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Investigating Novel Traits for Pregnancy Loss in Norwegian Red Cattle

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Abstract

The task was to identify potential traits to describe the incidence of pregnancy loss in Norwegian Red cows. These novel traits needed to consider the different biological stages of pregnancy and the risk of loss throughout the cow's gestation. In addition, these traits had to use available field data, insemination, and culling events. As a result, several traits formed: insemination return dates, from 0 to 48 days, between 49 to 80 days and 81 plus days. While culling traits were also evaluated, with culling due to miscarriage and culling due to fertility problems in general. The data set included 1 215 005 observations of 536 641 Norwegian Red cows from 4 999 herds from the year 2014 to 2022, sired from 793 bulls. The pedigree for these cows went back four generations and consisted of 1 025 393 individuals. Regarding trait observation, 25.95% of cows returned for insemination within 0 to 48 days, 5.34% returned within 49 to 80 days, and 3.62% returned after 81 days. For culling, 20.32% were culled due to various fertility problems, and 2.98% were culled specifically due to miscarriage.

To evaluate the trait, variance components and heritability were estimated. The calculations used linear animal models, with fixed effects of age-parity, year-month of insemination, and random effects of herd-year (plus the permanent environment when applicable). As expected for a reproductive trait, the heritability of the traits was low. The highest heritability was culling due to fertility problems (0.05), followed by the control return of 56 (0.01) and return between 0 to 48 days (0.01). The heritability of return 49 to 80 and 81 plus was very small (0.002) and did not correlate with returning interval 0 to 48. However, the lack of observed events may have influenced the later gestation trait evaluations.

Subsequently, a genome-wide association analysis was employed for a brief investigation to identify the potential of genetic and phenotype associations. The genome-wide association analysis was carried out on all defined traits, using the estimated breeding values of the sires from the previous calculations as the

phenotype. All sires with available phenotype and genotype data were used in the analysis. In the genome-wide association analysis, all traits had identified suggestive associations, with a few significant and convincing peaks in the return traits on BTA 12, 23, 24 and 26. The most convincing peaks appeared to be within or nearby genes that would affect the pregnancy stage investigated. These genes may be compelling candidates. However, taking the results cautiously and further investigation, including methods such as fine-mapping, is required.

The novel traits appear to capture the different biological stages of pregnancy. The different stages were represented by the return intervals, with the genetic background differing and a medium to low correlation between the stages.

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EBV: estimated breeding values.

PVE: proportion of variance in phenotype explained by a given SNP.

P4: progesterone.

QTL: quantitative trait loci.

SNP: single nucleotide polymorphism.

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1. Introduction

Production on a cattle farm must be economically viable. To ensure the viability of production, we can look at the contributing factors that affect financial performance. Many factors contribute to financial performance; however, a crucial component for cattle farms is the functional and production traits of the herd. In particular, one functional trait significantly contributes to profitability; this trait is a cow's reproductive success (Oliver *et al.,* 2019; Wijma *et al.,* 2022).

Reproductive success is the production of offspring over a lifetime or per breeding event. An unsuccessful breeding event can happen due to many factors, with reproductive failure resulting in spontaneous abortion or stillbirth. Reproductive failures, especially later when in gestation, significantly affect a farm's production efficiency and profitability (Fonseca, Schenkel, & Canovas, 2022; Sidgel *et al.,* 2022). It reduces efficiency and incurs financial losses due to the loss of a calf and subsequent lactation, the cost of maintaining the cow during pregnancy and the price of rebreeding or replacement, among other things (Cole, Null, & VanRaden, 2016; Oliver *et al.,* 2019; Ask-Gullstrand *et al.,* 2021; Wijma *et al.,* 2022). Moreover, pregnancy loss can harm animal health and welfare (Sigdel, Bisinotto & Penagaricano, 2021; Wijma *et al.,* 2022).

Previously, there was only the consideration of production traits when evaluating cattle for selective breeding. This exclusive selection of productive traits in breeding caused a decline in reproductive success due to their negative correlation. Once this decline was recognised, the breeding indexes were revised to incorporate reproductive traits (Lucy, 2019; Muttoranta *et al.,* 2019). The breeding indexes estimate the overall genetic merit of the cattle, enabling the selective breeding of genetically superior animals for chosen traits. By incorporating reproductive traits in the breeding indexes, selective breeding can gain permanent genetic improvement in reproductive success (Lucy, 2019; Ask-Gullstrand *et al.,* 2021). Despite the changes to the selection indices, pregnancy loss remains a persistent problem for

farmers (Sigdel, Bisinotto & Penagaricano, 2021; Wijma *et al.,* 2022). However, genetic improvement of reproductive success is feasible, with studies demonstrating genetic causes and sufficient genetic variability (Spencer, 2013; Ask-Gullstrand *et al.,* 2021; Sigdel *et al.,* 2022).

Ordinarily, the trait used in breeding indexes is the non-return rate 56 (Gershoni, Ezra, & Weller, 2020). The non-return rate is the proportion of cows that do not return to be re-inseminated within the specified interval, i.e. within 56 days (Gershoni, Ezra, & Weller, 2020). Those who return for re-insemination within the specified time interval have experienced reproductive loss. This reproductive loss can be due to an unsuccessful fertilisation event or early spontaneous abortion. Incorporating non-return 56 into the selection indexes has yet to have the desired effect. Furthermore, although fertilisation rate and early spontaneous abortion are indispensable traits, cows may experience pregnancy loss later in gestation. As a result, this requires further action.

The following action may be the development of a new selection trait(s) (Lucy, 2019; Muuttoranta *et al.,* 2019; Gershoni, Ezra, & Weller, 2020). When developing a new trait, it is essential to ensure its ability to achieve a significant amount of genetic gain.

Analysis can evaluate the potential of a trait for selection. Evaluation for a phenotypic trait is through estimating the additive genetic component of the phenotypic variance (Kruuk & Hadfield, 2007). The calculation of these variance components is through the use of models. The model used to evaluate traits can vary, so it is essential to consider which modelling approach and effects would be most appropriate to decipher the desired information (Sidgel *et al.,* 2022). The information deciphered by modelling can help determine how much trait diversity may be due to genetic variation, thus its usefulness as a trait in selective breeding (Kruuk & Hadfield, 2007; Muuttoanta *et al.,* 2019).

The trait analysis can go another step further, and this can be through a genome-wide association study. The genome-wide association study allows us to identify genetic markers significantly associated with the trait. By identifying markers significantly associated with the trait, thus, we can pinpoint significant genomic regions and find genetic candidates. Eventually, these regions and genes can be fine-mapped to identify causal SNPs (single nucleotide polymorphisms) and help us better understand the mechanisms behind pregnancy loss (Bamber *et al.,* 2009; Gershoni, Ezra, & Weller, 2020).

It is, therefore, beneficial to develop novel traits to reduce embryo and foetal loss incidence in Norwegian Red cows. Once these prospective traits are defined, they must be thoroughly investigated and evaluated for their potential. Consequently, evaluation calculates the traits' variance components and heritability. Subsequently, the traits may be analysed using genome-wide association analysis. These findings could eventually lead to new traits and markers for breeding decisions and help expand our understanding of pregnancy loss.

2. Literature

2.1. Pregnancy Loss in Cattle Breeding

Reproductive efficiency is an important characteristic and a problem in cattle due to selection for production traits. Consequently, there must be a reduction in the incidence of unsuccessful events. One way to reduce the incidence of unsuccessful events is to breed for reproductive success. The recent advances in breeding methodology with technological innovations in these breeding programs enable the achievement of more considerable genetic gain.

Selective breeding is the methodology used when breeding animals for genetic gain leading to trait improvement. This method selects superior individuals based on an

animal's estimated breeding value. The estimated breeding value measures an animal's genetic potential for a specific trait, enabling the selection of animals with the highest genetic merit. By selecting the animals with higher genetic merit for breeding, there is population genetic gain, leading to the long-term improvement of the trait (Tiezzie *et al.,* 2015; Spencer, 2013).

There are several measures of cattle fertility, but typically, the trait used to estimate breeding values for reproductive success is the non-return rate 56. Although the non-return rate may be implemented in the selection indexes, pregnancy loss remains an issue in farming. As pregnancy loss continues to be a problem, it indicates that additional or new reproductive success traits should be applied to evaluate an animal's genetic merit (Gerhsoni, Ezra & Weller, 2020; Wijma *et al.,* 2022).

Trait improvement for functional traits, such as reproductive success, has proven difficult (Spencer, 2013). This difficulty in breeding for progress is due to several factors, and the main factor is that reproductive success is a complex trait with very low heritability. As a complex trait with low heritability, many genes may affect this trait, and environmental factors can easily influence it. These influences lead to a lack of uniformity in incidences and further difficulties in phenotyping (Gerhsoni, Ezra & Weller, 2020; Toghiani *et al.,* 2017; Wijma *et al.,* 2022).

Consequently, there needs to be more understanding of reproductive success in terms of the physiological mechanisms and genomic structure. It is especially lacking understanding later on in pregnancy. Despite the knowledge gaps in reproductive mechanisms and genomic structure, there is evidence of a genetic role and observed variation. The evidence of a genetic role and variation indicates that control of reproductive success can be achieved partly through selective breeding (Spencer, 2013; Oliver *et al.,* 2019; Gerhsoni, Ezra & Weller, 2020; Wijma *et al.,* 2022).

Moreover, integrating genotype data in selective breeding is beneficial and becoming increasingly popular (Cole, Null, & VanRaden, 2016; Lima *et al.,* 2020). Genotype data is collected using SNP microarrays ("SNP chips"), which can provide valuable genetic information. This genetic information is the carrier status of a marker or quantitative trait loci, which aids in selection decisions (Cole, Null, & VanRaden, 2016; Lima *et al.,* 2020). This aid is especially beneficial for lowly heritable traits and traits that can only be measured later in life. Thus, it can dramatically increase the rate of genetic progress for reproductive success (Spencer, 2013; Cole, Null, & VanRaden, 2016; Lima *et al.,* 2020).

2.2. Pregnancy and Pregnancy Loss

In a reproductive event, a cow will carry their offspring from fertilisation till birth, and this period is pregnancy. The average gestation length for dairy cows is 282 days (with breed variation) and is generally split into three stages (VanRaden, 2004; Valadao *et al.,* 2019). These stages are the first, second, and third trimesters and indicate the developmental steps of pregnancy. Many genes and pathways govern each development stage, so theoretically, every event within pregnancy is heritable (Lonergan & Forde, 2014; Lucy, 2019). Despite this being a heritable trait, our understanding of the genes and pathways governing pregnancy is still developing (Lonergan & Forde, 2014; Lucy, 2019). Notwithstanding, pregnancy loss is typically caused by embryo mortality or the cow's failure to establish or maintain pregnancy (Bamber *et al.,* 2009).

2.2.1. Oestrus and Fertilisation

The first step in pregnancy is the fertilisation of the oocyte. For fertilisation of an oocyte to ensue, the oocyte first needs to be released from the ovary. An oocyte is released from the ovary once the ovarian follicle has matured. Once matured, ovulation will occur. Ovulation is when the ovary releases the oocyte into the oviduct, and the oviduct is where fertilisation occurs (Lucy, 2019; Valadao *et al.,* 2019). Fertilisation begins when spermatozoids come into contact with the oocyte; the

fertilisation process happens through a sequence of events, ending with the fusion of a spermatozoid and oocyte nuclei (Lonergan & Forde, 2014; Valadao *et al.,* 2019). If a fertilisation event has not occurred, another ovulation event will occur approximately 21 days later. This cycle of events is known as the oestrus cycle.

When an individual is expressing oestrus, there will be artificial insemination of the cow. If the insemination is successful, fertilisation of the oocyte will occur. Conversely, if the insemination is unsuccessful, oestrus expression would be expected 21 days later. Then 21 days later, another insemination attempt would take place. Moreover, an attempt may occur later if the oestrus is unobserved (Gershoni, Ezra, & Weller, 2020).

2.2.2. First Trimester

The First Stage of the First Trimester

Once fertilised in the oviduct, the zygote moves towards the uterus. This movement happens via peristalsis and beating cilia, enabling the zygote to enter the uterus on day 4 - 5 of pregnancy (Lonergan & Forde, 2014; Valadao *et al.,* 2019; Mazzaerlla *et al.,* 2021). During these first 4-5 days, the zygote undergoes the first cleavage divisions before passing into the uterus. The zygote passes into the uterus at the 16-cell stage, called a morula (Lonergan & Forde, 2014; Mazzaerlla *et al.,* 2021). Then, once the morula has entered the uterus, it floats freely there for several more days. Over these several days, the morula forms a blastocyst. The blastocyst then hatches from the zona pellucida on days 9 - 10 (Lonergan & Forde, 2014; Valadao *et al.,* 2019; Lonergan & Sanchez, 2020).

