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Genetics of new phenotypes of pregnancy loss in dairy cattle

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Abstract

This thesis examined use of novel phenotypes of pregnancy loss to improve genetic progress in female fertility in dairy cattle. Genomic information was used to identify candidate genes associated with pregnancy loss traits, and to estimate the extent and effect of genetic defects on pregnancy outcome. Automatically recorded progesterone (P4) data from 14 Herd Navigator™ herds and pregnancy-associated glycoprotein (PAG) levels recorded in 1119 herds affiliated to the Swedish milk recording scheme were used to evaluate embryo, fetal and total pregnancy loss. The extent of pregnancy loss was considerable according to both diagnosis methods, ranging from 30 to 60%. In most cases, Swedish Red cows showed better pregnancy maintenance than Swedish Holstein. The P4 and PAG concentrations in milk were significantly lower in initially pregnant cows after losing their pregnancy compared with full-term pregnant cows, which indicates the importance of continuously high P4 and PAG levels during gestation to support embryo and fetus development. Similarly to calving and insemination-based fertility traits, heritability estimates of pregnancy loss traits were low (0.00-0.07). A single-step genome-wide association study identified 19 candidate genes associated with pregnancy loss traits, several of which are known to influence embryonic and fetal development. Mating two carriers of genetic defects adversely affected fertility and caused 14-15% higher mortality compared with non-carrier matings. Considering pregnancy loss in future routine genetic and genomic evaluations of fertility in dairy cattle could genetically improve cow fertility by reducing pregnancy losses in milk production, while also preventing economic losses arising from extended service period and calving interval, and involuntary culling due to infertility.

Keywords: pregnancy loss, embryo loss, fetal loss, progesterone, pregnancy-associated glycoproteins, single-step genome-wide association study, genetic defects

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Sammanfattning

Denna avhandling utforskade användningen av nya mått för dräktighetsförlust för att förbättra det genetiska framsteget för fruktsamhet hos mjölkkor. Genomisk information användes för att identifiera kandidatgener som kan orsaka dräktighetsförluster, och för att skatta omfattningen och effekten av genetiska defekter på dräktighetsresultatet. Automatiskt registrerade progesterondata (P4) från 14 Herd Navigator^{TM}-besättningar, och registreringar av dräktighetsspecifika glykoproteiner (PAG) i 1119 besättningar i den svenska Kokontrollen användes för att utvärdera embryo-, foster- och totala dräktighetsförluster. Omfattningen av dräktighetsförlusterna var betydande baserat på data från båda analysmetoderna, från 30 till 60 %. I de flesta fall var svenska röda kor bättre på att behålla dräktigheten än holstein. P4- och PAG-koncentrationen var signifikant lägre hos kor efter en dräktighetsförlust jämfört med dräktiga kor, vilket indikerar betydelsen av kontinuerligt höga P4- och PAG-nivåer under dräktigheten för att stödja utvecklingen av embryo och foster. I likhet med kalvnings- och inseminationsbaserade fertilitetsegenskaper var arvbarhetskattningar av egenskaperna låga (0,00-0,07). En genomisk studie identifierade 19 kandidatgener kopplade till dräktighetsförluster, varav flera är kända för att påverka embryo- och fosterutveckling. Parning av två bärare av genetiska defekter påverkade fertiliteten negativt och orsakade 14-15 % högre dräktighetsförlust jämfört med icke-bärarparningar. Att inkludera dräktighetsförlust som en egenskap i framtida rutinmässiga genetiska och genomiska utvärderingar av fruktsamhet hos mjölkkor skulle kunna genetiskt förbättra kors fertilitet och minska dräktighetsförluster i mjölkproduktionen, samtidigt som det förhindrar ekonomiska förluster till följd av förlängda inseminationsperioder och kalvningsintervall, och för tidig utslagning på grund av infertilitet.

Keywords: dräktighetsförlust, embryoförlust, fosterförlust, progesteron, dräktighetsspecifika glykoproteiner, genomisk analys, genetiska defekter

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Ask-Gullstrand, P., Strandberg, E., Båge, R., Christensen, J. M., & Berglund, B. (2021). Genetic parameters for reproductive losses estimated from in-line milk progesterone profiles in Swedish dairy cattle. J. Dairy Sci. 104(3):3231-3239. https://doi.org/10.3168/jds.2020-19385

II. Ask-Gullstrand, P., Strandberg, E., Båge, R., & Berglund, B. (2023). Genetic parameters of pregnancy loss estimated from pregnancy-associated glycoproteins in milk. J. Dairy Sci. 106(9):6316-6324. https://doi.org/10.3168/jds.2022-23007

III. Ask-Gullstrand, P., Strandberg, E., Båge, R., Rius-Vilarrasa, E., & Berglund B. (2024). Single-step genome-wide association study of pregnancy loss in dairy cattle based on pregnancy-associated glycoproteins. (submitted)

