importance of additional random effects, especially the effect of the service sire on NRR56 and DO. As a novelty in this regard, we developed a customised R script (see Appendix S1), overcoming limitations in modelling further random effects with respective (co)variance structures for longitudinal GWAS.

4.2 | Candidate Genes and Their Roles in Biological Pathways

We considered as candidate genes those within 100 kb of significant or suggestive SNPs identified in the longitudinal GWAS. The SNP ARS-BFGL-NGS-104247 with significant effects on NRR56 is located in the intron of the *TMEM132C* gene. *TMEM132C* encodes the two transmembrane proteins

ENSBTAP00000055174 and *ESBTAP00000072407*, and is related to the family of genes that are often associated with human genetic disorders, particularly neurological disorders (Sanchez-Pulido and Ponting 2018). Additionally, this gene has been recently reported in relation to lung function in Tibetans (Zheng et al. 2023), suggesting that *TMEM132C* plays a predominant role with regard to adaptive processes at high altitudes. Furthermore, *TMEM132C* has been associated with the development of the nervous system in rats (Wang et al. 2022), growth traits in pigs (Gong et al. 2019), milk production in Thai multi-breed cattle (Yodklaew et al. 2017), and milk protein production in Holstein cattle (Liu et al. 2022). In Sarabi cattle, an Iranian taurine breed, the chromosomal segment including *TMEM132C* revealed strong selection signatures (Moradian et al. 2020), suggesting the role of adaptation and natural selection. The study by Souza et al. (2024) indicated associations between *TMEM132C* and body weight in Nellore cattle. Expression of *TMEM132C* in different reproductive organs suggests involvement in reproductive processes by affecting cell–cell interactions within the reproductive tract (Human Protein Atlas 2025). Differential expression of *TMEM132C* has been reported for the mouse nervous system (Wang et al. 2022), where this gene has been shown to influence hormonal signalling pathways that regulate reproductive functions.

IMPG1 was identified as a potential candidate gene for NRR56. *IMPG1* mutations were causal in 8% of families with adult-onset vitelliform dystrophy, characterised by moderate visual impairment and drusen-like lesions in humans (Meunier et al. 2014). Ebrahimi et al. (2023) reported significant downregulation of *IMPG1* in the sperm of infertile men, suggesting a potential role of this protein in sperm functions and male fertility. Although research on *IMPG1* in dairy cattle is limited, its involvement in sperm integrity and expression in reproductive tissues could imply a similar role in bovine fertility. Research in mice suggested a sex-biased expression in the pituitary gland, indicating a possible regulatory role in reproductive processes, particularly the hypothalamic–pituitary–gonadal axis (Oyola and Handa 2017). Moradian et al. (2020) highlighted *IMPG1* in the context of milk fat percentage, pregnancy rate and milk riboflavin content in Iranian Holstein cattle. Moreover, according to the results from the present study, expressions of *IMPG1* in the fornix of the vagina, uterine cervix and uterine horn, and isthmus of fallopian tube suggest the involvement of *IMPG1* in female fertility processes.

The *DCHS2* gene (identified as a potential candidate gene for CTFS) has been associated with a range of biological processes across different species, particularly in the context of production and reproduction in Simmental cattle (Wang et al. 2025). Hence, our findings align with previous research that highlights its significance in various physiological and reproduction-related traits. Lodge et al. (2020) reported that mutations in *DCHS2* lead to developmental defects in the pituitary and hypothalamus, resulting in hormone deficiencies and associated health problems in humans. This underscores the fundamental role of *DCHS2* in endocrine regulation, which is crucial for reproductive performance. Similarly, Murugesan et al. (2021) found *DCHS2* to be active during the craniofacial development of buffalo embryos, highlighting its involvement in early developmental processes. Studies in other species support that *DCHS2* influences reproductive and production traits. Gu et al. (2017) and Wu et al. (2022) demonstrated the effects of *DCHS2* variants on reproductive performances in pigs and on weights in humans, respectively. Our results are consistent with Wang et al. (2025), supporting the impact of *DCHS2* on reproductive traits in Simmental cattle. Given its known roles in endocrine functions, craniofacial development and reproductive traits in other species, *DCHS2* could be a potential candidate gene for improving fertility and production efficiency in dairy cattle.

CSNK1A1 (Casein Kinase 1 Alpha 1), identified as a potential candidate gene for DO, plays a particularly important role in cellular processes such as cell division, differentiation and apoptosis. *CSNK1A1* has been implicated in milk composition traits, including milk fat and protein percentages as well as milk yield, suggesting a potential link to metabolic regulations in high-producing dairy cows (Ameri et al. 2024). The antagonistic relationship between milk production traits and reproductive performances in cows suggests that *CSNK1A1* may also influence fertility (Ameri et al. 2024). Recent studies have highlighted the role of *CSNK1A1* in uterine gland development, where it regulates epithelial cell apoptosis and the expression of *Foxa2*, a critical factor for uterine function (Zhang et al. 2024). These mechanisms could influence implantation and embryo development, impacting fertility and days open. Additionally, *CSNK1A1* has been shown to regulate the postpartum quiescent state and reproductive cycle in yaks, involving processes such as oocyte maturation, oestrogen and Gonadotropin-Releasing Hormone (GnRH) signalling (Yang et al. 2024). This suggests that *CSNK1A1* plays a crucial role in reproductive timing and overall fertility, supporting our results from the longitudinal GWAS in dairy cows.

CSMD1 was identified as a potential candidate gene for DO. Dilower et al. (2023) and Liu et al. (2018) have shown that mice lacking specific protein ligands in granulosa cells fail to develop theca cells, leading to impaired steroidogenesis and infertility. In humans, Jaillard et al. (2016) identified the role of *CSMD1* in ovarian failure in patients under 40 years old, and they proposed this gene as a potential candidate implicated in reproductive functions. Lee et al. (2019) indicated that rare mutations of *CSMD1* cause male and female infertility in humans. Zheng et al. (2023) identified *CSMD1* as a candidate gene related to the body condition score and subcutaneous fat deposition trait in Holstein cattle. Liu et al. (2017) defined *CSMD1* as a candidate gene for fertility traits in Nordic Holstein cows. Gonzalez et al. (2020) found associations between variants of *CSMD1* with conformation traits in dairy cows. Li et al. (2022) highlighted the role of *CSMD1* in fertility mechanisms of Chinese indigenous goat breeds. *CSMD1* was expressed in cumulus cells and oocytes with an expression score larger than 55 (Bastian et al. 2021). The gene expressions in cumulus cells and oocytes further support its role in female fertility, potentially affecting oocyte maturation and quality, which are critical determinants of DO.

Investigations of the biological pathways indicated key biological processes which are directly or indirectly linked to fertility traits, including cell adhesion, hormonal regulation and metabolic functions (Tang et al. 2023). Similar findings were reported in viviparous species, where downregulation of cell adhesion molecules was associated with increased uterine plasticity and preparation for embryo implantation (Whittington et al. 2018). The involvement of *DCHS2* in the Hippo signalling pathway, indicated by an enrichment score of 2.2, further

supports its role in reproductive organ development and hormonal regulation. Clark et al. (2022) related Hippo pathway components with important roles in follicle growth and activation as well as with steroidogenesis, by regulating several key biological processes through mechanisms of cell proliferation, migration, differentiation and cell fate determination. In a study addressing the bovine Hippo signalling pathway, Plewes et al. (2019) highlighted the pathway's role in proliferation and estradiol synthesis, which is necessary for maintaining normal follicle development.

CSNK1A1, another candidate gene, has been implicated in a broad range of pathways. For example, Yang et al. (2024) described the role of *CSNK1A1* in postpartum quiescence and reproductive cycles of yaks, and upregulation during the seasonal light cycle, which may influence the reactivation of reproductive functions postpartum. Jiang et al. (2018) highlighted that CK1α (the protein encoded by *CSNK1A1*) is active in both the Hedgehog and Wnt pathways, and exhibits high expression in human female reproductive tissues. Taken together, these studies suggest that *CSNK1A1* may enhance reproductive outcomes by regulating processes such as cell division and protein phosphorylation, and the importance of signalling pathways for oocyte development.

Overall, by identifying genes found within genomic regions associated with longitudinal fertility traits, this study enhances our understanding of the underlying biological mechanisms influencing these traits. These traits are influenced by various factors, including gene regulatory mechanisms, hormonal interactions, and metabolic adaptations, highlighting the multifaceted and time-dependent nature of reproductive performance.

5 | Conclusions

The longitudinal GWAS enabled a clear separation of SNP effects along the time-dependent lactation trajectory, that is, for the intercept, for the slope and for the combination of both. The correlation coefficients between SNPs effects for the slope (middle layer) and for the intercept (outer layer) ranged from 0.83 to 0.91 for three fertility traits, reflecting changes in SNP effects over time. These results are supported by declining genetic correlation estimates between the same traits with increasing parity distances. The significant SNPs for the inner layer and for the middle layer, reflecting the dynamic processes of female fertility, explained 0.10%–4.03% of the total genetic variation. These small genetic variances support the polygenic nature of female fertility traits. From the GWAS for the intercept, slope and combined information of the same traits, we identified five potential candidate genes. Gene expression scores of these genes were related to tissues with functions in female fertility processes. Overall, the results from this longitudinal GWAS underscore the importance of specific SNPs, and associated genes, in relation to specific lactations.

Author Contributions

S.S. performed formal data analysis and wrote the manuscript; T.Y. conceptualised the longitudinal GWAS and supported in programming and statistical analyes, S.K. supervised, developed data anaysis strategies and wrote the manucript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data supporting this study are openly available from the figshare repository at https://figshare.com/s/3b596720d1bbd9282328.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Appendix S1:** age70078-sup-0001-AppendixS1.R. **Appendix S2:** age70078-sup-0002-AppendixS2.docx.

CHAPTER 4

MODELLING APPROACHES FOR THE ESTIMATION OF GENETIC PARAMETERS FOR CALVING EASE AND STILLBIRTH IN GERMAN HOLSTEIN DAIRY CATTLE

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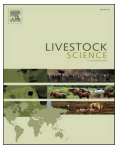

Modelling approaches for the estimation of genetic parameters for calving ease and stillbirth in German Holstein dairy cattle

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HIGHLIGHTS

- Allocation of calving traits to the dam generated a longitudinal data structure across parities as a basis for innovative modelling approaches.
- Maternal genomic multiple-trait models and maternal random regression models on a continuous parity scale displayed similar genetic parameter pattern.
- Additive genetic variances and maternal heritabilities declined with increasing lactation number.
- Largest genetic correlations between same traits from different parities were found between parities 1 and 2.
- Direct-maternal genetic associations were close to zero or of antagonistic nature.