In this period, the embryo stays in contact with the oviductal epithelial cells of the mother. This contact between embryo and maternal cells means the start of embryo-maternal crosstalk (Mazzaerlla *et al.,* 2021). As a result, the crosstalk causes changes in gene expression patterns of the oviductal epithelial cells, modifying the fluid secreted by the cells. This fluid secreted by the epithelial cells provides the ideal

environment for embryo development (Wiltbank *et al.,* 2016; Mazzaerlla *et al.,* 2021).

At this stage of embryo development, there is only local embryo-maternal crosstalk. While communication is only localised to the oviductal epithelial cells, if embryo loss occurs, it will not be recognised maternally. Therefore, the oestrus cycle should remain unchanged without full maternal embryo recognition. As the oestrus cycle is unaffected, the subsequent insemination will happen at the expected interval of about 21 days (Wiltbank *et al.,* 2016). For this reason, when records show a re-insemination at the expected time interval, it is impossible to differentiate whether the reproductive failure was due to unsuccessful fertilisation or early embryo loss.

The Second Stage of the First Trimester

After the blastocyte hatches, the hatched blastocyst begins the elongation stage to form the conceptus and begin implantation. This developmental stage of the blastocyte occurs in the uterine lumen and depends on maternally derived factors in the uterine luminal fluid (Forde *et al.,* 2009; Spencer, 2013; Lonergan & Sanchez, 2020). Given that this step depends on maternally derived factors, it concomitates maternal pregnancy recognition (Spencer, 2013; Lonergan & Forde, 2014). Consequently, this pregnancy recognition ensures pregnancy continuation.

During elongation, the blastocyte has rapid trophectoderm growth and development (Spencer, 2013). During this growth and development, the trophectoderm cells begin to secrete various factors. These factors include prostaglandins, pregnancy serum protein B and interferon tau (IFNT), which signal maternal pregnancy recognition (Forde *et al.,* 2009; Spencer, 2013; Valadao *et al.,* 2019; Lonergan & Sanchez, 2020). Once the signalling of maternal recognition has occurred, the implantation process can start (Valadao *et al.,* 2019).

As stated, factors from the conceptus cause maternal pregnancy recognition. The factors from the conceptus induce recognition, as they direct the corpus luteum to survive and enlarge. Consequently, the corpus luteum survives to produce oestrogen and progesterone (P4) (Forde *et al.,* 2009; Wiltbank *et al.,* 2016; Valadao *et al.,* 2019). This continued production of P4 from the corpus luteum causes changes in gene expression. As a result, changes in gene expression occur in the epithelium and superficial glandular epithelium to generate the production and transportation of secretions into the uterine lumen. These secretions aid in the conceptus elongation, implantation, and pregnancy establishment (Wiltbank *et al.,* 2016; Lucy, 2019; Lonergan & Sanchez, 2020). Furthermore, pregnancy establishment may also require immune molecular crosstalk between the mother and embryo. This molecular crosstalk is thought to promote infection prevention and tolerance for pregnancy establishment (Oliveira *et al.,* 2012).

Maternal pregnancy recognition occurs at approximately day 16 (Spencer, 2013; D'Occhio *et al.,* 2020; Lonergan & Sanchez, 2020). However, if maternal recognition of pregnancy fails, the pregnancy will be lost. Provided pregnancy loss occurs before maternal recognition, there will be no change in the oestrus cycle (Wiltbank *et al.,* 2016; Lucy, 2019). Conversely, there will be changes in the oestrus cycle if pregnancy recognition does occur. As a result of recognition, there is an extension of the luteal phase, so oestrus expression and re-insemination will have a longer interval than expected. For this reason, the interval is longer, and it is now possible to distinguish between unsuccessful fertilisation and a pregnancy loss event. In this case, the delay of oestrus expression is until day 25 - 50 (Bamber *et al.,* 2009; Wiltbank *et al.,* 2016; Lucy, 2019).

The Third Stage of the First Trimester

Once the embryo has finished implanting, officially, pregnancy is established. The establishment of pregnancy in cows happens by day 42, and henceforth the foetal period begins (DesCoteaux *et al.,* 2009; Spencer, 2013).

At this time, conceptus continues growth and differentiation. The inner cells of the blastocyst develop into the embryo, then the foetus. While the outer layer, the trophectoderm, develops into the foetal part of the placenta and from a yolk sac to a chorionic sac (Wiltbank *et al.,* 2016; Valadao *et al.,* 2019). Due to this growth and development, the foetus requires a greater diffusion of nutrients and gases. For this reason, the endometrium also undergoes extensive remodelling. Notably, the foetal and maternal placentomes connect, forming the allantoic placenta by day 60 (Wiltbank *et al.,* 2016). This remodelling increases blood flow and is the start of uteroplacental circulation (Neto *et al.,* 2009; Wiltbank *et al.,* 2016).

The Fourth Stage of the First Trimester

The rest of the first trimester, days 60 to 90, is characterised by sustaining the continued growth and development of the foetus. In order to sustain this growth, there is an increasing amount of amnion, chorioallantoic and placentomes. Furthermore, numerous growth factors and hormones are secreted from the placenta, significantly affecting maternal physiology (Wiltbank *et al.,* 2016).

2.2.3. Second and Third Trimesters

Subsequently, come the second trimester and the third trimester of pregnancy. The second trimester starts on day 90 of pregnancy, and the third trimester starts on week 27, which finishes at parturition.

Now that the pregnancy is established and recognised, these two periods focus on the continued development and growth of the foetus. There is a considerably large amount of growth; during the second trimester, the foetus grows more by length and then more by weight in the third trimester. In addition, organogenesis is eventually completed (Crosier *et al.,* 2002; DesCoteaux *et al.,* 2009; Valadao *et al.,* 2019).

Though the risk of pregnancy loss drops during trimesters 2 and 3, these stages are still critical. This growth and development during this period require many resources. So the dam has significant requirements to provide resources, and the placentome does not finish growth until day 190 (Wiltbank *et al.,* 2016; Valadao *et al.,* 2019).

2.2.4. Factors of Pregnancy Loss

The success or loss of a pregnancy can be down to many factors. These factors can be environmental or genetic, but it is a significant challenge to know which factor(s) is the cause. As a result, finding the cause is challenging, so pregnancy loss typically goes undiagnosed (Gerhsoni, Ezra & Weller, 2020; Wijma *et al.,* 2022).

Genetic components can underlie pregnancy loss and can be individual genes or result from genetic interactions. Despite the difficulties in phenotyping, the list of identified genetic factors contributing to pregnancy loss is increasing (Oliver *et al.,* 2019; Gerhsoni, Ezra & Weller, 2020; Sigdel, Bisinotto & Penagaricano, 2021; Wijma *et al.,* 2022). Typically, the genes identified as a pregnancy loss factor contribute to establishing and maintaining pregnancy. These establishment and maintenance genes can be from the dam or the embryo; therefore, the genes often affect the uterine environment or are an embryo chromosomal abnormality (Bamber *et al.,* 2009; Tiezzi *et al.,* 2015; Sigdel, Bisinotto & Penagaricano, 2021; Wijma *et al.,* 2022).

Moreover, when analysing the cause of pregnancy loss, it is essential to consider factors other than genetic causes. These causes are environmental factors. Environmental factors significantly affecting pregnancy loss include nutrition, diseases, and environmental stressors such as heat stress (Spencer, 2013; Wijma *et al.,* 2022). Furthermore, studies have also found a significant correlation between pregnancy loss and increased parity, age, fewer open days and a previous pregnancy loss (Thurmond *et al.,* 2005; Bamber *et al.,* 2009; Rafati *et al.,* 2010; Wijma *et al.,* 2022). Previous pregnancy loss has a significant correlation to an

unsuccessful reproductive event, and this is because previous loss can lead to fertility problems and health issues for the cow. These problems include retained placenta, metritis, endometritis, and pyometra (Wijma *et al.,* 2022).

2.3. Pregnancy Loss Traits

In animal breeding, pregnancy loss is one of the most complex traits (Lima *et al.,* 2020). Pregnancy loss is a complex trait in breeding as it has a low heritability, is tricky to record accurately and is notoriously difficult to evaluate (Lima *et al.,* 2020). Despite evaluation efforts to breed genetically superior animals, pregnancy loss remains a problem. In order to solve this problem, finding a better alternative or additional measure to the non-return rate 56 should be done (Oliver *et al.,* 2019; Wijma *et al.,* 2022).

2.3.1 Current Breeding for Fertility in Norwegian Red Cattle

Firstly, it is essential to consider the breeding for fertility traits, specifically in the Norwegian Red breed. Unlike other breeding programs, fertility has been included early on, with it included in the total merit index since 1971. Due to this early inclusion and sustained selection, the Norwegian Red is "likely the most fertile breed of dairy cattle in the world" (https://www.norwegianred.com/about-norwegian-red/norwegian-ebvs/daughter-fertility/)

The non-return rate of 56 days was the trait used when genetically evaluating an animal, with non-return 56 phenotyping marking the success of an animal's reproductive events. The success indicated is fertilisation and early embryo survival, both essential traits (Tiezzi *et al.,* 2015). Though they are important traits, recorded reproductive success is only based on early pregnancy success. As a result, if pregnancy loss occurs later in gestation and the cow is re-inseminated after 56 days, it will not be recorded. Furthermore, the 0 to 56-day interval has a significant proportion of returns due to fertilisation failure, resulting in an incorrectly documented pregnancy loss (VanRaden & Miller, 2006; Tiezzi *et al.,* 2015).

However, non-return 56 days for fertility has been switched recently to the number of inseminations (for a heifer or a cow). This switch was because Geno SA found the number of inseminations to have more genetic variation than non-return 56 https://www.norwegianred.com/about-norwegian-red/norwegian-ebvs/daughter-fertility/).

2.3.2. Considerations for New Pregnancy Loss Trait

When choosing a novel trait to record pregnancy loss, it is vital to consider various factors. These factors include ease of trait recording, genetic parameters such as heritability, and the trait being biologically meaningful, among others (Tiezzi *et al.,* 2015; Brito *et al.,* 2020; Gerhsoni, Ezra & Weller, 2020).

Recording Pregnancy Loss

The ease of trait recording is essential when deciding which trait to use in the analysis. For this reason, the decided pregnancy loss trait should allow for consistent recording and recording at a low cost (Tiezzi *et al.,* 2015; Gerhsoni, Ezra & Weller, 2020). These records can be direct records of pregnancy loss, or loss can be determined using indirect indicators (Sigdel, Bisinotto & Penagaricano, 2021).

Direct records can unambiguously determine a pregnancy loss diagnosis. In this case, a pregnancy loss can be determined by a confirmed pregnancy, followed by a subsequent diagnosis of pregnancy loss. The pregnancy diagnosis can be made using a couple of techniques, either confirmation of pregnancy using ultrasound or testing for pregnancy-associated glycoproteins. This pregnancy confirmation is possible approximately 30 days after the initial insemination (Bamber *et al.,* 2009; Wiltbank *et al.,* 2016; Ealy & Seekford, 2019). On the one hand, records will accurately document the later foetal losses; on the other, the record will be incorrect

when embryo loss occurs earlier than 30 days. Furthermore, the documentation of losses using direct techniques comes at an extra cost, requiring personnel and an exceptional recording system (Sigdel, Bisinotto & Penagaricano, 2021).

The alternative option is to use an indirect indicator for pregnancy loss, and this would be insemination records. The idea of insemination records to indicate pregnancy loss is that records will show no calf and a subsequent re-insemination. With re-insemination of the cow ideally happening at the following oestrus expression, it may also be possible to get a rough timeline of pregnancy loss. The upside to using the indirect indicator of inseminations is that this data comes at no extra cost as it is routinely collected data (Tiezzi *et al.,* 2015). So, data from recording schemes are ideal for new traits in genetic parameter estimates. In addition, recorded health data may also be used for selection (Pryce *et al.,* 2010).

Genetic Parameters

When considering a new trait, a few genetic parameters are used to analyse it, including genetic variation and heritability. To be able to select animals for genetic gain, there needs to be genetic variation. Therefore, the genetic variance indicates whether a pregnancy loss trait allows for management through selective breeding (Tiezzi *et al.,* 2015; Gerhsoni, Ezra & Weller, 2020). Furthermore, these variances can calculate the heritability of a trait. The heritability of a trait tells us how much of that variation is explained by genetic factors. A higher heritability means a higher explanation is due to genetic factors, so a higher heritability for a trait is ideal (Brito *et al.,* 2020). However, it is worth noting that all reproductive traits are lowly heritable and significantly affected by environmental factors (Tiezzi *et al.,* 2015; Gerhsoni, Ezra & Weller, 2020).