IV. Ask-Gullstrand P., Strandberg, E., Båge, R., Rius-Vilarrasa, E., & Berglund B. (2023). The effect of genetic defects on pregnancy loss in Swedish dairy cattle. J. Dairy Sci. (in press)

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Abbreviations

AI	Artificial insemination
BTA12	*Bos taurus* autosome 12
CFS	Interval from calving to first service, number of days
CIN	Calving interval, number of days
CLS	Interval from calving to last service, number of days
CR	Conception rate
DIM	Days in milk
EEL	Early embryonic loss
EL	Embryonic loss
FL	Fetal loss
FLS	Interval between first and last service, number of days
GWAS	Genome-wide association study
HH3	Holstein Haplotype 3
LEL	Late embryonic loss
NAV	Nordic Cattle Genetic Evaluation
NINS	Number of inseminations per series
P4	Progesterone
PAG	Pregnancy-associated glycoproteins
RDC	Red Dairy Cattle

SH	Swedish Holstein cattle
SNP	Single nucleotide polymorphism
SR	Swedish Red cattle
ssGWAS	Single-step genome-wide association study
TPL	Total pregnancy loss

1. Introduction

Pregnancy loss is an important form of infertility in dairy cows. Despite fertilization rates after artificial insemination (AI) of approximately 90%, calving rates are 30-50%, indicating extensive losses during gestation (Santos et al., 2004; Diskin et al., 2011; Nyman et al., 2018). However, few studies have attempted to determine the genetic variation in pregnancy loss and its potential usefulness as a trait to be considered in routine genetic and genomic evaluations in order to improve female fertility in dairy cattle and reduce pregnancy loss in dairy production systems.

Fertility traits generally have low heritability, which affects possible genetic progress. Improving phenotyping and creating new phenotypes for fertility using novel measurements that are closer to the underlying physiological background of fertility could be one way to achieve higher genetic gain. Compared with the classical calving and insemination-based fertility traits in use today, these novel fertility traits might have higher heritability or higher genetic correlation with the true breeding goal traits (Petersson et al., 2006; Tenghe et al., 2015). Endocrine fertility traits have been proposed as alternative indicators for fertility for these reasons.

Fertility is complex, polygenic and influenced by management and environmental factors, which has consequences for phenotyping and the possibility for improvement in fertility traits (Wijma et al., 2022). Identifying candidate genomic regions associated with pregnancy loss traits could improve selection efficiency of female fertility in dairy cattle (Höglund et al., 2009). Some of the observed embryonic and fetal losses may also be due to lethal recessive genetic defects accumulating in the population, but accurate phenotypic recording is limited at this stage of gestation. However, the increase in genotyping during the past decade has resulted in development of genomic analysis and high-density single nucleotide

polymorphism (SNP) chips with which to infer haplotypes of animals on a large scale. It is now possible to identify a deficit in homozygotes, which may indicate genetic defects affecting pregnancy maintenance and an associated decline in fertility (VanRaden et al., 2011; Fritz et al., 2013).

Deficiencies in fertility also lead to extra costs in production (Höglund et al., 2015). Pregnancy loss and inadequate fertility affect annual production per cow, hampering sustainable production with high animal welfare. This highlights the importance of establishing clear breeding strategies aiming to improve fertility on herd level, where distinct breeding plans and reproductive management are necessary in order to maintain an effective level of production, reduce involuntary culling, and increase herd profitability (e.g., Pursley et al., 2023). This would require e.g., management strategies to improve reproductive performance, identifying non-pregnant animals early, regulating herd dynamics and optimizing resource allocation, which would minimize the number of unproductive days in a cow's life. The environmental impact of production could also be reduced as a result of more efficient production owing to improved fertility performance.

1.1 The Nordic fertility evaluation

The Nordic countries have a long history of including fertility in their breeding goals. In Sweden, fertility traits were included in the evaluation as early as the 1970s by the breeding organization at the time, Svensk Avel, enabling continuous selection for reproductive performance (Lindhé et al., 1994). Using a balanced breeding goal, fertility has remained relatively stable and continued to improve in Swedish Red cattle (SR). However, use of imported semen in Swedish Holstein cattle (SH) from countries that mainly focused on milk production traits in their breeding programs, in combination with an antagonistic relationship between yield and fertility, have led to a subsequent decrease in fertility. Over time, this has been remedied through applying greater weight to fertility in the Swedish breeding goal (Nordic Cattle Genetic Evaluation, 2022). More recently, other countries have also adopted their own balanced breeding goals including fertility traits, facilitating continued import and use of high-quality breeding material. The genetic trend for fertility has thus rebounded in SH and it is equal in both breeds today (Figure 1). This change is reflected in the phenotypic development for fertility traits in the two breeds, although