ARTICLE INFO

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ABSTRACT

The aim of the present study was to apply alternative modelling approaches for genetic evaluations of stillbirth (SB) and calving ease (CE) from the dam perspective, enabling consideration of a longitudinal data and genetic covariance structure across lactations. We considered a comprehensive dataset including 435,489 calf records for CE and 477,800 calf records for SB from the birth years 2001 to 2017, and genotype data including 41,304 SNPs from 24,133 animals. The calves with phenotypes were offspring from 184,012 Holstein Friesian (HF) cows for SB, and from 177,162 HF cows for CE. The calves and cows were kept in 45 large-scale German dairy contract herds. The applied three genetic-statistical models based on single-step methodology considering both pedigree and genomic relationship matrices. In the "classical" model 1, we allocated SB and CE observations to the calf by considering direct and maternal genetic effects with their respective covariances. Model 2 was a multiple-trait model (MTM) by allocating the observations to the dam and considering same traits in different parities as different traits. Accordingly, in the random regression model (RRM), SB and CE were defined as a trait of a dam and analyzed on a continuous parity scale by considering random regression coefficients for additive-genetic effects of intercept and slope. From both models MTM and RRM, we observed a gradual decrease of additive genetic variances and maternal heritabilities with increasing parity. Genetic correlations between same traits from different parities were larger than 0.80 for adjacent parities, but declined with increasing parity distance. Correlations between maternal genomic breeding values (GEBV) from the two different models MTM and RRM for the same trait and parity were throughout larger than 0.80, and in the range from 0.68 to 0.88 with the maternal GEBV from model 1. Genetic and breeding value correlations close to zero were found between the direct and maternal genetic effects. Correlations between maternal GEBV from the RRM and maternal GEBV from official genetic evaluations were throughout larger than 0.82, and the large rank correlations indicate only minor changes in top lists for sires.

1. Introduction

Calving ease (**CE**) and stillbirth (**SB**) are economically important traits, and respective improvements are also imperative from an animal welfare perspective. Specifically, dystocia and SB were associated with increased veterinary and labor costs (Cole et al., 2007), impaired female fertility (Ghiasi et al., 2014) and declines in milk production (Sdiri et al., 2023). The importance of CE and SB is due to the detrimental effects on

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both animal categories calves and their dams. Thus, Lompard et al. (2007) and Lompard et al. (2003) reported an increasing risk for respiratory and digestive disorders in calves due to dystocia, and increased incidences for retained placenta, uterine diseases and mastitis in their dams, respectively.

Effects on both calf and dam traits indicate the complexity of genetic analyses for CE and SB. From a genetics perspective, CE and SB are dependent on three genetic groups including the direct effect of the calf, the maternal effect of the dam and the paternal effect of the service sire. Complex theoretical models to infer the respective effects for such "synergistic traits" considering the interplay of all genetic groups were developed by Wilham (1963) and extended by König et al. (2007) for pregnancy traits after embryo transfer additionally considering the genetic effect of the recipient. However, the genetic-statistical models for CE and SB as applied in official genetic evaluations are rather simple. Usually, the trait observations are assigned to the calf by simultaneously considering the direct-genetic effect of the calf and the maternal-genetic effect of the dam (Wiggans et al., 2003; Vanderick et al., 2014). Accordingly, official genetic evaluations have a long history, e.g., since 1978 for Holstein cattle in the US (Berger, 1994), but in most studies, the direct and maternal heritabilities were small with estimates close to 1 % or even below 1 % (Fürst and Egger-Danner, 2003; Eriksson et al., 2004). The small genetic variances and heritabilities for SB and CE from the "standard" genetic modelling approaches might explain the only minor improvements throughout decades with stable prevalences for SB up to 10 % and unfavorable CE classifications up to 50 % in some regions or herd clusters (Waurich, 2013).

Both traits, SB and CE, follow categorical data distributions. In the past, statistic model comparisons for genetic evaluations focused on the comparison between linear and threshold models (e.g., Varona et al., 1999). Most of the conducted model comparisons revealed superiority of threshold over linear models based on model evaluation criteria, but the rank correlations between estimated breeding values (**EBV**) from both models were close to 1, as recently indicated by Cesarani et al. (2025) for genetic evaluations in pigs. Also from a theoretical perspective, results from threshold and linear models are expected to be very similar when avoiding extreme data distributions (Freund and Walpole, 1980), as shown for ranks of sires according to their EBVs based on large phenotype datasets in Holstein dairy cattle (König et al., 2008) and in exotic cattle breeds (Camargo-Júnior et al., 2025). Regarding genetic correlations, Vinson and Kluwer (1976) proved that the estimates should be the same based on real binary trait or on Gaussian trait definitions.

Nowadays, genetic-statistical modelling approaches for SB and CE address the question of how to allocate the trait observations, i.e., to treat SB and CE as observation of the calf or of the cow, and how to perform longitudinal data analyses. Especially for SB, the prevalence differences across parities are very obvious (Cole et al., 2007). Different SB and CD incidences in different parities indicate alterations of genetic effects with progressing time. However, time series analyses based on a longitudinal data structure are not possible when assigning the observation to the calf. Alternatively, Axford et al. (2024) recently phrased modelling perspectives treating SB as an observation of the cow (= the dam). Such data structure with repeated measurements per cow enable the application of random regression models (**RRM**) for genetic analyses. Classically, RRM have been developed for genetic evaluations of dense test-day production traits (e.g., Jensen, 2003) or for growth curves based on weight data in narrow intervals (e.g., Mota et al., 2013). Random regression models have the property to better account for environmental alterations compared to multiple-trait or repeatability models (e.g., Swalve, 2000), and a larger number of data enables the estimation of a broad pattern of parameters and (co)variance components (Jensen, 2001). Consequently, Schaeffer (2004) indicated a broader range for RRM applications, additionally for traits which are measured in wide intervals, e.g., in different parities. Such RRM approaches were considered for cow survival (Veerkamp et al., 1999) and for female fertility (Sakhaeifar et al., 2025) across parities, or by Yin et al. (2014) for production traits along distinct classes of climatic gradients. Modelling genetic regressions along the parity gradient for calving traits is feasible when defining CE and SB as a trait of the cow. Such perspective also reflects the biological background, because cow associated factors including uterine characteristics, pelvic area, gestation length, blood parameters as health status indicators, body condition score (Bahrami-Yekdangi et al., 2022; Mahmoud et al., 2017) and maternal calving behavior (Behren et al., 2023), contribute to calving processes and to ongoing calf health.

The overall aim of this study was to estimate genetic (co)variance components for CE and SB by applying both modelling perspectives, i.e., from the calf and as a novelty from the dam perspective. As a second objective, the "dam perspective modelling approach" enabled RRM applications to infer genetic parameters based on a longitudinal SB and CE data structure along the parity-time scale. The third objective addressed practical validations with focus on correlations between genomic breeding values (**GEBV**) from all models for CE and SB with sire breeding values from the official German national genetic evaluations.

2. Materials and methods

2.1. Traits and pedigree structure

Editing of the raw phenotype data considered the following criteria: exclusion of twin births (exclusion of 1.3 % of all births) and exclusion of cows with only a single birth from one specific parity (18.5 % of all cows) to generate a dense longitudinal data structure across lactations. The final phenotype dataset for the ongoing genetic analyses comprised 477,800 calf records for SB and 435,489 calf records for CE from the birth years 2001 to 2017. The calves with phenotypes were offspring from 184,012 Holstein Friesian (**HF**) cows for SB, and from 177,162 HF cows for CE. Most of the cows had calves in all three parities reflecting the average values, i.e., 2.60 calves per cow for SB and 2.46 calves per cow for CE. A minor fraction of cows (7 % for SB and 4 % for CE) had only two calves from parities 1 and 2. These cows were genotyped which was the reason for their consideration in the ongoing analyses.

The calves and cows were kept in 45 large-scale dairy contract herds located in the German federal states of Hesse, Mecklenburg-West Pomerania and Berlin-Brandenburg. Stillbirth was defined as a binary trait with a "0" for alive calves and a "1" for dead calves within the first 48 h after birth. Calving ease phenotypes based on producer categorizations considering the four classes easy (sore = 1), moderate (score = 2), difficult with assistance (score = 3) and surgery (score = 4). The distribution of phenotypic records for SB and CE across classes is given in Table 1. The slightly smaller no of records for CE compared to SB was due to inconsistent trait recording in some cases and partly missing data. All calvings were from dams in parities 1, 2 or 3. The average no of calves per sire was 3986 for SB, and 3947 for CE. The 184,012 dams for SB were daughters of 5059 different sires, and the 177,162 dams for CE were daughters of 5011 different sires. For the ongoing genetic analyses, we considered a very deep pedigree which could be traced back to oldest

Table 1
Number of records for stillbirth (SB) and calving ease (CE) in the different scoring categories (SB: 0 = born alive, 1 = death with 48 h after birth; CE: 1 = easy, 2 = moderate, 3 = difficult with assistance, 4 = surgery) and with the respective number of dams.

Trait	Score	No. of records	Percentage (in %)	No. of dams
SB	0	450,055	94.19	177,983
	1	27,745	5.81	25,988
	Total	477,800		184,012
CE	1	321,554	73.84	150,505
	2	89,673	20.59	70,521
	3	23,226	5.33	20,910
	4	1036	0.24	1030
	Total	435,489		177,162

founder animals born in 1920. For the animals with phenotypic records, at least three complete generations backwards were available.

2.2. Genotypes

Among the dams with phenotypic records for CE and SB, a subset of 21,316 animals was genotyped using the *Illumina Bovine SNP50 v2 Bead Chip* (5403 cows) or the *Illumina Bovine Eurogenomics 10* K *low-density chip* (15,913 cows). In consequence, for the modelling approaches from the calf perspective, 21,316 female calves with phenotypic records for SB and CE were genotyped. The calves with phenotypic records for SB were offspring of 4273 sires, implying an average of 111.8 calves per sire. The average no of calves per sire for CE of the 3886 different sires was 112.7. For both traits SB and CE, the same 2150 cow sires and the same 1551 calf sires were genotyped with the *Illumina Bovine SNP50 v2 Bead Chip*. Females genotyped with the 10 K chip were imputed to 50 K by the national center for official genetic evaluation (vit, Verden), using the algorithm as defined by Segelke et al. (2012).