Other factors to consider

Pregnancy loss is complex, as pregnancy is a complicated biological process. The pregnancy process has many stages, each relying on multiple genes and pathways. Any disruption in one of these genes or pathways could result in an increased incidence of pregnancy loss. As a result, the genetic cause of incidence differs for each critical event or physiological period. Consequently, each period has a different potential cause of loss, and evaluating a genetic cause of pregnancy loss is more complex (VanRaden & Miller, 2006; Wiltbank *et al.,* 2016). For this reason, a multifaceted method that targets the physiological causes of pregnancy loss at various time points would be ideal (Gonzalez-Recio & Alenda, 2005; VanRaden & Miller, 2006; Wiltbank *et al.,* 2016; Gershoni, Ezra, & Weller, 2020).

The genetic cause may not even be the dam, as the embryo, sire of the embryo and environmental factors can also cause loss (Bamber *et al.,* 2009). In addition, finding the real cause of pregnancy loss is also problematic due to the limited number of records. Typically, a cow has one or few pregnancy records, and each pregnancy's outcome may differ (Bamber *et al.,* 2009). With a limited number of repeated records and potentially differing results, evaluations may provide little indication of genetic causes (Fonseca, Schenkel & Canovas, 2022; Wijma *et al.,* 2022). Evaluating pregnancy loss in terms of the sire's daughter is a valuable predictor of a future pregnancy outcome and may improve genetic gain at a greater rate (Bamber *et al.,* 2009; Lucy, 2019; Madureira *et al.,* 2022).

2.4. Statistical Analysis

There is the implementation of statistical genetics to select cattle and analyse the potential of new traits (Averill, Rekaya, & Weigel, 2004). The potential of a trait is the estimation of the genetic parameters, which are a trait's variance components (heritability) (Alijani *et al.,* 2011). In order to calculate these parameters, the estimations require a statistical model. Determining the suitable statistical method

and models for estimation is crucial for the results' accuracy, as a model's accuracy is essential to increasing the genetic gain (Averill, Rekaya, & Weigel, 2004; Alijani *et al.,* 2011; Brito *et al.,* 2020). Furthermore, the identification and integration of genotype information for selection also increase the accuracy of breeding values for individuals and their relatives (Brito *et al.,* 2020).

2.4.1. Genetic Parameters

Models

The models most commonly used in the statistical modelling of animal traits are threshold and linear models. Both threshold and linear models are suitable, with many studies having successful results estimating variance components and heritability of reproductive traits with each of them (Blangero *et al.,* 2013; Averill, Rekaya, & Weigel, 2004; Muuttiranta *et al.,* 2019).

In principle, threshold models are theoretically better for analyses of binary traits. Furthermore, in some situations, such as analysis of disease traits, they can be significantly better than simple linear models (Jamrozil *et al.,* 1991; Kadarmideen *et al.,* 2000; Averill, Rekaya, & Weigel, 2004; Tiezzi *et al.,* 2015), however, the computational time and demand can be overwhelming (Kadarmideen *et al.,* 2000; Muuttiranta *et al.,* 2019).

Conversely, a threshold model may not always be advantageous, and a linear model can have similar results. So, when there are many observations, a trait is binary and relatively homogenous across fixed effects, a linear model would be of choice (Jamrozil *et al.,* 1991; Muuttoranta *et al.,* 2019). Linear models are also the chosen standard modelling approach, widely used in animal breeding (Blangero et al., 2013; Muuttoranta *et al.,* 2019).

Animal Model

The model can also be a sire, dam, or animal model. Though the genetic prediction ability of the models may be close, the animal model provides estimates of quantitative genetic parameters with higher precision. Consequently, these higher precision results make it the superior choice (Akesson *et al.*, 2008; Sun *et al.,* 2009; Kruuk & Hadfield, 2007; Tiezzi *et al.,* 2015).

The animal model considers all relationships in a pedigree, making it more robust and less likely to be biased by complicating factors (Akesson *et al.*, 2008). These factors include uneven data sets, selective breeding, and inbreeding. Furthermore, it reduces the bias of animals with shared environmental effects, especially when the pedigree contains many generations (Akesson *et al.*, 2008; Sun *et al.,* 2009). Increasing the number of generations also improves parameter accuracy (Kruuk & Hadfield, 2007).

Environmental Effects and Repeated Records

Trait diversity may also be due to environmental variation or a combination of environmental and genetic variation (Kruuk & Hadfield, 2007). The animal model can accommodate these variance components by including environmental sources of variation as fixed or random effects (Kruuk & Hadfield, 2007). These effects can be chosen based on known and proven sources of trait variation.

An incidence or measurement can be recorded multiple times for particular animal traits. These repeated records for an individual can be used when evaluating the trait's genetic parameters. As a result of using repeated measurements to evaluate the parameters, there is an increase in estimation accuracy (Akesson *et al.*, 2008). However, when using multiple records from an individual, there may be permanent environmental effects. The definition of *Permanent environmental effects* is the "Environmental effects on an individual's phenotype that are constant across (or

common to) repeated measures on that individual" (Kruuk & Hadfield, 2007). These permanent environmental effects should be accounted for in the model, which can cause a drop in heritability; however, failure to include the effect can derail analysis and increase bias in the estimate (Kruuk & Hadfield, 2007).

2.4.2 Genome-wide Association Analysis

A genome-wide association analysis identifies single nuclear polymorphisms (SNP) markers significantly associated with a quantitative trait. These markers may be the cause of trait variation or, most likely, in linkage with one source of trait variation. As a result, GWAS can be used to identify significantly associated regions and subsequently, with further analysis, identify causal mutations underlying trait variation.

There can be many causes of trait variation, with complex traits such as fertility influenced by many genes, each having minor effects. As these effects are minor, they often go undetected due to the stringent significant threshold required in GWAS when avoiding false positives. Larger samples with higher marker density may provide more power when identifying significant associations, but the availability of genotype and phenotype information can be limited (Gaddis, Null & Cole, 2016; Ma & Zhou, 2021). However, genomic selection is widely implemented, so genomic information is available on many progeny-tested bulls. Furthermore, use of fertility trait predictions based on sire daughter fertility, can remove outliers and have more power (Spencer, 2013; Wijma *et al.,* 2022).

3. Objectives and Aims

This study aims to develop novel traits for selective breeding to reduce embryo and fetal loss incidence in Norwegian Red cows. The objective was to investigate the use of current field data for prospective traits and to evaluate their potential. These prospective traits were to examine pregnancy loss throughout gestation and confirm

the differences between pregnancy stages. Consequently, evaluation calculated the traits' variance components and heritability using a linear animal model. Subsequently, the traits were analysed using genome-wide association analysis. These findings could eventually lead to new traits and markers for breeding decisions and help expand our understanding of pregnancy loss.

4. Materials and Method

4.1. Data

4.1.1. Data Cleaning

The data used in the study was field data, which was provided by Geno SA (www.norwegianred.com). The raw data set contained 2,496,645 records of one record per cow per lactation, January 2010 to February 2023, from the Norwegian Dairy Herd Recording System. This data was pre-cleaned, for example, in relation to the minimum age of insemination and the number of days from calving to insemination.

Nevertheless, further restrictions were also put on the data set:

1. The records of insemination were restricted from years 2014 to 2022.
2. The records were only from the Norwegian Red cattle breed.
3. Records only use single insemination in the first and, if applicable, second insemination. (Removal of double inseminations (where there has been initial artificial insemination and a second artificial insemination in consecutive days to increase the availability of sperm for fertilisation to increase chances of successful fertilisation), embryo transfer, and mating).
4. Sire of cow known as Norwegian Red Ai Bull, and each sire has a minimum of 25 daughters.
5. There are at least 20 individuals in a herd-year group.

The traits were defined, and the data was reviewed in R.

4.1.2. Definition of Traits

Six traits were defined, one control trait and five novel embryo and fetal loss traits. These performance traits are binary measurements.

Return 56. If, after initial insemination, a cow is returning to be re-inseminated within a 56-day interval. This interval is a standard measurement of fertility and acts as a control measure (1 = return, 0 = non-return).

Return from 0 to 48 days: If, after initial insemination, a cow is returning to be re-inseminated within 48 days. When a cow returns within this period, it may indicate unsuccessful fertilisation, loss of embryo before maternal recognition or problems with maternal recognition and the uterine environment.

Return from 49-80 days. If, after initial insemination, a cow returns for insemination between 49 to 80 days. When a cow returns within this period, it may indicate embryo loss after maternal recognition and problems with implantation.

Return from 81+ days. If, after initial insemination, a cow returns for insemination after 81 days. When a cow returns within this period, it indicates later pregnancy loss. During this period, the risk of pregnancy loss is significantly reduced (end of first trimester, second trimester and third trimester), and the number of records is limited.

Culling reason Miscarriage: Miscarriage was the number one culling reason for the culling of the cow.

Culling reason fertility. A fertility problem was the number one culling reason for the culling of the cow. These fertility problems included miscarriage, low fertility, non-return rate, poor heat and other fertility causes.

4.1.3. Description of Data Set

That data set included 1,215,005 observations of Norwegian Red cows, see Table 1. Each record consisted of an insemination event, followed by a subsequent insemination event if the first insemination event had failed. Additional information was collected, such as lactation number, cow's sire, birthdate, insemination dates, owner Id and pedigree for all animals.

The data used in the current study were from 4,999 herds with inseminations from 2014 to 2022. Within this time, records on 536,641 cows, sired from 793 bulls, were observed. Geno SA also provided pedigree data on all cows with records in the data set. Due to computational restrictions, the pedigree file goes back only four generations and includes 1,025,393 individuals.

Table 1. *Summary statistics of the data set, including the number of records, number of cows, number of sires (with at least 25 daughters in the data set), number of herds, the number of animals in the pedigree and years of insemination.*

	n
Number of records	1, 215, 005
Number of cows	536,641
Number of sires	793
Number of herds	4999
Year of Insemination	2014 - 2022
Pedigree	1,025,392

The observations of inseminations were summarised into one record per lactation, and given in Table 2 is a summary description of each trait. The percentage of

observations showing the trait ranges widely. Within the return rate traits, it ranges from 3.62% to 27.39%, with the percentage of the population experiencing loss decreasing as pregnancy progress; this decrease in percentage is the expectation for later pregnancy stages. For the culling traits of cows with records, 2.98% were culled due to miscarriage, and 27.39% were culled due to fertility problems in general (miscarriage included).

T**able 2.** *Summary statistics for the number of trait records (return 56, return from 0 to 48 days, return from 49 to 80 days, return from 81+ days, Culling reason fertility, Culling reason miscarriage) and observations of events in the analysed data.*

Trait	**n = 1**	**n = 0**	**Percentage return/culled**
Return 56	332,838	882,167	27.39%
Return from 0 to 48 days	315,057	899,948	25.93%
Return from 49-80 days	64,836	1,150,169	5.34%
Return from 81+ days	43,999	1,171,006	3.62%
Culling reason fertility	109,045	427,596	20.32%
Culling reason miscarriage	15,971	520.670	2.98%

n = 1 event occurred in the record; n = 0 event did not occur in the record.

Some traits also had a yearly trend, as seen in Figure 1. The number of culling due to fertility, the number of daughters returning before 56 days and from 0 to 48 days is decreasing, and this is a favourable trend. This positive trend may be due to improved management and/or selective breeding. On the other hand, culling due to miscarriage, daughters returning between 49 to 80, and 81 days plus, appears to go unchanged.

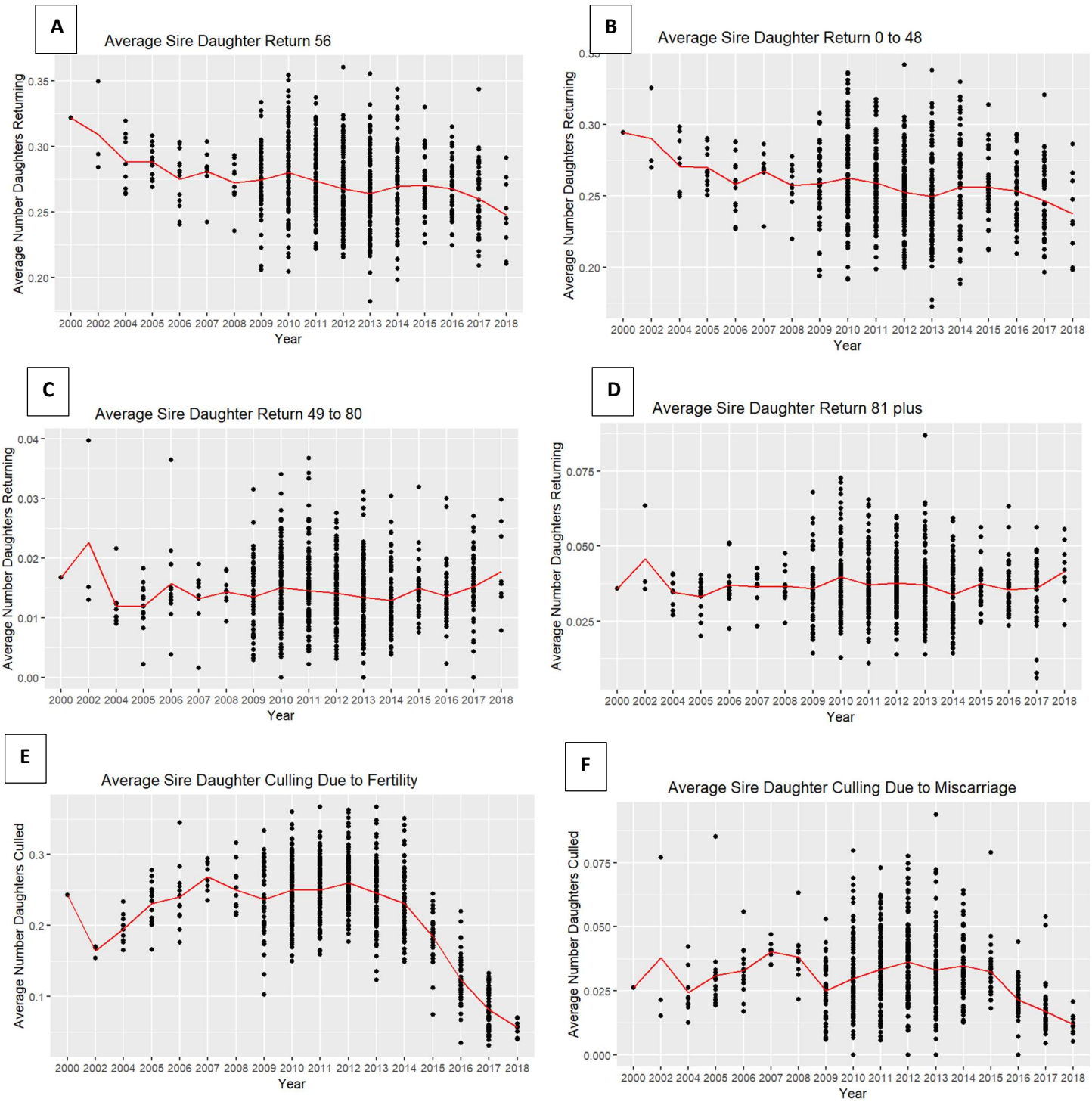

Figure 1. *Plots for sire-daughter trait trends (with at least 25 daughters in the data set) for insemination intervals and culling reasons: A, return within 56 days; B, return within 0 to 48 days; C, return within 49 to 80 days; D, return after 81 days; E, culling due to fertility problems; F, Culling due to miscarriage. Sires are plotted based on the birth year of the sire. The red line indicates the annual average percentage of a sire's daughter having observed return or culling.*

4.2. Statistical Analysis

4.2.1. Estimation of Variance Components

Univariate linear animal models were used when analysing the traits. The calculation of (co)variances was done with DMU, using the DMUAI package (Madsen & Jensen, 2000). The estimation of (co)variance components used Average information restricted likelihood (AI-REML), combined with Expected Maximisation (EM) with no scaling of data.

4.2.2. The Models

The following two linear animal models were used for calculating the (co)variance and heritability of the traits. Model 1.1 is an animal model for repeated measurements within the reproductive traits and used for control return 56 trait and return from 0 to 48, 49 to 80 and 81+ days. Model 1.2 is for traits without repeated measurements, which are the traits regarding culling reasons.

$$Y_{ijklmn} = AP_i + IYM_j + hy_k + a_l + pe_m + e_{ijklmn} \quad (1.1)$$

Where Y_{ijklmn} is an observation of all phenotypic observations across individuals, for a cow with additive genetic effect l, inseminated at the age-parity group i, in year-month j and herd-year class k; AP_i is fixed effect of age and parity, in 12 classes; IYM_J is fixed effect of month and year j of first insemination, in 108 classes; hy_k is a random effect, 31135 classes, where hy ~ $N(0,\mathbf{I}\sigma_{hy}^2)$, where **I** is the identity matrix, and σ_{hy}^2 is the herd-year variance; a_l is the random animal additive genetic effect of animal **l**, a ~ $N(0,\mathbf{A}\sigma_a^2)$, where σ_a^2 is the additive genetic variance, and **A** is the relationship matrix containing pedigree information four generations back for 1,025,392

animals; pe is the permanent environmental effect of cow m due to repeated observations, pe ~ N(0,**I**σ_{pe}^2), where **I** is the identity matrix, σ_{pe}^2 is the permanent environmental variance; e_{ijklmn} is the random effect of residual, of observation n with distribution e ~ N(0, σ_e^2), where σ_e^2 is the residual variance.

$$Y_{ijklm} = AP_i + IYM_j + hy_k + a_l + e_{ijklm} \quad (1.2)$$

The model is the same as model 1.1, except without the pe effect.

Herd-year was made a random effect due to low frequency in classes. While for better accuracy, the fixed effects of age and parity were merged and split into 12 groups, with a substantial number of observations. The heifers have four age groups, >13 months, 14 to 15 Months, 16 - 17 months, and 18< months. The one-parity cows were split into four age groups: >23 months, 24 to 25 months, 26 to 27 months, and 28< months. The two-parity age groups, >39 and 40<. The three-parity plus were also split into age groups, >54 and 55<.

4.2.2. Heritability, Repeatability and Correlation

For each defined trait, there was a heritability calculation (h^2). Traits under model 1.1, the return traits, had heritability defined using equation 2.1. Traits under model 1.2, the cull traits, had heritability defined using equation 2.2.

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2}$$

(2.1)

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2}$$

(2.2)

Where σ_a^2 is the additive genetic, σ_{pe}^2 is the permanent environmental, σ_e^2 residual variance components calculated for each trait.

Under model 1.1, the return traits had repeatability (R) calculated, which is defined using equation 3.1.

$$R = \frac{\sigma_a^2 + \sigma_{pe}^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2}$$

(3.1)

Where σ_a^2 is the additive genetic, σ_{pe}^2 is the permanent environmental, σ_e^2 residual variance components calculated for each trait.

There was also the calculation of the correlation of estimated breeding values between the traits. This correlation calculation used Spearman-rank correlations methodology.

4.3. Genome-Wide Association analysis

4.3.1. Genome-Wide Association Analysis

The genome-wide association was done as a quick analysis to identify any potential genetic causes behind the traits and, as a result, confirm whether these traits may be attractive for future investigations.

The variance component analysis included calculations of the estimated breeding values (EBV), thus the phenotypic values for sires in the genome-wide association analysis. Geno SA (www.geno.no) provided the genomic data. Genotypes from 4170 Norwegian Red animals with 617 739 SNP located on 29 chromosomes passed the quality control.

The Genome-wide association analysis was performed using the GCTA (Genome-wide Complex Trait Analysis), a software package developed for complex traits, using MLMA (mixed linear model association analysis using dense GRM (estimating genetic relationships among individuals in GWAS data) (Yang *et al.,* 2011; Yang *et al.,* 2014).

Upon the performance of the genome-wide association analysis, if the analysis revealed a definitive SNP peak over the significance threshold, it went under further investigation. From the most significant SNP in a peak, the region 1 Mb upstream and downstream (Berg *et al.,* 2020) was investigated on the NCBI genome viewer within the current cattle genome build (ARS-UCD1.2 genome) to identify genes in linkage disequilibrium with the SNP marker (Rangwala *et al.,* 2020). Genes within this region were listed then investigated for functional perspective in Ensembl (GO functions) and the literature; in order to identify potential candidate genes and to compare regions of significance with other studies (Wolfsberg, 2010).

4.3.2. Computing Proportion of Variance in Phenotype Explained by a Given SNP

The variance in the phenotype can be decomposed into two components (4.1):

$$Var(Y) = \beta^2 Var(X) + \sigma^2 \tag{4.1}$$

β is the effect size of genetic variant X, $\beta^2 Var(X)$ captures the variance explained by the genetic variant X, and σ^2 is the remaining variance (environmental factors or other genetic variants).

The $\beta^2 Var(X)$ can be estimated by $2\,\hat{\beta}^2\,MAF\,(1 - MAF)$ (4.2):

$$Var(\hat{\beta}) = (se(\hat{\beta}))^2 \approx \frac{\sigma^2}{2\,N\,MAF\,(1 - MAF)} \tag{4.2}$$

$\hat{\beta}$ is the size effect estimate and MAF the minor allele frequency for the genetic variant X.

From a simple linear regression model, therefore, X and Y as covariate and response (4.3):

$$PVE = \frac{\beta^2 Var(X)}{Var(Y)} = \frac{\beta^2 Var(X)}{\beta^2 Var(X) + \sigma^2}$$

Therefore, the proportion of variance in phenotype explained by a given SNP (PVE) was estimated using equation 4.4 (Teslovich *et al.,* 2010).

$$PVE = \frac{2\,\hat{\beta}^2\,MAF\,(1-MAF)}{2\,\hat{\beta}^2\,MAF\,(1-MAF) + (se(\hat{\beta}))^2\,2\,N\,MAF\,(1-MAF)} \quad (4.4)$$

$\hat{\beta}$ is the effect size of the estimate, MAF minor allele frequency for the genetic variant X, N is the number of individuals in the analysis, se() is the standard error of effect size for the genetic variant X.

For the most strongly associated SNPs of each peak, there was the calculation of PVE.

5. Results

5.1. Statistical Analysis

5.1.2 The Variance Components and Heritability

Estimates of variance components and heritability were calculated for the defined traits and given in Table 3. The highest heritability was culling due to fertility (0.05). While the control trait return by 56 days (0.01) and the trait return within 0 to 48 days (0.01), both with low heritability. The traits, return by 49 to 80 days (0.002) and return 81 days plus (0.002), had a calculated heritability of even lower values. The repeatability values were also low for all traits.

Table 3. *The variances components (SE) calculated heritability and repeatability of return rate 56, return 48, return 49 to 80, return 81+, miscarriage and culling due to fertility problems.*

	Variance components						
Trait	**σ_a^2 (SE)**	**σpe^2 (SE)**	**σ_{hy}^2 (SE)**	**σ_e^2 (SE)**	**σ_p^2**	**h^2**	**R**
56	0.228 -02 (0.177 -03)	0.376 -02 (0.224 -03)	0.480 -02 (0.825-04)	0.187 (0.301 - 03)	0.193	0.0118 1.18%	0.031
0 - 48	0.206 -02 (0.164 -03)	0.356 -02 (0.213 -03)	0.443 -02 (0.778 -04)	0.181 (0.291 - 03)	0.187	0.0110 1.10%	0.030
49 - 80	0.779 -04 (0.130 -04)	0.483 -03 (0.500-04)	0.482-03 (0.144-04)	0.494 -01 (0.801-01)	0.050	0.0016 0.16%	0.011
81+	0.671-04 (0.103-04)	0.303 -03 (0.367-04)	0.910-03 (0.154-04)	0.335 -01 (0.559-04)	0.034	0.0020 0.20%	0.011
CullF	0.713 -02 (0.391 - 03)		0.171 -01 (0.221 - 03)	0.131 (0.373 - 03)	0.138	0.0517 5.17%	
CullM	0.751 -04 (0.147 - 04)		0.801 -03 (0.205 - 04)	0.278 -01(0.564 -04)	0.028	0.0027 0.27%	

56 = Return rate 56; 0 - 48 = Return from 0 to 48 days; 49 - 80 = Return from 49 to 80 days; 81+ = Return after 81 days; CullF = Culling due to fertility reasons; CullM = Culling due to miscarriage.
σ_a^2 = Additive genetic variance; σ_{pe}^2 = permanent environmental variance; σ_{hy}^2 = herd-year variance; σ_e^2 = residual variance; σ_p^2 = phenotypic variance ($\sigma_a^2 + \sigma_{pe}^2 + \sigma_e^2$) or ($\sigma_a^2 + \sigma_e^2$); h^2 = heritability, R = repeatability.

5.1.2 The Fixed and Random Effects

The model calculated all animals' estimated breeding values (EBV) as a random effect. In all animals, the breeding values ranged from return rate 56 -0.069 to 0.105, with a standard error (SE) of 0 to 0.051; Culling due to fertility reasons -0.167 to 0.225, with an SE from 0 to 0.09; culling due to miscarriage -0.008 to 0.019, with SE from 0 to 0.01; return 0 to 48 -0.069 to 0.105, SE 0 to 0.051; return 49 to 80 -0.019 to 0.016, SE 0 to 0.01; return 81 plus -0.025 to 0.015, SE 0 to 0.009. The calculated sire breeding values had a lower SE rate across all traits than all the animal breeding values (the dams). Breeding values for all sires calculated showed a normal distribution (some slightly skewed), and population variance, which can be observed

in Figure 2. Culling due to fertility problems shows the largest variance in breeding values, followed by the control and return 0 to 48.