Quality control of the SNP genotypes was performed using the software package PLINK (Purcell et al., 2007). Quality criteria considered a minor allele frequency (**MAF**) of 0.05 (exclusion of 3550 markers), a minimum call rate of 0.9 (exclusion of 816 markers) and a significant deviation (p-value $< 1 \times 10^{-6}$) from Hardy-Weinberg equilibrium (**HWE**) (exclusion of 4 markers). Genomic relationships among all genotyped animals were smaller than 0.95. We identified no single Mendelian conflict, i.e., inconsistencies when comparing the individual genotype with the parental genotypes. Finally, after filtering, 41,304 SNPs from 24,133 genotyped animals were available for ongoing genomic studies.

2.3. Genetic-statistical modeling approaches

2.3.1. Model 1: direct-maternal genetic model from the calf perspective

Model 1 represented the classical genetic evaluation for SB and CE, i.e., allocating the observations to the calf. We applied single-trait animal models considering the direct-genetic effect of the calf and the maternal-genetic component. The statistical model 1 in matrix notation was defined as follows:

$$y = Xb + Zu + Wm + Q_{pe} + e \quad (1)$$

with $\boldsymbol{y}$ = the vector for observations for SB and CE, $\boldsymbol{b}$ = the vector for fixed effects including herd-year-month of birth, sex of the calf and calving age of the dam, $\boldsymbol{u}$ = the vector for random additive genetic effects, $\boldsymbol{m}$ = the vector for random maternal genetic effects, **pe** = the vector for random maternal permanent environmental effects, $\boldsymbol{e}$ = the vector for random residual effects, and **X, Z, W** and $\boldsymbol{Q}$ = the respective incidence matrices for random effects. The (co)variance structure for random effects was the following:

$$\text{var}\begin{bmatrix} u \\ m \\ pe \\ e \end{bmatrix} = \begin{bmatrix} g_{11}\mathbf{H} & g_{12}\mathbf{H} & 0 & 0 \\ g_{21}\mathbf{H} & g_{22}\mathbf{H} & 0 & 0 \\ 0 & 0 & \mathbf{I}\sigma^2_{pe} & 0 \\ 0 & 0 & 0 & \mathbf{I}\sigma^2_{e} \end{bmatrix}$$

with g_{11} the direct additive genetic variance, g_{22} = the maternal genetic variance, g_{12} and g_{21}, $\boldsymbol{H}$ = the combined relationship matrix (see Legarra et al., 2009) considering the pedigree relationship matrix (**A**) and the genomic relationship matrix (**G**) which was constructed according to van Raden (2008), σ^2_{pe} = the random maternal permanent environmental variance, σ^2_e = the random residual variance, and **I** = identity matrices for n dams and m calves, respectively. Compatibility between the **G** and **A** matrices was ensured by blending the raw genomic relationship matrix with the pedigree-derived submatrix for genotyped animals (A22) as described by Aguilar et al. (2014).

2.3.2. Model 2: multiple-trait model from the dam perspective

In model 2, SB and CE were defined as a trait of the dam. According to the multiple-trait modelling approach by Falconer and Mackay (1996), we defined same traits from different dam parities as different traits. In matrix notation, the multiple-trait animal model (**MTM**) for 3 traits (i.e., the same trait from the first 3 cow parities) was defined as follows:

$$\begin{bmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \\ \mathbf{y}_3 \end{bmatrix} = \begin{bmatrix} \mathbf{X}_1\mathbf{b}_1 + \mathbf{Z}_1\mathbf{u}_1 + \mathbf{S}_1\mathbf{s}_1 + \mathbf{e}_1 \\ \mathbf{X}_2\mathbf{b}_2 + \mathbf{Z}_2\mathbf{u}_2 + \mathbf{S}_2\mathbf{s}_2 + \mathbf{e}_2 \\ \mathbf{X}_3\mathbf{b}_3 + \mathbf{Z}_3\mathbf{u}_3 + \mathbf{S}_3\mathbf{s}_3 + \mathbf{e}_3 \end{bmatrix} \quad (2)$$

where $\mathbf{y_1}$, $\mathbf{y_2}$ and $\mathbf{y_3}$ = the observation vectors for SB or CE in the 3 different cow parities, $\mathbf{b_1}$, $\mathbf{b_2}$ and $\mathbf{b_3}$ = the vectors for fixed effects for the 3 traits including herd-year-month of calving, sex of the calf and cow calving age, $\mathbf{u_1}$, $\mathbf{u_2}$ and $\mathbf{u_3}$ = the vectors for additive genetic effects for the 3 traits, $\mathbf{s_1}$, $\mathbf{s_2}$ and $\mathbf{s_3}$ = the vectors for random calf sire effects for the 3 traits, $\mathbf{e_1}$, $\mathbf{e_2}$ and $\mathbf{e_3}$ = the vectors for the random residual effects for the 3 traits. $\mathbf{X_1}$, $\mathbf{X_2}$, $\mathbf{X_3}$, $\mathbf{Z_1}$, $\mathbf{Z_2}$, $\mathbf{Z_3}$, $\mathbf{S_1}$, $\mathbf{S_2}$ and $\mathbf{S_3}$ = incidence matrices for $\mathbf{b_1}$, $\mathbf{b_2}$, $\mathbf{b_3}$, $\mathbf{u_1}$, $\mathbf{u_2}$, $\mathbf{u_3}$, $\mathbf{s_1}$, $\mathbf{s_2}$ and $\mathbf{s_3}$, respectively. The variance-covariance structure for random effects was as follows:

$$\text{var}\begin{bmatrix} u_1 \\ u_2 \\ u_3 \\ s_1 \\ s_2 \\ s_3 \\ e_1 \\ e_2 \\ e_3 \end{bmatrix} = \begin{bmatrix} g_{11}\mathbf{H} & g_{12}\mathbf{H} & g_{13}\mathbf{H} & 0 & 0 & 0 & 0 & 0 & 0 \\ g_{21}\mathbf{H} & g_{22}\mathbf{H} & g_{23}\mathbf{H} & 0 & 0 & 0 & 0 & 0 & 0 \\ g_{31}\mathbf{H} & g_{32}\mathbf{H} & g_{33}\mathbf{H} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & \sigma^2_{s1}\mathbf{I}_{s1} & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \sigma^2_{s2}\mathbf{I}_{s2} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & \sigma^2_{s3}\mathbf{I}_{s3} & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & r_{11} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & r_{22} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & r_{33} \end{bmatrix}$$

where $\mathbf{g_{ii}}$ = the additive-genetic effects for the 3 traits and the respective additive genetic covariances for all trait combinations, $\boldsymbol{\sigma^2_{s_1}}$, $\boldsymbol{\sigma^2_{s_2}}$ and $\boldsymbol{\sigma^2_{s_3}}$ = the variances for the calf sire effects for the 3 traits, $\boldsymbol{H}$ = the combined relationship matrix as explained above, $\mathbf{I_{s_1}}$, $\mathbf{I_{s_2}}$ and $\mathbf{I_{s_3}}$ = the identity matrices for the calf sires for the 3 traits, $\mathbf{r_{11}}$, $\mathbf{r_{22}}$ and $\mathbf{r_{33}}$ = the random residual variances for the 3 traits.

2.3.3. Model 3: random regression model from the dam perspective

Model 3 was a single-trait RRM. Following model 2, SB and CE were defined as a trait of the dam by modelling random regressions on cow parities. In matrix notation, model 3 was defined as follows:

$$y = Xb + Zu + Wp + Ss + e \quad (3)$$

where $\boldsymbol{y}$ = a vector of records for SB and CE from the 3 cow parities; $\boldsymbol{b}$ = a vector for fixed effects including herd-year-month of calving, sex of the calf, cow calving age and fixed linear regressions on cow parity considering the coefficients for intercept and slope, $\boldsymbol{u}$ = a vector for random regression coefficients for additive genetic effects on cow parity considering the coefficients for intercept and slope, $\boldsymbol{p}$ = a vector for permanent environmental effects for the cows, $\boldsymbol{s}$ = a vector for random calf sire effects, and $\boldsymbol{e}$ = a vector of random residual effects. **X, Z, W** and **S** were incidence matrices for **b, u, p** and **s**, respectively. The variance-covariance structure for random effects was:

$$\text{var}\begin{bmatrix} u \\ p \\ s \\ e \end{bmatrix} = \begin{bmatrix} \mathbf{G} \otimes \mathbf{H} & 0 & 0 & 0 \\ 0 & \sigma^2_p\mathbf{I}_p & 0 & 0 \\ 0 & 0 & \sigma^2_s\mathbf{I}_s & 0 \\ 0 & 0 & 0 & \sigma^2_e\mathbf{I}_n \end{bmatrix}$$

where $\boldsymbol{G} = a2 \times 2$ (co)variance matrix for random regression coefficients for the additive genetic effect, $\boldsymbol{H}$ = the combined relationship matrix as explained above, σ_p^2, σ_s^2 and σ_e^2 = variances for the permanent environmental, calf sire and residual effect, respectively, $\mathbf{I_p}$, $\mathbf{I_s}$ and $\mathbf{I_n}$ = identity matrices for p cows, s calf sires and n observations, respectively, and $\otimes$ denotes the Kronecker product. Genetic variances for each parity and covariances between the same trait from different parities were calculated as a function of the covariables in the model, i.e., intercept and slope. Parity specific heritabilities were computed considering the parity specific genetic variances and the variance components for the permanent environmental and residual effect.

In models 2 and 3, the calf trait is considered as an observation of the dam and the genetic relationship matrix was constructed from the dam perspective. From the genetic-modelling perspective, it is a direct genetic effect, but from the biological perspective, we denote this effect as "maternal" in the ongoing presentation of results and in the discussions.

For models 1 and 2 we used GBLUP methodology, and for the RRM ssGBLUP, as implemented in the BLUPF90 software packages (Mistzal et al., 2014) to estimate (co)variance components and breeding values. The convergence criterion for all three models was the change of the log-likelihood function between iterations. For the RRM (model 3), the run was finalized when the relative change was smaller than 10^{-12}, and smaller than 10^{-10} for models 1 and 2.

2.3.4. Breeding value correlations

Parity specific GEBV (results from the MTM (model 2) and the RRM (model 3)) for SB and CE were standardized to relative breeding values with a mean of 100 and a SD of 12 points. These relative breeding values indicate the genetic effects on SB and CE from the cow (maternal) perspective in parities 1, 2 and 3, and were denoted for SB as R_SB_p1_m2, R_SB_p2_m2, R_SB_p3_m2, R_SB_p1_m3, R_SB_p2_m3, R_SB_p3_m3, and for CE as R_CE_p1_m2, R_CE_p2_m2, R_CE_p3_m2, R_CE_p1_m3, R_CE_p2_m3, R_CE_p3_m3 with p = the parity and m = the model. The maternal genetic perspective is also depicted through the maternal genetic effect as considered in model 1 (i.e., the model from the calf perspective). In consequence, we considered the solutions for the maternal genetic component from model 1, and we also standardized these GEBV to a mean of 100 and a SD of 12 points. The relative breeding values for SB and CE from model 1 were denoted as R_SB_m1 and R_CE_m1, respectively.