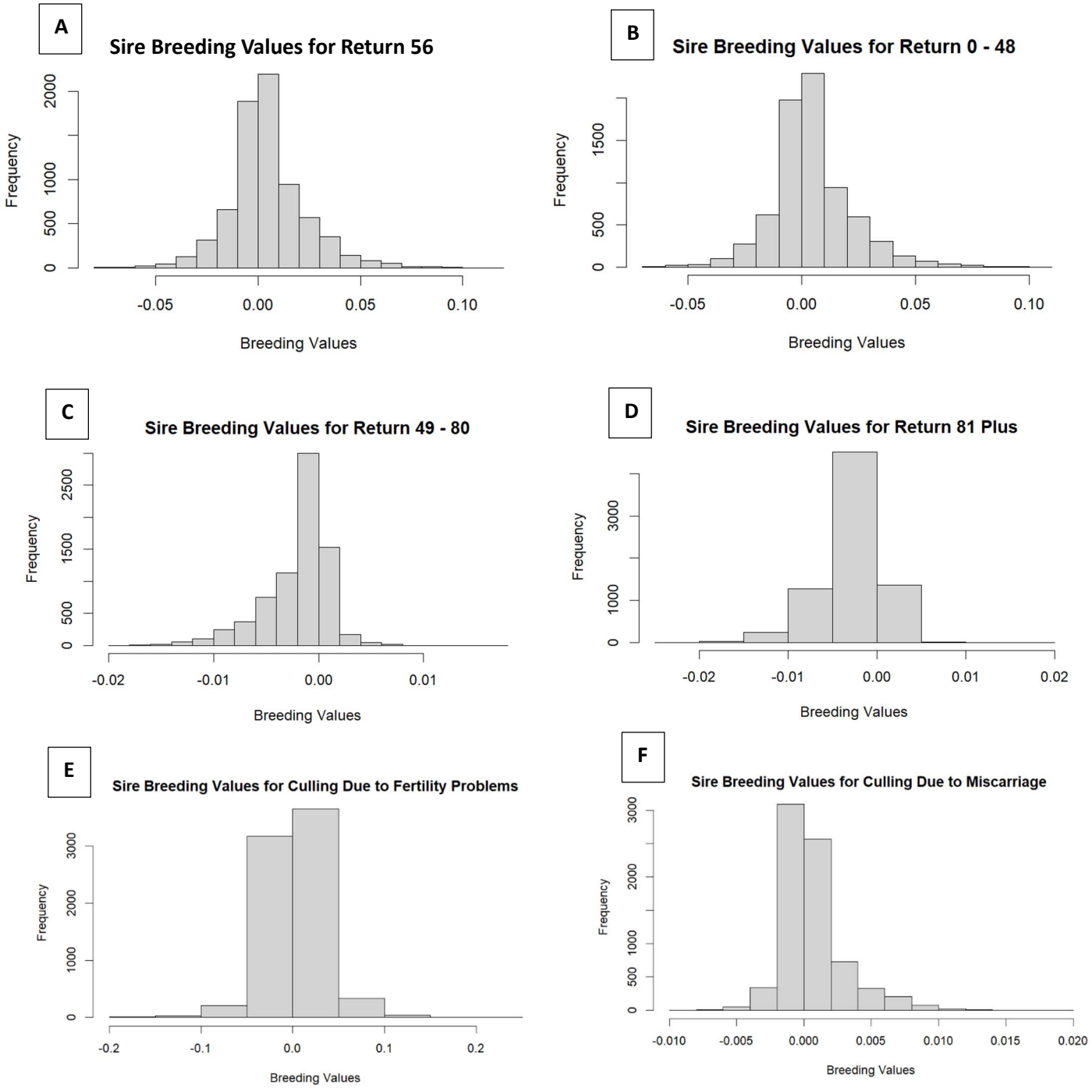

Figure 2. *Histogram of Norwegian Red sire (with at least 25 daughters in the data set) breeding values for return for insemination and culling traits: A, return within 56 days; B, return within 0 to 48 days; C, return within 49 to 80 days; D, return after 81 days; E, culling due to fertility problems; F, culling due to miscarriage.*

A fixed effect included in the model was month-year effects, as illustrated in Figure 3, showing significant differences between month-years for all traits. There is a favourable trend for the return traits, with the number of returns decreasing over the past ten years. Furthermore, there appears to be a seasonal variation across all return traits. On the other hand, there is no significant trend for either of the culling traits. Apart from a trending decline from 2021/2022, which is likely because the cows are young.

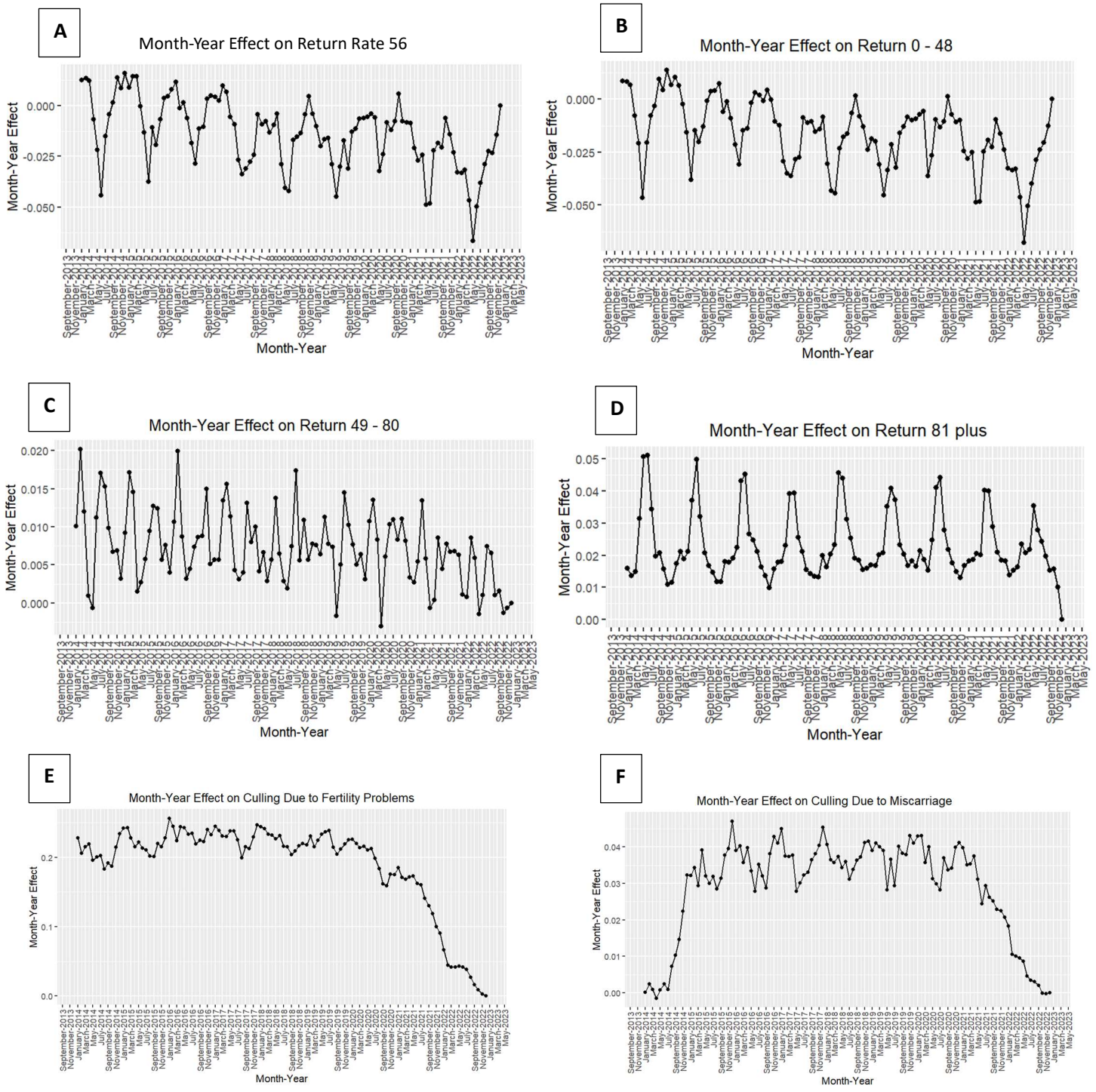

Figure 3. *A graph to represent the month-year effect for traits for return for insemination and culling in Norwegian Red cattle: A, return within 56 days; B, return within 0 to 48 days; C, return within 49 to 80 days; D, return after 81 days; E, culling due to fertility problems; F, culling due to miscarriage.*

The other fixed effect was Age-Parity, Figure 4. Looking at the effect on the return by day 56 and 0 to 48, we can see that a return is more likely as the animal has more offspring and a younger age within parity. This trend is reflected in the effect of culling due to fertility. Conversely, there appears to be no significant trend for the interval 49 to 80, and only an increased risk during the first parity for return 81 days.

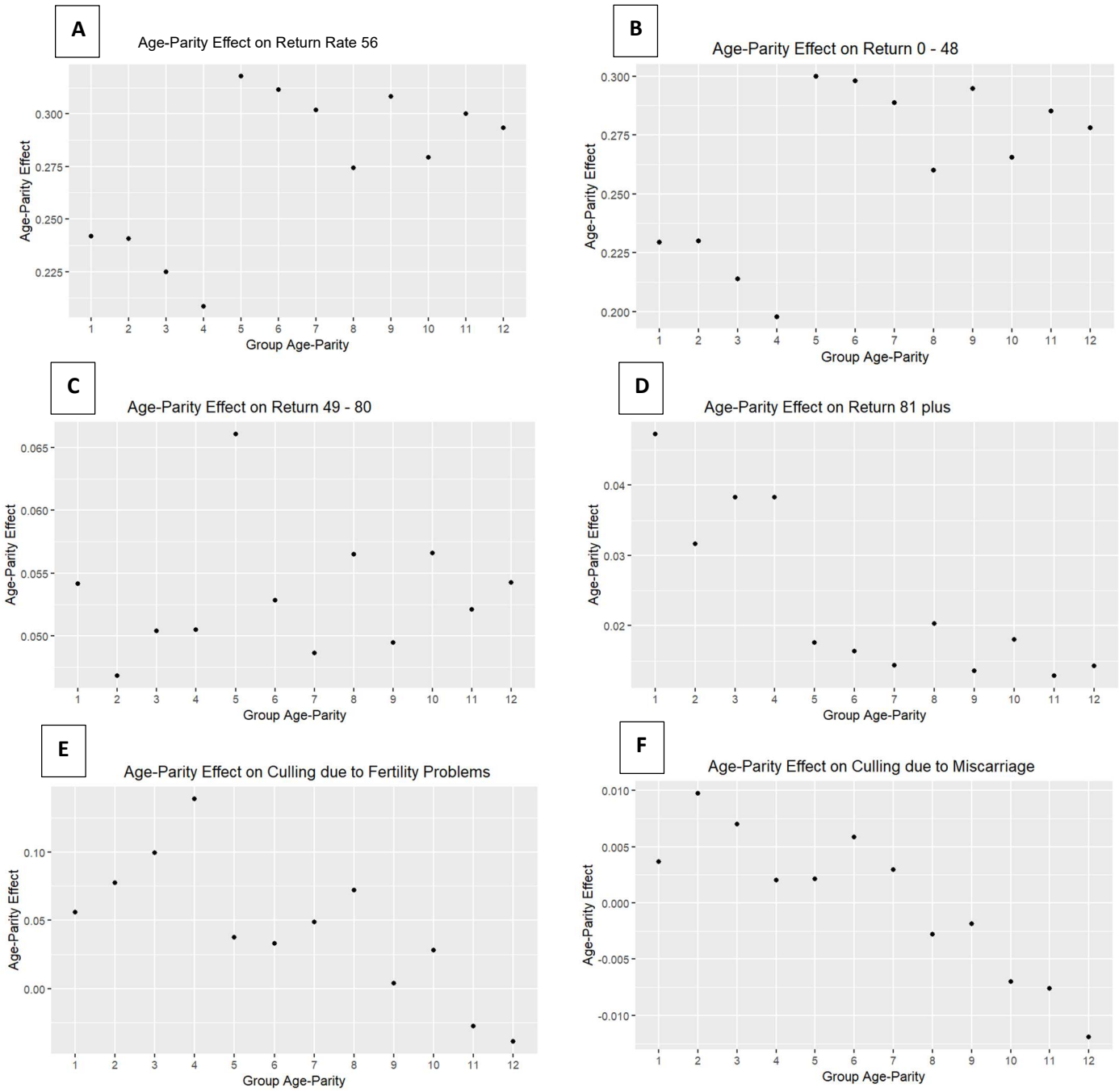

Figure 4. *A graph to represent the group Age-parity effect in Norwegian Red cattle for the novel return and culling traits: A, return within 56 days; B, return within 0 to 48 days; C, return within 49 to 80 days; D, return after 81 days; E, culling due to fertility problems; F, culling due to miscarriage.*

Regarding genetic trends (Figure 5), there appears to be no significant trend for estimated genetic merit regarding culling for all fertility problems, as the average breeding values of sires born over the years appear to experience no change. However, there appears to be a change in average sire breeding value across the other traits. There is a favourable decrease in daughter return traits from 49 to 80 and return 81 plus. Conversely, the opposite may be true for return 56, 0 to 48 and culling due to miscarriage, with an increasing trend in the last few years.