Correlations among the relative breeding values from the 3 models were computed considering 890 cow sires with at least 10 daughters for both traits SB and CE. Furthermore, all these cow sire relative breeding values were correlated with relative breeding values for the direct calving component for SB and CE (**RZ_SB_dir, RZ_CE_dir,** respectively) and for the maternal calving component (**RZ_SB_mat**, RZ_**CE_mat**) from the official national German genetic evaluation from 04/2022 (vit, 2025). In addition to all GEBV correlations, we ranked the 890 sires according to their respective GEBV, and we computed Spearman rank correlations.

3. Results

3.1. Heritabilities and variance components for same calving traits in different parities

Variance components and heritabilities for CE and SB from model 1 addressing the calf perspective and model 2 addressing the cow perspective are given in Table 2. The maternal heritabilities for CE and SB from model 1 reflecting the genetic effect of the dam were 0.045 and 0.038, respectively. These maternal estimates agree with the maternal heritabilities from model 2 which ranged from 0.035 (parity 3) to 0.086 (parity 1) for CE, and from 0.032 (parity 3) to 0.074 (parity 1) for SB. Hence, for both traits CE and SB, a gradual decline of genetic variances and heritabilites was observed with increasing lactation number. The direct heritabilities (estimates from model 1) reflecting the genetic effect of the calf were 0.053 for CE and 0.076 for SB, and were marginally larger than the corresponding maternal heritabilities. The variance component related to the service sire for CE and SB from model 2 was small and in a narrow range across all parities. The residual variance gradually increased with increasing parity for CE from 0.344 to 0.638, but was stable (0.01 to 0.02) for SB.

The maternal heritabilites from the MTM (model 2) reflect the heritability pattern along the parity scale from the RRM (Fig. 1). Accordingly, from the RRM, the heritability for CE was 0.111 in parity 1 and declined from 0.067 (parity 2) to 0.047 (parity 3). For SB, the maternal heritability gradually decreased from 0.063 in parity to 0.023 in parity 3. The decreasing heritabilities for CE and SB with increasing lactation number were due to the decline of additive genetic variation with aging. Across all models and parities, genetic variances and heritabilties for the maternal component were larger for CE than for SB. Due to the large sample size, all direct and maternal heritability estimates from all models had small standard errors (Table 2).

Table 2
Variance components for direct genetic, maternal genetic[1], maternal permanent environmental, service sire and residual effects and direct and maternal[1] heritabilities with corresponding SE (in brackets) for calving ease (CE) and stillbirth (SB) from the direct-maternal genetic model (model 1 = allocation of observations to the calf) and from the multiple-trait model (model 2 = allocations of observations to the cow).

Model	Trait	Parity	Variance components						
			Direct genetic	Maternal genetic	Maternal pe	Service sire	Residual	h^2-direct	h^2-maternal
1	CE	all	0.014	0.012	0.008		0.223	0.053 (0.003)	0.045 (0.003)
2	CE	1		0.037		0.050	0.344		0.086 (0.002)
2	CE	2		0.027		0.049	0.581		0.042 (0.001)
2	CE	3		0.025		0.042	0.638		0.035 (0.001)
1	SB	all	0.001	0.001	<0.001		0.009	0.076 (0.001)	0.038 (0.001)
2	SB	1		0.001		<0.001	0.011		0.074 (0.004)
2	SB	2		0.001		<0.001	0.016		0.043 (0.003)
2	SB	3		0.001		<0.001	0.018		0.032 (0.003)

[1] In model 2, the calf trait is considered as an observation of the dam and considering the genetic relationship matrix from the dam perspective. From the genetic modelling perspective, it is a direct genetic effect, but from the biological perspective, we denote this effect as "maternal".

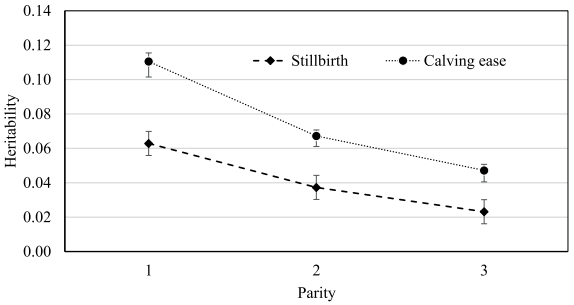

Fig. 1. Maternal genetic heritabilities in parities 1, 2 and 3 (i.e., the additive genetic variations from Fig. 1 in relation to the total trait variation) with respective SE (indicated as stripes) for calving ease and for stillbirth from the random regression model (model 3).

3.2. Genetic correlations between same calving traits from different parities

The multiple-trait modelling approach considering same traits from different parities as different traits and the RRM considering a continuous parity scale enabled the estimation of genetic correlations for all parity combinations. The genetic correlations from the MTM were 0.88 between CE from parity 1 with CE from parity 2, 0.83 between CE from parity 1 with CE from parity 3, and 0.92 between CE from parity 2 with CE from parity 3. The genetic correlations for the respective parity combinations for CE from the RRM were 0.90 (parity 1 with parity 2), 0.85 (parity 1 with parity 3) and 0.93 (parity 2 with parity 3). Also, measurements for SB in neighboring distance were higher correlated than measurements being far apart, i.e., 0.84 (parity 1 with parity 2) and 0.88 (parity 2 with parity 3) compared to 0.76 (parity 1 with parity 3) as outlined for the RRM in Fig. 2. The respective genetic correlations for SB from the MTM were 0.81 (parity 1 with parity 2), 0.90 (parity 2 with parity 3) and 0.72 (parity 1 with parity 3).

Accordingly, we correlated the parity specific maternal relative breeding values of the 890 cow sires with at least 10 daughters. The breeding value correlations for SB are depicted in Table 3, and for CE in Table 4. Generally, the breeding value correlations reflect the pattern of genetic correlations, i.e., 0.85 between the relative breeding value for SB in parity 1 with the relative breeding value for SB in parity 2, 0.89 between SB in parity 2 with SB in parity 3, and the smallest value of 0.69 between SB in parity 1 with SB in parity 3 (results from the MTM = model 2). The respective correlations for SB from the RRM were 0.80 (parity 1 with parity 2), 0.86 (parity 2 with parity 3) and 0.78 (parity 1 and parity 3). Regarding CE and model 2 (Table 4), the breeding value correlations were 0.91 (parity 1 and parity 2), 0.81 (parity 1 with parity 3) and 0.97 (parity 2 and parity 3). The respective breeding value correlations from the RRM were 0.84 (parity 1 with parity 2), 0.79 (parity 1 with parity 3) and 0.95 (parity 2 with parity 3). Hence, the largest GEBV and genetic correlations were always found when correlating same traits from parity 2 and parity 3.

3.4. Breeding value correlations between same calving traits from different models

The most important issue in the context of genetic-statistical modelling approaches addresses breeding value correlations for same traits in same parities. In consequence, we correlated the relative breeding values of the 890 sires for SB from model 2 (MTM) in parities 1, 2 and 3 with the respective parity specific relative breeding values from model 3 (RRM), and with the maternal genetic breeding values from model 1 (Table 3). The same correlation calculations were performed for CE (Table 4). The sire breeding value correlations between SB from different models (MTM and RRM) and same parties were substantial with 0.88 in parity 1, 0.82 in parity 2 and 0.84 in parity 3. Regarding model 1, overall maternal breeding values comprising all parities simultaneously were estimated. Also, the correlations between R_SB_m1_mat (= the relative maternal breeding value from model 1) with all other parity specific sire breeding values from models 2 and 3 were large in the range from 0.76 to 0.88 (Table 3).

Regarding sire breeding value correlations for CE from models 2 and 3 (Table 4), the correlation coefficients in same parities were 0.90

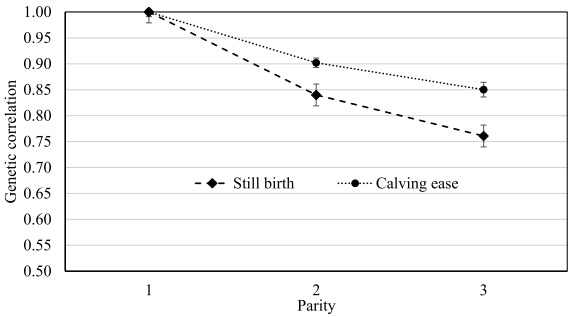

Fig. 2. Genetic correlations for direct genetic effects (referred to as "maternal genetic component) with respective SE (indicated as stripes) between calving ease in parity 1 with calving ease in parities 2 and 3, and between stillbirth in parity 1 with stillbirth in parities 2 and 3 from the random regression model (model 3).

Table 3
Correlations[1] among cow sire genomic breeding values (890 cow sires with at least 10 daughters) for stillbirth (SB) from model 1 (for the maternal component = m1_mat) and from models 2 and 3 (for the additive genetic component of the cow denoted as "maternal") in same parities and the respective rank correlations (in brackets).

	Relative breeding value						
	m1_mat	p1_m2	p2_m2	p3_m2	p1_m3	p2_m3	p3_m3
m1_mat		0.83	0.78	0.77	0.88	0.76	0.77
		(0.85)	(0.79)	(0.79)	(0.91)	(0.78)	(0.78)
p1_m2			0.85	0.69	0.88	0.65	0.62
			(0.88)	(0.70)	(0.89)	(0.68)	(0.65)
p2_m2				0.89	0.66	0.82	0.69
				(0.92)	(0.68)	(0.86)	(0.73)
p3_m2					0.73	0.75	0.84
					(0.77)	(0.76)	(0.85)
p1_m3						0.80	0.78
						(0.83)	(0.79)
p2_m3							0.86
							(0.89)

[1] All correlation coefficients differed significantly from zero at $P < 0.01$.
[2]p1, p2, p3 indicates the parity 1, 2 and 3, respectively; m1, m2, m3 indicates the statistical model 1, 2 and 3, respectively.

Table 4
Correlations[1] among cow sire genomic breeding values ((890 cow sires with at least 10 daughters) for calving ease (CE) from model 1 (for the maternal-genetic component = m1_mat) and from models 2 and 3 (for the additive genetic component of the cow denoted as "maternal") and the respective rank correlations (in brackets).