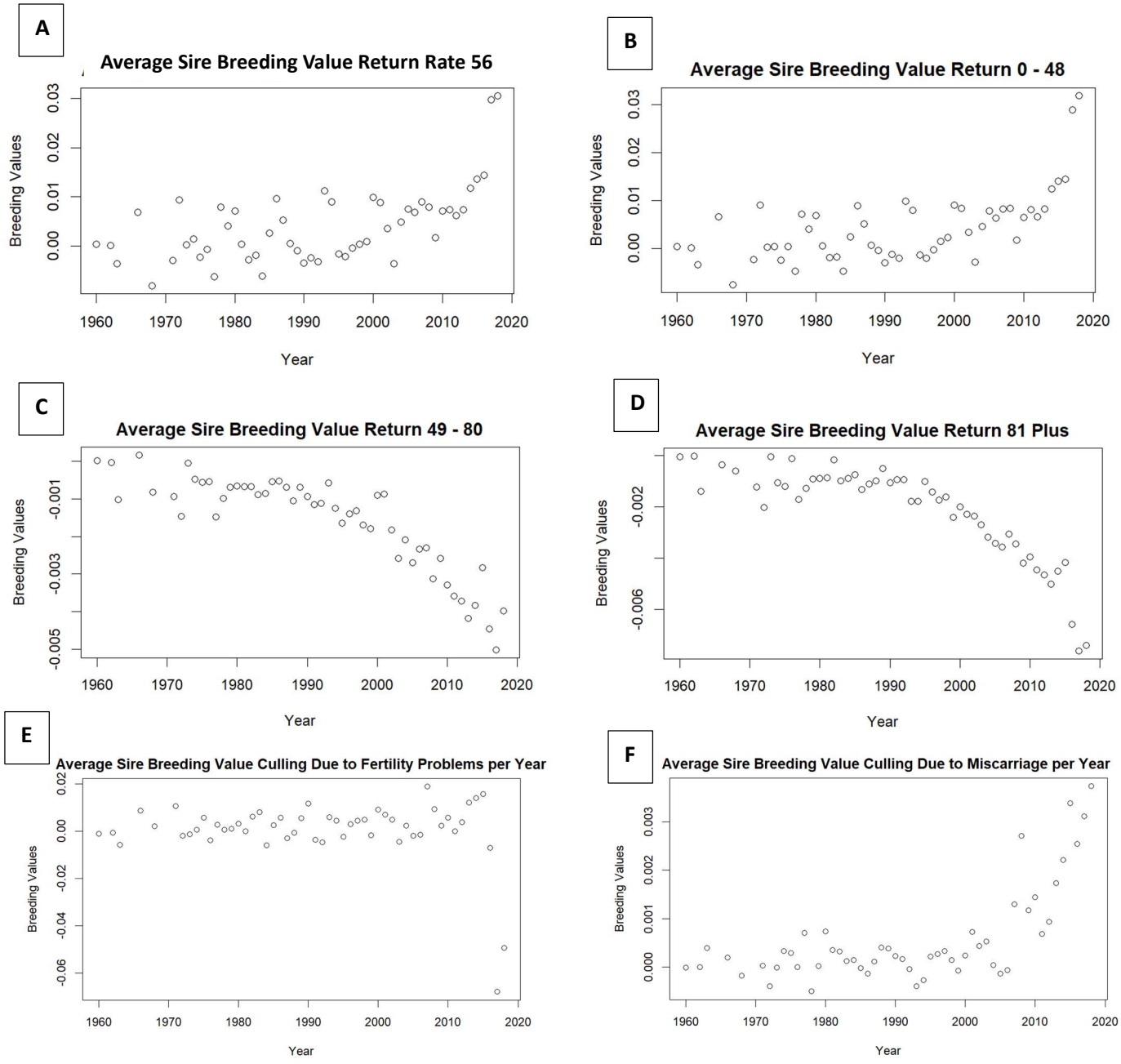

Figure 5. *Scatter plot of average sire (with at least 25 daughters in the data set) breeding value per year for traits in Norwegian Red cattle, for novel traits in return for insemination and culling traits: A, return within 56 days; B, return within 0 to 48 days; C, return within 49 to 80 days; D, return after 81 days; E, culling due to fertility problems. F, culling due to miscarriage.*

The observed phenotype of the sire's return and culling (based on percentage) vs the predicted values (predicted additive genetic variance) were plotted to confirm the results. As Figure 6 shows, there is a strong correlation between the observed and

predicted values of the control, return 0 to 48 days, and culling due to fertility. Regarding the other traits, return 81 days plays and culling due to miscarriage shows moderate correlation. However, there appears to be weak to no correlation with the trait return 49 to 80.

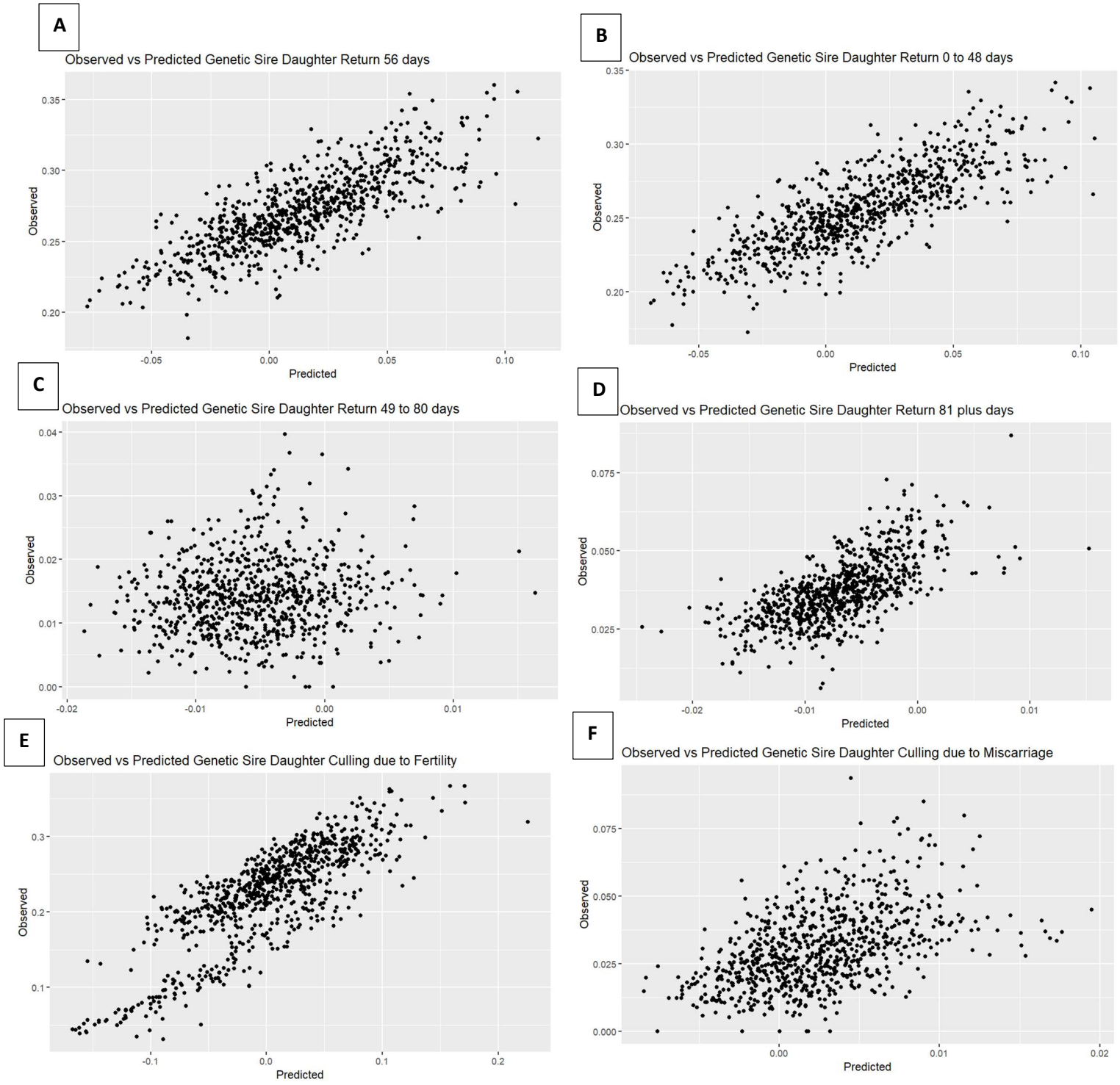

Figure 6. *Observed sire daughter phenotype vs predicted sire additive genetic variance (sire with at least 25 daughters in the data set) for the novel traits in return for insemination and culling: A, control return within 56 days; B, return within 0 to 48 days; C, return within 49 to 80 days; D, return after 81 plus days; E, culling due to fertility; F, culling due to miscarriage.*

The correlation between all sire EBV was also calculated, with Spearman-rank correlations done between the control and novel traits, and the novel return traits. The highest correlation was between the control return 56 and return trait 0 to 48 (0.976), which is expected as most of the trait data is the same. The correlation between control rate 56 and culling due to fertility problems was slightly lower but also positive (0.277). However, there was little and a slightly negative correlation between the control 56 and return 49 to 80 (0.119) and 81 plus (-0.117). As anticipated, the correlation of return 0 to 48 has similar genetic correlations to the other traits as the return 56, with the return 49 to 80 (0.048) and 81 plus (-0.149) having little correlation. Furthermore, the correlation between return 49 to 80 and return 81 plus is the second highest at 0.594.

Table 4. *Correlation of sire breeding values between the traits control and novel traits, and between the novel trait return traits (control return 56 days. Novel traits: within 0 to 48 days; within 49 to 80 days; after 81 days; culling due to fertility problems; Culling due to miscarriage).*

Correlation

Variable	**56**	**Trait048**	**Trait4980**	**Trait81**	**CullF**	**CullM**
56		0.976	0.119	-0.117	0.277	0.141
Trait048			0.048	-0.149		
Trait4980				0.594		
Trait81						

56 = Return rate 56; Trait048 = Return from 0 to 48 days; Trait4980 = Return from 49 to 80 days; Trait81+ = Return after 81 days; CullF = Culling due to fertility reasons; CullF = Culling due to Miscarriage.

5.2. Genome-Wide Association Study

For all the traits, control, and novel traits (return within 0 to 48 days, return within 49 to 80 days, return after 81 days, culling due to fertility problems, and culling due to miscarriage), a genome-wide association analysis was done. The association analysis results are in Figure 7, represented in Manhattan Plots. The plots have a blue line indicating suggestive significance, while the red line indicates significance above the threshold. All traits showed SNP peaks above the suggestive significance, while the return traits showed SNP peaks of interest above the threshold. The SNP peaks for the return traits were peak at BTA26 for return 0-48 days, BTA23 and 24 for 49-80, and return after 81 days on BTA 12. It is worth noting that due to the methodology for the brief analysis, spurious associations were higher than wanted, so the results should be interpreted with caution. However, the SNP peaks and the differentiation between the traits are positive indications for the novel traits.

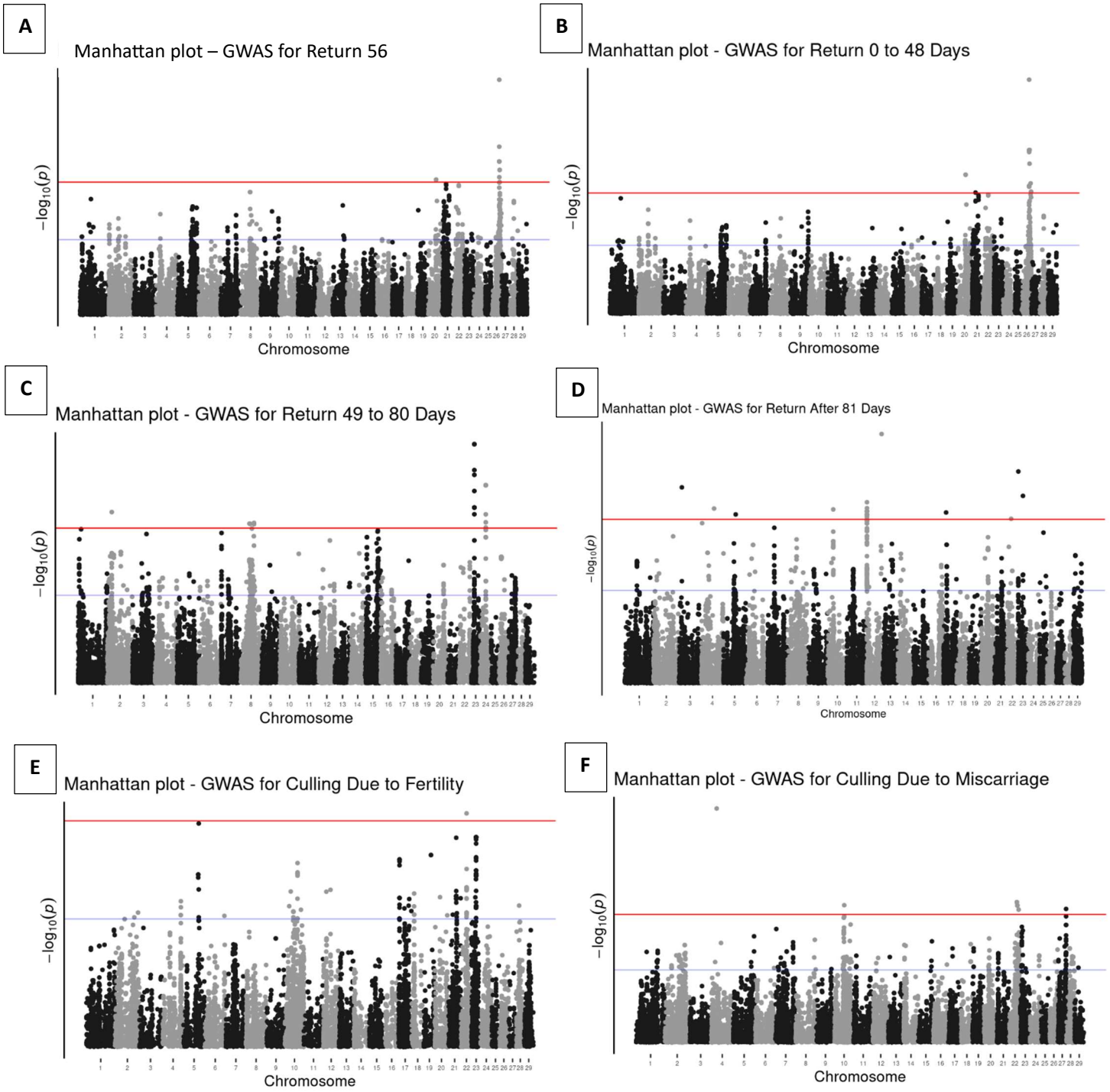

Figure 7. *Manhattan plots for the novel traits in return for insemination and culling in the Norwegian Red cattle population: A, return within 56 days; B, return within 0 to 48 days; C, return within 49 to 80 days; D, return after 81 days; E, culling due to fertility problems; F, culling due to miscarriage. The red line is the threshold significance line, and the blue line is suggestive significance.*

5.2.1. Return 0 to 48 Days

The genome-wide association on the novel trait return 0 to 48 illustrates a significant SNP peak on BTA 26, with the most significant SNP at bp 40608203. This SNP is inside the gene phospholipid phosphatase 4 (PLPP4). The PLPP4 gene has GO:0001835 blastocyst hatching, so the gene plays a role in hatching the cellular blastocyte from the zona pellucida. This genetic role of the gene links to the trait of interest, and thus this trait may be a good candidate gene. However, despite the potential of being a candidate gene, there is little information regarding the gene and no identification in other studies.

There was also an investigation of the region around the SNP. Within this region was the Fibroblast growth factor receptor (FGFR2). Although there was no identification of FGFR2 in other GWAS studies, literature studies support it as a potential candidate. These literature studies are within mutant mice models and cattle. In the mutant mice study by Filant *et al.* (2014), the mice suffered from peri-implantation pregnancy loss.

Interestingly, the mice were initially subfertile and infertile, with increasing parity. Regarding cattle studies, the gene enhances uterine receptivity to implantation and placentation, critical to maternal conceptus interactions during early pregnancy (Lim *et al.,* 2017). Furthermore, Okumu *et al. (2014)* found the receptor essential for signalling during early pregnancy, and its expression significantly increased by day 16. These studies indicate a clear biological link to the return trait 0 to 46, suggesting that the FGFR2 may be a good candidate gene.

Other fertility studies also identified significance within this region. However, the closest gene noted in the study did not link to fertility or was not likely to be biologically linked to the trait. These genes included the TIAL1 (associated with the development of primordial germ cells) and BAG3 (proximity to QTL affecting daughter pregnancy rate in Nellore beef cattle) in other studies (Nascimento *et*

al., 2018; Grigoletto *et al.,* 2020). In addition, the gene WRD11 is causal of hypogonadotropic hypogonadism in humans, but no mention in cattle (Kim *et al.,* 2010). The other genes within the region did not affect fertility (RGS10, INPP5F, MCMBP, SEC23P).

5.2.2. Return 49 to 80 Days

The first significant SNP peak for trait return 49 to 80 was the SNP bovineHD2300005876 on BTA 23 on gene LOC112443810 which was uncharacterised. The gene closest to the SNP was phosphoglycerate kinase 2, PGK2. Although expressed in the female reproductive system, the GO biological process was flagellated sperm motility and only documented concerning bull fertility. The gene's role in bull fertility was a trend for other genes in the region. These genes were CRISP1/2/3 gene and beta-defensins (DEFB114, DEFB133, DEFB110, DEFB112) and identified as a role in male fertility in livestock (Diether & Dyck, 2017; Lui *et al.,* 2019; Solanki *et al.,* 2023).

Fortunately, the region is also significantly associated with female reproductive genes in cattle. These genes are TFAP2D (Tahir *et al.,* 2021), TFAP2B (Liu *et al.,* 2017; Tahir *et al., 2021*) from the AP-2 family, and identified as positional candidate genes that contribute to gestation progress (Tahir *et al., 2021*). The AP-2 family plays a crucial role in placetogenesis and may be "involved in trophoblast cell differentiation, remodelling of the endometrium, implantation and regulation of the bovine placenta". Although the exact roles of the genes are unclear, it links biologically to a return of 49 to 80 days and would be suitable candidate genes (Ushizawa *et l.,* 2007).

The other genes within the region appeared not to affect fertility or had little information referencing them to make any conclusions (RHAG, C23H6orf141, GLYATL3, CENPQ, MUT, OR5M10, OR9G1).

The next SNP peak for return 49 to 80 was on BTA 24, with the most significant SNP BovineHD2400008764. The closest gene to the SNP was zinc finger protein 521, ZNF521. The ZNF521 has been identified as a female fertility candidate gene in multiple GWAS in cattle and Norwegian Red cattle (Hoglund *et al.,* 2015; Mohammadi *et al.,* 2020; Vineeth *et al.,* 2020; Rahman *et al.,* 2023). Although this repeated association with female fertility indicates association in this region, it is known if/how it affects fertility.

The other genes within the region did not affect fertility or had little to no information (TRNAC-GCA, SS18, LOC112444180, HRH4, IMPACT, OSBPL1A, TTC39C, LAMA3). However, the CABYR was associated with male fertility (Cai *et al.,* 2017; Gross *et al.,* 2019).

5.2.3. Return 81 Days

The final trait was return after 81 days, with a SNP peak on BTA 12. The region around this peak is poorly defined (with no information on LOC789865, LOC100140262, LOC112449029, LOC540096, LOC112449031, and LOC112449024); however, the chromosome and the region are associated with fertility traits (Li *et al.,* 2018; Fernandez *et al.,* 2019). The two genes within the region are DIAPH3 and TDRD3. The TRDR3 gene has implications for female fertility, but nothing definitive (Tarekegn *et al.,* 2021). The other gene is DIAPH3, in which the SNP is within. This gene causes chromosome instability under high-temperature conditions, increasing or decreasing the number of chromosomes passed on to daughter cells (Kazama *et al.,* 2020).

There is also another potential cause for this peak. Although not within 1Mb, nearby, a well-known 660-Kb deletion in Norwegian Red cattle may be the cause. This deletion is a cause of fertility problems in Norwegian Red cattle and is associated

with late returns (Kadri *et al.,* 2014). As it is a large deletion, it may be why the region is slightly off; however, this study cannot confirm or deny this.

Table 5: *The most significant SNP at peaks identified in GWAS (for traits return from 0 to 48 days, return 49 to 80 days, return 81 days) with the closest gene and genes within the 1MB downstream and upstream*. In addition to frequency, regression coefficient and computed proportion of variance in phenotype explained by a given SNP (PVE).*

Trait	chr	SNP	bp	Freq	b	PVE	Closest Gene	Genes Within Region
Return 0 to 48	26	BovineHD2600011310	40608203	0.173	0.0034	0.013 (1.3%)	**PLPP4**	RGS10, TIAL1, BAG3, INPP5F, MCMBP, SEC23P, WDR11, **FGFR2**.
Return 49 to 80	23	BovineHD2300005876	22505580	0.441	-0.0003	0.010 (1.0%)	LOC112443810 PGK2 (closest)	CRISP3, CRISP2, RHAG, C23H6orf141, GLYATL3, CENPQ, MUT, OR5M10, OR9G1, CRISP1, DEFB133, DEFB114, DEFB113, DEFB110, DEFB112, **TFAP2D, TFAP2B.**
	24	BovineHD2400008764	31795004	0.524	0.0003	0.010 (1.0%)	No information for the region.	ZNF521, TRNAC-GCA, SS18, LOC112444180, HRH4, IMPACT, OSBPL1A, CABYR, TTC39C, LAMA3
Return 81 days	12	BovineHD1200000750	2490117	0.152	-0.0008	0.038 (3.8%)	DIAPH3	LOC789865, LOC100140262, TDRD3, LOC112449029, LOC540096, LOC112449031, LOC112449024,

**Genes related to fertility are underlined, and the genes related to the trait phenotype are highlighted in bold.*

6. Discussion

This study aimed to develop novel strategies to investigate pregnancy loss throughout gestation and identify traits to reduce the incidence of loss in Norwegian Red cattle. This development of novel strategies to reduce pregnancy loss uses currently available field data insemination records and culling information. Firstly, the insemination records indicate unsuccessful fertilisation and loss of pregnancy; used to create traits return 0 to 48, 49 to 80 and 81 plus, indicating loss at different developmental stages. Secondly, the culling reason indicates fertility problems observed by the farmers. This observation led to the traits culling due to fertility problems and culling due to miscarriage. In addition, a standard fertility indicator, (non-)return 56, was also included as a control.

Subsequently, statistical analysis of the novel traits took place. This analysis involved calculating variance components and followed by a genome-wide association analysis.

6.1 The variance Components and Heritability

6.1.1. Initial Observations

The paper started with initial observations on the traits and yearly trends. These observations were taken over a ten-year period, it would not have been possible to extend this due to computational restrictions.

The control trait revealed that over the ten-year period, on average 27.39% of cows were returning for re-insemination within 56 days (or 72.61% non-return rate 56). Other studies had also found similar results, for example, Resdal (2007) and Garmo*et al.*, (2008), calculated the non-return rate of 72.7% and 72.5% in Norwegian Red cattle, respectively. In addition, the non-return rate 56 showed a

favourable decline for sire's daughters average return rate. Although this decline is not reflected between this study and the two previously mentioned, an estimate of non-return rate in 1985 was 68.1%, indicting a favourable trend in Norwegian Red cattle fertility (Resdal 2007). Fertility has been included in the selection index of Norwegian Red cattle since 1971 which most likely aided the favourable increase in non-return rate across these periods, in addition to changes in management.

For the novel traits, apart from return 0 to 48 day which overlaps with return 56, there appeared to be no significant trends in sire's daughter average return traits. As these are novel traits, there are no other studies of these traits in Norwegian Red cattle for comparison. However, other studies reported that rate of still births remined unchanged from 1978 to 2004 despite being part of selection index (Heringstad *et al.,* 2007) and the rate of culling due to fertility problems has also remined steady in Norwegian Red cattle (Resdal 2007).

Despite this favourable increase for the non-return rate 56, a significant percentage of Norwegian red cows, one in five, are still being culled to due fertility problems. These fertility problems included miscarriage, low fertility, non-return rate, poor heat, or other fertility causes. Furthermore, many of these cows were culled within their first few parities meaning that there were potentially lack of repeated records for cows with poor performance.