	Relative breeding value[2]						
	m1_mat	p1_m2	p2_m2	p3_m2	p1_m3	p2_m3	p3_m3
m1_mat		0.83	0.71	0.79	0.68	0.75	0.80
		(0.86)	(0.75)	(0.80)	(0.69)	(0.79)	(0.84)
p1_m2			0.91	0.81	0.90	0.73	0.64
			(0.94)	(0.82)	(0.92)	(0.74)	(0.67)
p2_m2				0.97	0.71	0.84	0.74
				(0.98)	(0.75)	(0.85)	(0.75)
p3_m2					0.69	0.80	0.88
					(0.73)	(0.82)	(0.89)
p1_m3						0.84	0.79
						(0.87)	(0.80)
p2_m3							0.95
							(0.95)

[1] All correlation coefficients differed significantly from zero at $P < 0.01$.
[2] p1, p2, p3 indicates the parity 1, 2 and 3, respectively; m1, m2, m3 indicates the statistical model 1, 2 and 3, respectively.

(parity 1), 0.84 (parity 2) and 0.88 (parity 3). The breeding value correlations between the overall maternal breeding value for CE from model 1 (R_CE_m1_mat) with all other parity specific breeding values were in a range from 0.68 to 0.83. Accordingly, the correlations between sire breeding values across different models and parities were large, displaying a smallest estimate of 0.62 (R_SB_p1_m2 with R_SB_p3_m3). In agreement for CE, the smallest correlation coefficient (0.64) was found when correlating R_CE_p1_m2 with R_CE_p3_m3, i.e., the maternal breeding value from the MTM in parity 1 and the maternal breeding value from the RRM in parity 3. Hence, both aspects, different modelling approaches and parities in greater distance, contributed to the smallest breeding value correlations.

3.5. Breeding value correlations with official breeding values for maternal and paternal stillbirth and calving ease

The correlations between the maternal breeding values from models 1, 2 and 3 for the 890 sires with their official breeding values from the German national genetic evaluations for maternal and paternal SB and CE are given in Table 5. The correlations between the maternal breeding values with the respective official breeding value for SB (RZ_SB_mat) were substantial and ranged from 0.82 to 0.90. The well-known close genetic relationship between maternal SB and maternal CE is reflected through the breeding value correlations between the maternal breeding values from all models with RZ_CE_mat in a range from 0.61 to 0.71. The correlations between the maternal breeding values for SB from models 1, 2 and 3 with the official direct breeding value for SB (RZ_SB_dir) were close to zero or even negative, indicating an unfavorable (antagonistic) genetic relationship between the maternal and direct SB component. A similar pattern of breeding value correlations was found for the correlations between the maternal breeding values for SB with the official relative direct breeding value for CE (RZ_CE_dir).

In agreement with SB, the correlations between the maternal breeding values for CE from models 1, 2 and 3 with the official maternal relative breeding value for CE (RZ_CE_mat) were larger than 0.80, and in a moderate to large range from 0.66 to 0.76 with RZ_SB_mat. Accordingly, for CE, the breeding value correlations between the maternal component from the own modelling approaches with official direct breeding values for CE were unfavorable or very close to zero. The same trend of correlation pattern was found for maternal breeding values for CE and the official paternal breeding value for SB (RZ_SB_pat).

Model 1 which related the CE and SB observation to the calf and simultaneously considering the genetic relationships for the direct and the maternal component enabled the estimation of genetic covariances and genetic correlations between direct and maternal genetic effects. The respective correlation between direct and maternal genetic effects was −0.11 for SB and −0.19 for CE. The respective breeding value correlations considering the 890 sires were −0.07 (SB) and −0.13 (CE). Hence, the most pronounced antagonistic relationships (but still close to zero) between paternal and maternal genetic effects over all models and

Table 5
Correlations between relative breeding values[1] considering 890 cow sires with at least 10 daughters for stillbirth (SB) and for calving ease (CE) from model 1 (for the maternal genetic component = m1_mat) and from models 2 and 3 (for the additive genetic component of the cow denoted as "maternal") with relative breeding values from the official genetic evaluation for the direct component for SB and CE (RZ_SB_dir, RZ_CE_dir, respectively) and for the maternal component (RZ_SB_mat, RZ_CE_mat, respectively), and respective rank correlations (in brackets).

		Relative breeding values from the German national official genetic evaluation			
Trait	Breeding values[1]	RZ_SB_dir	RZ_CE_dir	RZ_SB_mat	RZ_CE_mat
SB	m1_mat	0.03	0.05	0.87	0.69
		(0.08)	(0.06)	(0.90)	(0.74)
	p1_m2	−0.01	−0.11	0.84	0.71
		(0.00)	(−0.07)	(0.85)	(0.74)
	p2_m2	0.00	0.01	0.88	0.70
		(0.03)	(0.05)	(0.88)	(0.74)
	p3_m2	−0.03	−0.10	0.90	0.65
		(0.00)	(−0.07)	(0.91)	(0.68)
	p1_m3	−0.01	−0.06	0.82	0.69
		(−0.01)	(−0.02)	(0.84)	(0.73)
	p2_m3	0.11	0.03	0.83	0.61
		(0.14)	(0.06)	(0.84)	(0.65)
	p3_m3	0.02	0.01	0.84	0.63
		(0.05)	(0.02)	(0.84)	(0.67)
CE	m1_mat	−0.04	−0.10	0.76	0.90
		(−0.02)	(−0.07)	(0.79)	(0.92)
	p1_m2	0.00	−0.06	0.73	0.85
		(0.04)	(−0.02)	(0.75)	(0.89)
	p2_m2	−0.04	−0.10	0.67	0.84
		(0.00)	(−0.06)	(0.68)	(0.85)
	p3_m2	−0.10	−0.12	0.75	0.92
		(−0.07)	(−0.10)	(0.79)	(0.92)
	p1_m3	0.01	0.00	0.66	0.84
		(0.04)	(0.01)	(0.68)	(0.87)
	p2_m3	0.07	0.11	0.70	0.85
		(0.10)	(0.12)	(0.74)	(0.86)
	p3_m3	0.01	0.05	0.68	0.83
		(0.05)	(0.06)	(0.70)	(0.85)

[1] Correlation coefficients between maternal and direct relative breeding values were not significantly different from zero, correlation coefficients between maternal relative breeding values differed significantly from zero at $P < 0.01$.

parities were obtained from model 1.

4. Discussion

4.1. Genetic parameters for same calving traits in different parities

The small maternal heritabilities from the different modelling approaches in the present study for CE (range 0.035 to 0.086) and for SB (0.032 to 0.074) reflect parameter estimates in other populations and from alternative statistical models. The generally small maternal genetic influence on calving traits and the magnitude of the residual component was outlined in recent studies by Silva et al. (2020) and Marinho de Negreiros et al. (2024) in Nellore cows, and by Silvestre et al. (2019) in Portuguese dairy cattle. Marinho de Negreiros et al. (2024) estimated direct and maternal heritabilities for CE in primiparous Nellore cows using a threshold model, and reported values of 0.027 and 0.019, respectively. This threshold model as applied by Marinho de Negreiros et al. (2024) corresponds with our model 1 (i.e., allocating the CE record to the calf), apart from the threshold versus linear modelling perspective. Accordingly, we found smaller maternal heritabilites than direct heritabilities for the calving traits. In contrast, Silva et al. (2020) reported a higher estimate of 0.039 for the maternal CE heritability compared to the direct CE heritability (0.018) when applying a threshold sire-maternal grandsire model.

Our multiple-trait (model 2) and random regression (model 3) modelling approaches enabled the estimation of parity-specific genetic parameters. However, allocating CE and SB observations to the dam is rather unusual in cattle, but was practiced in swine. As one example, Klein et al. (2018) recorded fitness traits of piglets directly after birth, but genetic evaluations were performed from the sow perspective. In this study, Klein et al. (2018) defined parity (= litter number) as fixed effect, implying overall instead of parity-specific genetic parameter estimates. As an extension, the MTM and RRM modelling approaches from the present study enabled the definition of genetic covariance structures among the different cow parities, contributing to deeper insights into genetic parameters for distinct lactation numbers. In such context, we found declining genetic variances and maternal heritabilities with increasing parity, from the MTM as well as from the RRM application. Smaller heritabilities with increasing lactation number might be due to selection, narrowing additive genetic variation as theoretically outlined by Dempfle (1990). In a quantitative genetic study conducted in the dairy sheep breed Manech Tête Rousse, Macedo et al. (2020) estimated base population genetic variances for base populations from birth years 1981 to 2014. They found a reduction of genetic variation in milk yield in younger birth years due to increasing relationships and intensified selection. Genomically, reduced genetic variation due to selection was outlined via homozygosity measurements in French Holstein bulls (Doublet et al., 2019). However, in the French local cattle breeds Montbelliard and Normande characterized by less intensive selection, no significant changes over birth years were identified. In the past in dairy cattle breeding programs, effects of selection in Holstein populations were mainly found for production traits due to intensified selection on milk and protein yields (e.g., Powell et al., 2003), but not for low heritability functional traits. In recent years, the selection focus in Holstein dairy cattle has changed, explaining the narrowing of genetic variation for female fertility traits with increasing parity (Sakhaeifar et al., 2025).

One major finding from the present study is the agreement of maternal heritabilities from the MTM (model 2) and RRM (model 3). The very large dataset comprising 435,489 and 477,800 calf records for CE and SB, respectively, and 184,012 and 177,162 cow records for SB and CE, respectively, contributed to consistent genetic (co)variance components, irrespective of the statistical modelling strategy. All cows and calves from the present study were kept in large-scale dairy cattle farms. Stronger effects of the applied genetic statistical modelling approach on genetic parameter estimates were identified in small datasets and small contemporary groups for genetic evaluations, as outlined by Yin et al. (2012) in the case of organic dairy cattle farming in mountainous regions. In the present study, all models 1, 2 and 3 were linear models, considering the categorical data structure as Gaussian distributed. Theoretically, for such types of data, threshold models should be applied for genetic evaluations of CE and SB as done by, e.g., Cole et al. (2007) or Hansen et al. (2004). However, larger genetic parameter differences from threshold versus linear models are expected only for small datasets and extremely small incidences for SB, or a very limited number of observations for specific CE categories. Theoretical proofs were derived by Freund and Walpole (1980), and the incidence related effects are reflected in the "heritability transformation formula" (Dempster and Lerner, 1950). For SB, a substantial number of 27,745 calves were dead within the first 48 h after birth and allocated to the score 1, and all CE classes comprised many observations with 321,554, 89,673, 23,226 and 1036 records from parity 1, 2, 3 and 4 cows, respectively.

Genetic correlations between same traits from different parities were larger for neighboring parities compared to estimates between parity 1 and parity 3. This finding agrees with results based on dense longitudinal test-day production (Schaeffer, 2004) or health records (Gernand and König, 2014). The genetic correlations between same traits from parity 2 and parity 3 were larger than the correlations between parity 1 and parity 2. An explanation might be related to the physiological differences in first parity compared to older cows, with additional energy requirements for growth and differing energy balance profiles,

providing evidence for genetic driven body energy alterations (Friggens et al., 2007).

4.2. Breeding value correlations between same traits from different models

Very similar genetic parameters for same traits in same parities from models 2 and 3 as outlined above might explain the moderate to large respective breeding value correlations based on the 890 sires with at least 10 daughters. The correlations between parity-specific relative breeding values from the MTM with respective breeding values from the RRM were throughout larger than 0.80, indicating only minor re-rankings of sires due to different statistical modelling approaches. The high agreement between RRM-GEBV and MTM-GEBV indicates the suitability of random regression modelling approaches also for longitudinal data with measurements in great distance, i.e., the parities in the present study. However, an open question addresses the modeling of the most propriate covariance functions to model the variances and covariances of a longitudinal trait along the continuous age scale. In the present study, we modelled random regression coefficients for additive genetic effects and permanent environmental effects of cows considering the coefficients for intercept and slope from linear regressions, without evaluating further functions. Kirkpatrick et al. (1990) gave a broad overview for the modelling perspectives, whereas orthogonal polynomials of standardized units of time seem to be most appropriate. Gernand and König (2014) focused on deeper validations regarding RRM applications for novel traits and a broad variety of continuous gradients, and they generally favored Legendre polynomials of order 3. Accordingly, also for other continuous gradients than the "classic" days in milk, e.g., climatic scales (Yin et al., 2014) or rural-urban distances (Velayudhan et al., 2021), the best goodness of fit was achieved with Legendre polynomials of order 3 or 4.

A good agreement is also depicted for the maternal breeding values from model 1 with all parity-specific breeding values from models 2 and 3. The correlations between breeding values ranged from 0.76 to 0.88 for SB, and from 0.75 to 0.83 for CE. The classical genetic evaluation approach as reflected via model 1 only present one overall maternal breeding value, implying the assumption of stable gene effects across lactations. However, alterations of genetic parameters from RRM are an indication that different genes are active or switched off with progressing time (Schaeffer, 2004). Accordingly, in genome-wide associations for female fertility traits, SNP effects differed in different lactations (Pimentel et al., 2011), indicating the importance of the SNP × time interaction term (Sikorska et al., 2015). The molecular explanation for genetic parameter and SNP effect alterations with progressing time are the differentially expressed genes, as outlined by, e.g., McGrath et al. (2021). From a practical breeding perspective, we assume stronger genetic gain when basing mating and selection decisions based on parity-specific GEBV from the models 2 and 3. In Germany, herd age structures vary widely, with a large percentage of first parity cows in the large-scale commercial production herds due to the large intra-herd replacement herds, and in contrast, and an older cow age structure in the small family farms or especially in the organic herds (Shabalina et al., 2020). In consequence, for young-cow herd age structures, sires with high maternal breeding values for CE and SB in first and second parity should be preferred, but the sires with high R_SB_p3_m2 and R_SB_p3_m3, and high R_CE_p3_m2 and R_CE_p3_m3 to improve SB and calving ease, respectively, in the herds with high fractions of old cows in late parities.

4.3. Maternal and direct stillbirth and calving ease genetic relationships

Model 1 applications indicted genetic antagonistic relationships between direct and maternal effects on SB and CE. Specifically, the correlation between direct and maternal genetic effects was −0.11 for SB and −0.19 for CE. The respective breeding value correlations considering the 890 sires were −0.07 (SB) and −0.13 (CE). Small deviations between breeding value correlations and genetic correlations are due to breeding value accuracies which are smaller than 1. Only in case of very accurate EBV with an accuracy of 1, breeding value and genetic correlations are expected to be identical (Calo et al., 1973). The correlations between the maternal sire breeding values from models 2 and 3 with the respective direct breeding values from official national German genetic evaluations were negative or very close to zero, supporting the antagonistic relationship from model 1. In the literature, very strong negative (unfavorable) up to moderately positive correlations between direct and maternal effects for calving traits have been reported, depending on the breed, the population and the statistical modelling approach. For example, in Korean Holstein cows, the direct-maternal correlation for CE was pronounced negative for all applied models up to −0.81 in the herds with large contemporary groups, but close to zero with a largest coefficient of 0.08 in the small sized herds (Alam et al., 2024). The negative direct-maternal correlation for CE suggests that female calves with small body sizes (implying improved CE) turn out to be small dams with small pelvis width, implying deterioration in CE from the dam perspective.

In Dutch Holstein-Friesian dairy cattle, Eaglen and Bijma (2009) created different data subsets depending on the CE residuals between dam and offspring from different models, and they applied sire-maternal grandsire and animal models for genetic evaluations. The direct-maternal genetic correlations varied for the different subsets and models from −0.07 to −0.44. Eaglen and Bijma (2009) highlighted the very small covariance between direct and maternal genetic effects from all analyses, indicating that moderate antagonistic correlations are due to the extremely small variances for CE in the denominator.

For SB, the picture for direct-maternal genetic associations reflects the associations as presented above for CE. In US Holsteins, the mean genetic correlation coefficient between direct and maternal effects for SB was close to zero with −0.02 (Cole et al., 2007). In contrast, stronger antagonistic relationships between the two effects were found in other dairy cattle breeds from the US with −0.15 in Jersey and −0.35 in Brown Swiss (Sigdel et al., 2022). Negative (unfavorable) genetic associations between direct and maternal SB effects were also reported some decades ago in Canadian (Luo et al., 1999) and in Swedish Holstein (Steinbock et al., 2003). Steinbock et al. (2003) estimated direct-maternal genetic correlations for SB separately in first and second parity cows. In agreement with our present study, they found only minor genetic correlation differences between both effects along the parity trajectory.

Furthermore, the genetic correlations as estimated by Steinbock et al. (2003) were very similar from the linear modelling approach and from the threshold model application. This finding reflects the proof given by Vinson and Kluwer (1976), indicating that genetic correlations should be identical regardless of if the trait of interest is considered as a real categorical or as a Gaussian trait. In beef cattle, numerous studies intensively addressed direct-maternal genetic correlations, but with focus on weight traits (e.g., Bonifazi et al., 2020). Nevertheless, calf weights are a very good proxy for CE and SB (Johanson and Berger, 2003), and the within-country and between-country evaluations for beef weights (Bonifazi et al., 2020) confirmed the range of direct-maternal genetic associations from the present study.

4.4. Practical implications

From a more practical breeding perspective, the present study indicated the possibility to allocate SB and CE observations to the dam, allowing to estimate parity specific GEBV via RRM applications. Such approach might be an extension of official genetic evaluations for both traits SB and CE following the procedure for female fertility traits. In official genetic evaluations for female fertility traits in Germany, non-return rates for heifers and for cows from later parities are considered as different traits since almost two decades (Pasman et al., 2006).

Recently, RRM applications for female fertility traits indicated alterations of (co)variance components for non-return rates and days open across cow parities (Sakhaeifar et al., 2025). As an alternative to RRM, multiple-trait models have the potential to estimate parity specific EBV for SB and CE, but RRM have a greater flexibility for the modelling of appropriate covariance functions over time (Schaeffer, 2004). Respective advances of random regression modelling approaches in the genomic era have been outlined by Oliveira et al. (2019). From a theoretical perspective, RRM are most suitable for the genetic analysis of longitudinal data structures. However, we must state that the correlations between maternal GEBV from our RRM and maternal GEBV from official genetic evaluations were throughout larger than 0.82, and the large rank correlations indicate only minor changes in top lists for sires. The greatest advantage in presenting parity-specific GEBV for SB and CE is the possibility to select appropriate sires according to the age structure of the herd. Consideration of phenotypic, genomic and quantitative herd characteristics in the context of cow sire selection have been outlined by Yin and König (2018).

5. Conclusion

The alternative genetic-statistical modelling approach, i.e., allocating the SB and CE observations to the dam, enabled the estimation of genetic parameters and GEBVs for specific cow parities. We found very consistent direct and maternal heritabilities from the MTM and the RRM, both indicating a gradual decrease of additive genetic variances and heritabilities for CE and SB with increasing lactation number. The maternal heritability was smaller than the direct heritability, which was confirmed from the "classical" calf model. Genetic correlations between same traits in different parities were larger for neighboring parities than for parities in greater distances. All genetic correlations or breeding value correlations between direct and maternal genetic effects were close to zero. The new RRM modelling approach from the cow perspective has potential to optimize genetic evaluations for SB and CE by reflecting time-dependent genetic mechanisms in more detail. However, from a practical breeding perspective, only minor re-rankings of sires were observed when compared to rankings from official national genetic evaluations.

Author statement

thanks for you detailed comments, which helped us to improve our manuscript. We considered all your comments in the revised manuscript (highlighted in yellow). In addition, please see our response letter below. The only comment, which we did not address: One reviewer suggested to indicate confidence intervals for the heritabilities, but we presented the respective standard errors in the Table. Should make no difference.

CRediT authorship contribution statement

Sina Sakhaeifar: Writing – original draft, Investigation, Formal analysis. **Sven König:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Data curation, Conceptualization.

Declaration of competing interest

We declare that we have no conflicts of interest.

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CHAPTER 5

General Discussion

General discussion

The main objective of this study was to comprehensively evaluate the genetic architecture of female fertility and calving traits in German Holstein cattle using different modelling approaches. The research aimed to estimate genetic parameters, including heritabilities, genetic correlations, and breeding values across different reproductive traits, while accounting for the dynamic nature of fertility traits including none-return rate in day 56 (**NRR56**), calving to first service (**CTFS**), days open (**DO**) in chapter 2, and calving traits including calving ease (**CE**) and stillbirth (**SB**) in chapter 4. The study also compared the performance of different statistical frameworks conventional animal models, repeatability models, and random regression models (**RRM**) to identify the most appropriate methodology for longitudinal reproductive data for fertility traits and compared multiple trait model (**MTM**) models, maternal model and RRM for calving traits. A further, secondary objective was to apply longitudinal genome wide association study (GWAS) and integrate genomic and functional findings to complement the quantitative genetic results and provide biological context to the observed genetic patterns, which is completely explained in chapter 4.

Overall, this study aims to (1) estimate genetic parameters for fertility and calving traits using different model structures, (2) quantify the stability of genetic correlations across time, (3) assess the suitability of RRM for routine genetic evaluation, and (4) briefly link the most influential genomic regions to biological pathways relevant to fertility using longitudinal GWAS. The overarching goal was to enhance genetic improvement strategies for fertility traits in Holstein cattle while maintaining balance with production and health traits.