The novel return traits showed that the percentage of cows returning for re-insemination after 48 days significantly drops, and risk continues to fall after 80 days. This decrease in risk of pregnancy loss is expected, and aligns with previous studies (Bamber *et al.,* 2009). It also indicates that the majority of re-inseminations may be due to unsuccessful artificial insemination, unsuccessful fertilisation events, the uterine environment, or an issue with maternal recognition and blastocyte hatching. However, when returning before 48 days, it is still not possible to determine the proportion of return failures due to fertilisation failure or early embryonic loss.

6.1.2. Heritability and Repeatability

Using a linear animal model, variance components were calculated and used to estimate the heritability and repeatability of the control and novel traits. The calculations revealed small phenotypic variation, low heritability, and low repeatability for all traits, with some variation between them. This low heritability and repeatability would indicate that reproductive success has little to do with genetics and indicates that environmental effects are dominating (Bormann *et al.,* 2006). As environmental effects play such a large role, it highlights the importance of proper management; however, when reproductive success is not included in genetic evaluations it has led to fertility decline and indicates importance of inclusion despite the low heritability.

In general, fertility traits in cattle are typically lowly heritable, with values calculated at less than 0.1, with between breed differences (Shahinfar *et al.,* 2014; Sidgel, Bisinotto, & Penegaricano, 2022). In Norwegian Reds, the heritability of non-return rate 56 has been calculated at 1.2% to 1.4%, with models varying slightly (Ranberg *et al.,* 2004). These values are very similar to the 1.18% heritability of the control return rate 56 calculated in this paper.

For the novel traits of the paper, the return 0 to 48 had a similar heritability to return 56 as expected at 1.10%, and the later return traits had a much lower heritability at approximately 0.2%. While the culling traits had a heritability of 5.17% and 0.27%, for fertility problems and miscarriage respectively. The larger heritability of the culling trait for fertility problems is favourable for a novel trait and is higher than the control trait. This higher heritability may be caused by the observed reasons for poor reproductive health having greater genetic causes, and the novel trait may be improved through selection with greater genetic gain than the others.

The results also indicate that the heritability of later pregnancy loss is lower than early pregnancy loss. However, these traits have extremely low incidence rates which may have led to problems in the estimation of the variance components (Pryce *et al.,* 1998). Despite, combining later pregnancy losses, when possible, to increase the number of records after risk drops, the number of incidences was still very low. When estimating variance components, the binomial probability of the trait should be within 20% to 80%, however this is not valid for the aforementioned traits and is violating linear model assumptions (VanVleck, 1971; Gonzalez-Recio & Alenda, 2005). This distribution may therefore lead to bias, so although the advantages of a linear model are expected to outweigh the disadvantages, different modelling approaches may be considered for the future (Pryce *et al.,* 1998; Gonzalez-Recio & Alenda, 2005).

A modelling approach that may be beneficial is a threshold model. Likewise, it also has its disadvantages, including that it is less conservative, the extra computational time required and at least one incidence is required per subclass (Pryce *et al.,* 1998). Despite these disadvantages, multiple studies are proving its success for disease and fertility traits (Gonzalez-Recio & Alenda, 2005; Bamber *et al.,* 2009). In addition, it is also possible to model multiple traits simultaneously. By modelling multiple traits simultaneously, it can enable sounder modelling of the environment and calculate the genetic correlation of those traits (Muuttoranta *et al.,* 2019). Accordingly, simultaneously assessing pregnancy loss at different gestation points may improve estimation (Tiezzi *et al.,* 2015). Furthermore, another alternative is estimating the variance components using a single-step genomic evaluation methodology which was found to have higher accuracy (Cornelissen *et al.,* 2017). As this study confirms that these novel traits are heritable with a genetic background, further study with comparison between different methodologies for these novel traits may be beneficial. However, the threshold model is on a different scale, so it is not directly comparable.

Further considerations are field data is collected under commercial conditions, so the quality of this data, the calibre of heat detection and artificial insemination may vary (Bormann *et al.,* 2006). Furthermore, there may be trait variation due to farmers'

decisions for inseminations and culling of cows. The insemination records are determinants for return rates, and this means that insemination is always considered successful if there is no recorded breeding within the set interval. There may also be disparities between farmers' observational skills and decision processes for the culling traits. Despite this room for error, culling due to fertility problems had the highest heritability and thus appeared to be no issue with the re-inseminations and culling having a standard practice. However, direct recording of pregnancy loss through the use of confirmed pregnancy may have increased heritability estimates (Sidgel *et al.,* 2022).

Pedigree in the statistical analysis considers the nonindependence of records among relatives when calculating additive genetic effect. More information can limit the standard error and improve the magnitude of heritability (Akesson *et al.*, 2008). Conversely, adding more generations to the known lowly heritable trait may not have a prominent enough effect that makes it worth the extra computational task (Blangero *et al.,* 2013). Moreover, further computational restrictions were at play, with the pedigree data limited to four generations and no room for additional generations.

6.1.3. Trait Correlations

As expected, the return 56 control and return 0 to 48 EBV trait correlation revealed them to be almost identical. Notably, there appeared to be little to no correlation and small negative correlation between return 0 to 48 and the return traits 49 to 80 and return after 81 days, respectively. This lack of correlation between the return traits indicates that the traits may be under different genetic control. Furthermore, the traits return 49 to 80 and trait 81 appear to have some correlation. In addition, a study a by Sigdel *et al.,* (2022) also found that “fetal loss is largely independent of current fertility traits” as they capture other components of fertility, so advocates for use in selection.

For control and culling traits, there appears to be a weak but positive correlation. It means that some cows are being culled due to fertility problems. However again, they may be capturing different components.

6.2.4. The Fixed and Random Effects

The linear animal model accounts for various fixed and random effects for the trait, including age-parity, first insemination month-year, herd year, animal effect, and permanent environmental effect. In addition, various effect thresholds were set to ensure enough records per group to aid in the accuracy of the variance component calculations (Lush, 1931). Despite these restrictions causing a loss in half the data, the stringent editing is necessary and is typical for fertility traits (Averil, Rekaya, &Weigel, 2004)

Month-year effect was used as a fixed effect, as it helps to avoid confounding environmental and genetic effects (Ranberg *et al.,* 2003). Although there were no seasonal or yearly variation for culling traits, there was seasonal variation for all return traits with a favourable trend over years. The same seasonal variation for the return 56 and return 0 to 48 days is also similar in other studies in Norwegian Red cattle (Ranberg *et al.,* 2003; Anderson-Ranberg *et al.,* 2005). However, there were no comparisons for later losses in Norwegian Red cattle.

The inclusion of age-parity effect with grouping shows smaller prediction errors and higher correlations between observed and predicted values (Ranberg *et al.,* 2003). All the culling problems show decrease of culling due to fertility problems or miscarriage as age increases, likely because the cows not performing are culled early on, while cows that perform well are kept for later. However, there does appear to be age-parity effects on the novel traits, with a different age-parity trend for the return traits. Return 0 to 48 showing increase in an unsuccessful pregnancy event

after the first parity, and insemination at a later age for parity is beneficial (Ranberg *et al.,* 2003). For return 49 to 80, there appears to be an increase in risk of loss as parity increases. While for age-parity for 81 days plus, chance of loss decreases after the first pregnancy.

The EBV of sires were also investigated. In general, it appeared that there were no EBV trends for the culling traits, though there were some fluctuations. While the return rate 56 and return 0 to 48 days also showed fluctuations, but a potential unfavourable trend for sires born within the past few years. This is unexpected as this trait is under selection, observations indicated a favourable decline in return rates and observations had positive correlation to observed. The exact reason is unknown. Conversely, the other return traits showed a favourable trend. However, the correlation between observed and predicted genetic value for the return 49 to 80 days appeared weak indicating other environmental influences.

Nevertheless, the model does not account for all potential sources of variation, and different effects could be considered in the future to try improving the model (Averill, Rekaya, & Weigel, 2004). As a consequence of less unexplained variance or error variance, higher heritability could be expected (Pryce *et al.,* 1998). Other effects that could change the outcome are the person doing the insemination, service sire, disease, and embryo abnormalities (Bamber *et al.,* 2009; Spencer, 2013; Tiezzi *et al.,* 2015; Wijma *et al.,* 2022). For example, fertilisation success can also be affected by the insemination bull. However, the service sire was already considered a random effect but deemed too computationally expensive for its worth in this study. Service sire was found to have little influence on the model's ability (Ranberg *et al.,* 2003). Furthermore, it is expected that the most elite sires are used more often and can have thousands of daughters. However, their use is regulated, so there should be no bias from non-random effects across herds (Ranberg *et al.,* 2003).

Other sources of environmental effects may be considered. Another example is that the embryo may cause pregnancy loss, and embryo inbreeding may be considered. However, it is equally important to consider the availability of data, the computational expense and not to overfit the effects of the model.

6.2 Genome-wide Association Analysis

The genome-wide association analysis in the paper detected significant SNP peaks for the novel traits within the Norwegian Red cattle population. The significant SNP peaks within the novel traits are not alike, which suggests that different genes are involved in and affect the different biological steps of pregnancy. Furthermore, the genes within the region have a role in those pregnancy stages, which further supports the matter. This support is a positive indication that the novel return traits identify the genes affecting the different biological stages of pregnancy as desired.

Another good indication is that the genome-wide association results from this study are similar to that in other cattle fertility literature, as, at times, they identify the same regions as having a significant effect on fertility. In addition, other studies found strong associations with the same chromosomes over the suggest significance in Norwegian Red cattle (Olsen *et al.,* 2010; Olsen *et al.,* 2011).

Despite the seemingly positive results, it is vital to be cautious with them. This caution is required as the genome-wide association analysis was to indicate whether the traits could identify differential genetic backgrounds. However, re-estimation with a more stringent analysis and fine mapping is required for further investigations. For example, the more stringent analysis should include permutation testing (Pahl & Schafer, 2010). In addition, to consideration of other methodology including daughter yields deviations to replace the estimated breeding values. Subsequently followed by fine-mapping methods to identify the quantitative trait loci (QTL) as it is possible that linkage disequilibrium could be conserved over more than 1 MB (Berg *et al.,* 2020).

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The hope would be that the genome-wide association will result in a better understanding of pregnancy in Norwegian Red cattle and aid in identifying appropriate QTL for selection. Fertility for trait selection is often problematic because it is a complex trait of many genes. Being controlled by many genes means a gene will likely only explain a small fraction of the phenotypic variation. However, identifying the underlying causal variation is interesting if the genes explain a sufficient amount of phenotypic variation. According to the calculated PVE, the SNPs account for 1% to 3.8% with the return from 0 to 48 days and return after 81 days SNP having a lower allele frequency. Although there is the need for re-estimation of associations and further analysis, these results suggest potential.

6.3 Future Perspectives

Despite the low heritability of these novel return traits, these traits appear to capture the different biological stages of pregnancy. Because these traits showed little correlation and a difference in genetic background, further investigation may be fascinating. Firstly, comparing other modelling techniques to estimate variance components may be beneficial. In addition, a more stringent analysis and further investigation to try to pinpoint the QTL.

Furthermore, there may be an exploration into culling due to fertility problems, as culling due to fertility problems had the highest heritability. Understanding the traits causing this could be beneficial. In addition, despite this high heritability, there were no strongly significant SNP peaks. However, between the two culling traits, there was an indication of QTL, which may warrant further research. Conversely, these peaks were not over the threshold, and thus further investigation for QTL, the return traits may take priority.

7. Conclusion

To summarise, reproductive efficiency is a costly problem in cattle. This problem of reproductive efficiency, including the risk of pregnancy loss, can be reduced through selective breeding and management. Ordinarily, selective breeding of fertility traits is happening, but the trait is lowly heritable and current genetic progress is slower than desired. It may be possible to aid progress by adding novel traits that indicate reproductive success or new markers.

The paper evaluates a couple of novel strategies, trialling potential new traits not tested before and gaining a further understanding of pregnancy loss using field data. These traits are returned for re-insemination at 0 to 48 days, 49 to 80 days, and 81 plus days following pregnancy loss throughout gestation. The other strategy was using culling reason, in which farmers observe fertility problems, leading to cow culling. In addition, it uses a standard selection trait to act as a control measure. All traits undergo evaluation by calculating variance components and heritability using a linear animal model. The results indicate that early pregnancy loss is more heritable than later losses. However, the number of observations was unsuitable for the model in later pregnancy loss traits, so it should be noted and considered in further investigations. Furthermore, the trait of culling due to fertility problems showed a higher heritability than the control and other traits.

In addition, a genome-wide association was completed and revealed genetic differences between each novel trait. All the novel traits appeared to have significant peaks, identifying regions of interest and candidate genes biologically related to the trait. Despite this identification, the results for the genome-wide association should be taken with caution. The results need to undergo more rigorous analysis, and with fine mapping, the peaks and regions of interest may shift. However, the results are positive and, thus, may warrant further investigation.

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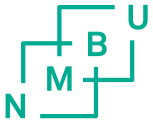

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