5.1 Reproductive performance

Reproductive performance is a cornerstone of dairy herd sustainability and economic efficiency. Improving reproductive performance in dairy cattle requires a comprehensive understanding of both female fertility traits and calving traits. These traits share biological pathways related to reproductive physiology and calving processes, and both are characterized by low heritability, strong environmental influence, and complex direct-maternal genetic relationships. Consequently, robust genetic evaluations benefit from longitudinal modelling approaches capable of capturing parity-specific dynamics, heterogeneous variances over time, and genetic correlations across lactations. Declining fertility in high-producing Holsteins has been widely documented (Jorjani 2006; Liu et al. 2008), and improving it genetically requires a robust understanding of heritability and correlations with other traits. Fertility and calving traits are typically characterized by low heritability, complex biological regulation, and strong environmental influences (Veerkamp et al. 1999). The use of RRM allows for a more precise estimation of time-dependent genetic variances and correlations, providing greater insight into how genetic control changes throughout lactation (Schaeffer 2004). This approach also facilitates the derivation of EBV at specific time points, offering more refined tools for selection.

5.1.1 Female fertility trait

The results of this thesis provided valuable insight into the heritable nature of fertility and calving traits in German Holsteins. Across models, heritability estimates for fertility traits were generally low to moderate, confirming that environmental and management factors exert substantial influence. Although the differences in heritabilities between parities in the present study were small, their upward trend in cows may initially seem surprising. Selection, both natural and artificial, often

reduces genetic variance by eliminating inferior individuals before they reach later parities. Artificial selection can also gradually diminish genetic diversity (Doublet et al. 2019), which should theoretically reduce additive variance with age. However, several alternative explanations may account for the observed pattern. First, comparing heifers to cows does not reflect changes within individuals across time; instead, genetic variances in heifers across years or in cows across consecutive parities may provide more biologically meaningful indicators of true evolutionary dynamics. Second, fertility in heifers and cows may reflect different underlying genetic architectures. Differences in gene expression across the lifespan have been shown in humans (Zhang et al. 2020), and comparable mechanisms may play a role in cattle and might contribute to variation in genetic parameter estimates. However, the magnitude of heritability varied across lactation stages, indicating that genetic control is not constant over time. These patterns were captured most effectively by the RRM approach, which demonstrated superior flexibility in modeling genetic variances and covariances along the reproductive trajectory.

For early postpartum intervals, when cows are under negative energy balance and physiological stress, heritability estimates were typically low, reflecting stronger environmental and metabolic effects (Roche 2006). Moreover López-Catalina et al. (2024) also suggested that calves gestated by lactating versus non-lactating cows exhibit different methylation profiles, supporting the broader concept that parity-specific physiological conditions may influence genetic parameter expression. As lactation progressed and animals re-entered estrous cycles, additive genetic variance increased, and heritability values rose accordingly. Such temporal changes align with previous reports that fertility heritability is dynamic and context-dependent (Jamrozik et al. 1997 & 2005; Haile-Mariam et al. 2003 & 2008).

The identification of these periods of elevated genetic variance has direct implications for targeted selection strategies, as breeding efforts may be more effective when focused on biologically stable phases. Kipp et al. (2021) demonstrated genotype-by-heat-stress interactions influencing NRR56 and altering (co)variance components. Their additional work (Kipp et al. 2021) showed that such environmental exposures can influence traits expressed later in life, such as longevity. Taken together, the consistency between RRM and MTM in this study strengthens confidence in the robustness of the genetic parameter estimates. While MTM remains intuitive for parity-specific evaluations, RRM offers greater flexibility for modelling continuous trajectories and handling missing data and environmental gradients.

5.1.2 Calving traits

Genetic evaluation of calving traits such as **CE** and **SB** is crucial for improving animal welfare, reducing economic losses and enhancing reproductive efficiency in dairy cattle populations. These traits, like female fertility, are characterized by low heritability, substantial environmental influence and complex direct-maternal relationships. The findings in the present study confirm the well-known challenges associated with CE and SB and provide important insights into how different statistical models influence estimates of genetic parameters along the parity trajectory.

Calving traits such as **CE** and **SB**, showed lower heritability, consistent with earlier literature (Cole et al. 2005; Berger 1994). Across all models, calving traits expressed through the cow, particularly maternal CE and maternal SB, showed heritabilities ranging between 0.035 and 0.086 for CE and between 0.032 and 0.074 for SB, consistent with previous studies applying linear and threshold models in Holstein and beef populations (Silvestre et al., 2019; Silva et al., 2020; Marinho de Negreiros et al., 2024).

Interestingly Marinho de Negreiros et al. (2024) estimated direct and maternal heritabilities similar to our result for CE in primiparous Nellore cows 0.027 and 0.019 using threshold models, apart we used linear model. These values confirm that maternal contributions, although modest, are meaningful and should not be ignored in genetic evaluations. Furthermore, results showed clear parity-specific patterns. Maternal genetic variances tended to decline from first to later parities, a result also reflected in additive genetic variances. This pattern is biologically plausible, as young cows differ in pelvic development, metabolic demands and hormonal environment compared with older cows. Declining variance with increasing parity may also reflect the effect of selection, as cows with severe calving difficulties in early parities are more likely to be culled before reaching higher parities, an argument also relevant for other reproductive traits (könig et al 2005).

Estimated breeding values derived from the random regression models provided additional insight. The temporal EBV patterns reflected differences in genetic merit expression across lactation, with certain sires exhibiting superior fertility performance during mid-lactation but lower values in early or late phases. This variability supports the concept that EBVs for reproductive traits are not static but evolve with physiological state. Such dynamic EBVs offer a refined selection tool compared with traditional aggregate fertility indices.

5.2 Methodological Insights: Model Comparison and Parameter Estimation

A central contribution of this study lies in comparing modeling strategies for reproductive traits. For fertility traits we applied a comparison between multiple-trait models (MTM) and RRM. Both models indicate slightly increase in all three traits with increasing parity. Liu et al. (2008) applied MTM model for NRR56 between heifers and cows and they obtained different heritability for heifers and cows. Moreover, their estimates for heritability were in consistence with our results.

The RRM, implemented using second-degree Legendre polynomials, allowed each individual's trajectory to vary flexibly across time (Li et al., 2020; Campbell et al., 2018). This indicates that RRM better represents biological reality, where environmental and genetic influences fluctuate across reproductive stages (Schaeffer, 2004; Strabel et al., 2001). The covariance functions estimated from the RRM demonstrated smooth changes in genetic variance across days in milk, capturing both peak and trough phases of genetic expression. Consequently, heritability curves obtained from RRM provided a more nuanced understanding than static estimates.

The genetic correlations across time points revealed that fertility records taken close together were highly correlated, while correlations declined as intervals widened demonstrating time-dependent genetic relationships. This autocorrelation structure supports the use of random regression in predicting breeding values for any day within the observed interval. Furthermore, the study confirmed strong genetic continuity between successive lactations, suggesting that fertility traits in first lactation provide useful indicators for later reproductive performance (González-Recio & Alenda, 2007; Yin & König, 2018).

The inclusion of maternal effects in calving models improved accuracy, as maternal genetic variance contributed notably to total phenotypic variance, particularly for traits like calving ease and birth weight. Negative direct-maternal genetic correlations indicated potential conflicts between fetal growth and maternal pelvic capacity, consistent with previous reports (Meyer, 1992; Phocas et al., 1998; Strabel et al., 2001). Accounting for these relationships is critical to avoid undesirable correlated responses when selecting for calving performance.

Overall, the methodological comparison confirmed that RRMs, complemented by appropriate consideration of maternal effects, provide the most realistic framework for modeling fertility and

calving traits. They allow the derivation of continuous heritability estimates and dynamic EBVs, enhancing selection precision and genetic evaluation efficiency.

5.3 Interpretation of Genetic Parameters

5.3.1 Heritability Patterns

The estimated heritabilities for fertility traits were within the range reported in previous studies typically between 0.02 and 0.15 (Pryce et al., 2004; Berry et al., 2014). Such low heritability values highlight the polygenic nature and environmental sensitivity of reproductive traits. However, the RRM revealed that heritability is not uniform across time. It tended to increase toward mid-lactation, suggesting that as cows stabilize metabolically, genetic differences become more pronounced. This supports the hypothesis that heritability estimates depend on physiological state, energy balance, and hormonal regulation. In other words, the changes in heritability across the different lactation numbers indicate the possibility of up/down regulation of expression of different genes.

Calving traits displayed lower heritability, ranging approximately between 0.004 and 0.074 for SB and 0.036 to 0.111 for CE, depending on the specific trait and model structure. These estimates are consistent with the literature and indicate that moderate genetic progress is achievable through selection. High repeatability estimates for calving traits also suggest that genetic merit remains relatively stable across parities, supporting the use of early records in selection programs.

The comparison between the three main modelling approaches, the classical calf-based maternal model, the multiple-trait model across parities (MTM), and the random regression model (RRM) shows notable differences and advantages. The traditional maternal model (Model 1), which assigns observations to the calf and separates direct and maternal effects, detected slightly unfavorable direct-maternal genetic correlations, estimated at approximately -0.11 for SB and -0.19 for CE.

These antagonistic relationships are consistent with earlier reports (Eaglen & Bijma, 2009) and reflect biological trade-offs: calves genetically predisposed for easy calving (direct effect) may develop into cows with narrower pelvic canals, increasing calving difficulty when they themselves become dams (maternal effect). While the maternal model remains central to many national genetic evaluation systems, it is limited in its ability to capture parity-specific behavior and longitudinal dynamics.

As an extension, the MTM and RRM modelling approaches from chapter 4 enabled the definition of genetic covariance structures among the different cow parities, contributing to deeper insights into genetic parameters for distinct lactation numbers. The MTM approach, in which each parity is treated as a distinct but correlated trait, more effectively captured heterogeneity across parities. Genetic correlations between parities were high, especially between adjacent parities, but declined noticeably as parity distance increased. This pattern reflects the underlying biology, as pelvic structure, fetal size, dam maturity and management conditions change substantially over the cow's lifetime. The MTM revealed that although CE and SB expressed in different parities share common genetic underpinnings, they cannot be treated as identical traits. This finding supports the need for parity-specific genetic evaluations, particularly for first and second parities, where calving problems are naturally more frequent. Similar behavior was observed in studies investigating parity-specific CE and dystocia traits, supporting the reliability of the MTM results.

The RRM provided an even more nuanced understanding of the genetic architecture of CE and SB. RRM model genetic and residual variances along a continuous parity trajectory, enabling estimation of how genetic parameters evolve across time. In this study, RRM produced heritability estimates similar to the MTM but offered smoother and more biologically realistic curves for additive and

residual variances. This suggests that calving traits exhibit underlying continuous genetic trajectories rather than discrete jumps between parities. EBV correlations between MTM and RRM were high, exceeding 0.80 across most parities, demonstrating that although the models differ in structure, they converge on similar genetic interpretations. The advantage of RRM becomes most apparent in handling heterogeneous variances, modelling non-linear changes across parities and providing EBVs at intermediate parities or points where phenotypic records are sparse. Similar advantages of RRM have been reported for calving traits, growth trajectories and production curves in other species (Arnal et al., 2019; Oliveira et al., 2019; Sakhaeifar & König., 2025), underscoring the value of RRM for calving trait evaluation.

5.3.2 Genetic and Phenotypic Correlations

The analysis of genetic correlations provided insight into the interrelationships among reproductive traits. Strong negative genetic correlations between production and fertility traits confirmed the antagonism observed in modern Holsteins (Veerkamp et al., 2007). Cows genetically predisposed for higher milk yield tended to exhibit extended calving intervals and reduced conception rates. This negative association underscores the need for balanced breeding goals that incorporate reproductive fitness alongside production efficiency.

Positive genetic correlations between fertility traits, such as days open and calving interval indicate that selection for improved performance in one trait will likely benefit the other. Similarly, favorable genetic correlations between calving ease and SB highlight the joint role of maternal conformation and calf viability. These relationships provide a framework for multi-trait selection strategies.

Phenotypic correlations followed similar patterns but were generally lower in magnitude due to environmental variance. The consistency between genetic and phenotypic associations suggests that

environmental management reinforcing genetic trends (for example, improving body condition and postpartum health) can accelerate overall reproductive improvement.

5.3.3 Estimated breeding values

The EBV analysis revealed substantial sire variability for fertility and calving performance (Oliveira et al., 2019). The RRM enabled estimation of EBVs at different lactation stages, showing how genetic merit changes dynamically (Pool et al., 2000; Schaeffer & Dekkers, 2000). Sires with persistently high EBVs across time were considered genetically robust for fertility, while others showed declining merit under physiological stress periods (Dzomba et al., 2010). This temporal resolution allows for more informed mating decisions selecting sires whose daughters maintain stable fertility performance over time (Ooi et al., 2023).

EBV accuracy improved under RRM compared to the repeatability model due to more precise variance-covariance estimation (Oliveira et al., 2019). Furthermore, the inclusion of maternal genetic effects in calving models allowed more accurate identification of sires contributing positively to both direct and maternal performance (Eaglen & Bijma, 2013). These dynamic EBVs could be integrated into selection indices to better capture reproductive resilience and efficiency (Kašná et al., 2025).

5.4 Genetic Relationships and Breeding Implications

The strong interdependence among fertility and calving traits has critical implications for breeding strategies (Eaglen & Bijma, 2013; Berry, 2014). The unfavorable correlation between high milk yield and reduced fertility requires that modern selection indices include reproductive parameters to maintain overall herd performance (Windig e al., 2006; Chafai et al., 2024). Incorporating fertility

EBVs derived from RRM into the total merit index can help counteract this antagonism and prevent further fertility decline (Shao et al., 2021).

The observed moderate heritability and favorable genetic correlations among certain fertility indicators suggest that genetic progress, though slow, is feasible (Shao et al., 2021; Chafai et al., 2024). Selection based on multiple traits, such as conception rate, interval from calving to first insemination, and non-return rate can yield cumulative improvements (Berry, 2014). Furthermore, because the RRM captures the temporal dynamics of heritability, selection can target periods when genetic variance is highest, improving efficiency (Windig et al., 2006).

Maternal genetic effects identified for calving traits imply that both sire and dam lines must be considered (Eaglen & Bijma, 2013). Negative direct-maternal correlations caution against unilateral selection for heavier calves or rapid fetal growth, as this may increase dystocia risk (Eaglen & Bijma, 2013; article on maternal genetic relationships, 2024). Balanced selection emphasizing both calf viability and maternal ease of calving is therefore recommended (Eaglen & Bijma, 2013).

The dynamic EBVs estimated in this study can support precision breeding programs by aligning sire selection with herd reproductive objectives. For example, sires demonstrating high EBVs during early lactation could be prioritized for herds emphasizing rapid postpartum recovery, whereas those with stable mid-lactation fertility performance may suit herds targeting consistent conception rates (Shao et al., 2021).

5.5 Practical and Industry Relevance

The practical relevance of these findings lies in improving both the genetic evaluation system and on-farm decision-making. Implementing RRM in national evaluation pipelines could refine the

prediction of fertility estimated breeding values (EBVs), increasing reliability for traits traditionally considered difficult to improve (Liu et al., 2007; Feltes et al., 2022). Dynamic EBVs allow breeding organizations to provide stage-specific fertility rankings, giving farmers more precise tools for sire selection (Arnal et al., 2019).

At the herd level, understanding genetic correlations between fertility and calving traits supports management strategies that minimize antagonistic effects (Mancin et al., 2023). For instance, balanced feeding and health management can mitigate the environmental components of negative production-fertility relationships (Kadarmideen et al., 2016). Incorporating genetic information into herd reproductive planning promotes greater efficiency and animal welfare, reducing involuntary culling and improving longevity (Mancin et al., 2023). For the dairy industry, these advancements contribute to sustainability by enhancing reproductive efficiency without compromising productivity. As breeding goals evolve to include welfare and environmental indicators, accurate fertility evaluations grounded in robust quantitative models will become increasingly essential (Berry, 2014; Mancin et al., 2023).

5.6 longitudinal Genomic study

This study provides the first longitudinal genome-wide association analysis (GWAS) for female fertility traits in dairy cattle, revealing dynamic genetic mechanisms that regulate reproductive performance over time. Unlike traditional GWAS, which assume static SNP effects, the longitudinal approach allowed detection of time-dependent genetic influences that shift across lactations (Das et al., 2011; Sikorska et al., 2015; Ning et al., 2018). The declining genetic correlations between parities confirmed that the same fertility trait is governed by partially different sets of genes as cows

age or transition through physiological states, supporting the concept of dynamic gene regulation (Sakhaei-far et al., 2025).

The significant loci identified across fertility traits *TMEM132C* and *IMPG1* for NRR56, *DCHS2* for calving-to-first-service interval CTFS, and *CSMD1* and *CSNK1A1* for DO, illustrate that fertility is influenced by a wide range of biological pathways, including cell adhesion, neuroendocrine regulation, and intracellular signaling. For example, *CSNK1A1* participates in the Wnt, Hedgehog, and Hippo signaling pathways, which are essential for follicular development, oocyte maturation, and hormonal regulation (Jiang et al., 2018; Clark et al., 2022; Plewes et al., 2019). Likewise, *DCHS2* is involved in cell-cell junction organization and the Hippo signaling cascade, influencing reproductive organ development and estrus cycling (Wang et al., 2025; Lodge et al., 2020). The functional annotation of *TMEM132C* suggests a role in cell adhesion and reproductive tract integrity, consistent with its expression in uterine and vaginal tissues (Zheng et al., 2023).

Although the proportion of variance explained by individual SNPs was small ranging from 0.10% to 4.03% these results are consistent with the polygenic nature of fertility traits reported in previous GWAS (Höglund et al., 2015; Nayeri et al., 2016; Wolf et al., 2021; Mohammadi et al., 2020). The integration of longitudinal GWAS with random regression modeling enhances the resolution of SNP effects across physiological time scales, allowing the identification of SNP × time interactions that contribute to fertility resilience under variable environmental and metabolic conditions (Schaeffer, 2004; Sikorska et al., 2015).

Importantly, in genomic analyses, the next logical step after estimating genetic (co)variance components through the RRM is to use these variance structures as the foundation for conducting longitudinal GWAS. This integrated workflow connects traditional quantitative genetic modeling

with genome-level inference, enabling the identification of time-dependent markers that explain dynamic genetic variation in fertility and related traits (Das et al., 2011; Ning et al., 2018; Sakhaeifar et al., 2025).

The enrichment of key signaling pathways, particularly Hippo, Wnt, and GnRH, underscores the interconnected roles of endocrine and cellular regulatory systems in reproductive function (Clark et al., 2022; Zhang et al., 2022; Hernandez Gifford, 2015). Such mechanistic insights are valuable for genomic selection programs aiming to balance productivity and fertility. Incorporating time-dependent estimated breeding values (EBVs) derived from longitudinal models could improve selection for reproductive resilience and reduce fertility decline associated with high milk production (Beerda et al., 2008; Berry, 2014).

Overall, this longitudinal GWAS highlights the importance of dynamic modeling for understanding fertility as a time-varying phenotype. The combination of genomic data with longitudinal modeling offers a framework for more accurate genomic evaluations and for identifying biologically meaningful candidate genes that regulate fertility across lactations, ultimately contributing to sustainable and welfare-oriented dairy breeding systems.

5.7 General Conclusions

This study demonstrated that fertility and calving traits in German Holstein cattle exhibit dynamic genetic variation across the reproductive cycle. Random regression models outperformed conventional models by accurately describing time-dependent heritability and genetic correlations, providing a deeper understanding of reproductive trait architecture. The inclusion of maternal genetic effects further refined parameter estimation and revealed important biological relationships between dam and offspring performance.

Heritability estimates confirmed that while fertility remains a low-heritability trait, meaningful genetic progress is achievable through balanced multi-trait selection. Moderate heritabilities for calving traits and strong genetic correlations among related reproductive indicators highlight the potential for coordinated improvement. Dynamic estimated breeding values derived from RRM provide a practical tool for identifying animals with stable fertility performance across lactation.

Brief genomic and functional analyses supported the quantitative findings, pointing to biological pathways consistent with reproductive physiology. Together, these results provide a solid foundation for enhancing fertility evaluation and breeding programs. The implementation of longitudinal modeling approaches will allow breeders to select for more resilient, fertile cows while maintaining productivity and welfare standards.

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FORMAL DECLARATION

Erklärung gemäß § 10 Absatz 6 der Promotionsordnung des Fachbereichs Agrarwissenschaft der Justus-Liebig-Universität Gießen vom 06.11.2012

Ich erkläre: Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten oder nicht veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis, wie sie in der „Satzung der Justus-Liebig- Universität Gießen zur Sicherung guter wissenschaftlicher Praxis“ nie dargelegt sind,
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Sina Sakhaei Far

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