management decisions and recommendations from the breeding organizations also influence the trends. The interval from calving to first service (CFS) was 88 days in SR and 95 days in SH in 2010 (Swedish Dairy Association, 2010), but was reduced to 82 and 84 days, respectively, in 2023 (Växa, 2023). Similarly, the interval from calving to last service (CLS) has decreased from 122 to 116 days in the SR breed and from 136 to 120 days in SH (Swedish Dairy Association, 2010; Växa, 2023). Calving interval (CIN) was 13.1 and 13.6 months in SR and SH, respectively, in 2010 (Swedish Dairy Association, 2010), and has now been reduced to 13.0 and 13.1 months, respectively (Växa, 2023).

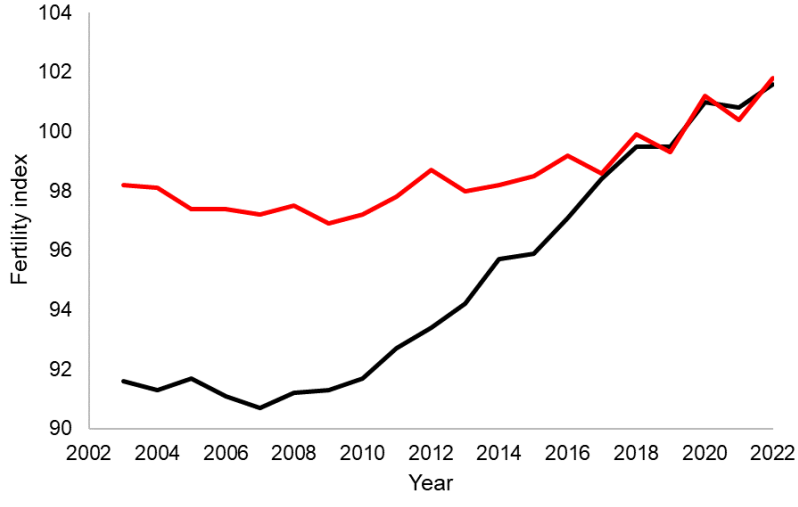

Figure 1. Genetic trend in female fertility in Nordic Red Dairy Cattle (red line) and Swedish Holstein (black line). Adapted from Nordic Cattle Genetic Evaluation (2023a).

Genetic evaluation has continued to evolve over the years. In 2002, the Nordic Dairy Cattle Evaluation (NAV) was founded for cooperative genetic evaluation in Sweden, Denmark and Finland. The joint breeding goal, called Nordic Total Merit Index, has been used since 2008 (Nordic Dairy Cattle Evaluation, 2023b). It describes the total economic potential determined by genetics for production, functional and health traits (Nordic Dairy Cattle Evaluation, 2022).

Female fertility is genetically improved through a fertility index in the breeding goal which consists of three sub-indices of classical calving and

insemination-based fertility traits to describe the genetic potential of the cow to return to cyclicity after calving, express estrus and conceive when inseminated at the correct time. These sub-indices are: CFS (only for cows), interval from first to last service (FLS, both heifers and cows) and number of inseminations (NINS, both heifers and cows). Thus the fertility index does not include the cow's ability to maintain a pregnancy until term, even though high incidences of embryonic and fetal losses have been reported (e.g., Nyman et al., 2018). Apart from the traits defined in the fertility index, breeding values are also estimated for non-return rate, conception rate (CR) and estrus intensity, for use as indicator traits.

Increasing the genetic progress of these classical traits is an important step towards improving overall fertility, but they are low heritability traits, which restricts genetic gain (Berry et al., 2014; Muuttoranta et al., 2019). They can also be influenced by recording errors and management practices (e.g., heat detection, voluntary waiting period, preferential treatment) that affect the perceived fertility of the cow (Tenghe et al., 2015). The increased automation of production provides interesting opportunities in breeding, e.g., taking advantage of automatic recordings for higher-precision phenotyping and creation of novel phenotypes for fertility. Finding biomarkers associated with novel fertility traits is also an important step in improving genomic evaluation of fertility in dairy cattle.

1.2 Dairy cattle fertility

1.2.1 The estrous cycle and early pregnancy

Non-pregnant cows are permanently polyestrous, having regular estrous cycles, approximately 21 days long, throughout the year. The estrous cycle consists of two main phases: the follicular phase (3-5 days) and the luteal phase (16-18 days) (Figure 2). The follicular phase is characterized by high estrogen concentrations, and the luteal phase by high progesterone (P4) concentrations. During each estrous cycle, the cow has usually two to three waves of developing follicles, where one follicle in each wave will become dominant (Crowe, 2008). The last wave develops when the P4 concentration is decreasing, allowing the follicle to grow larger and produce more estrogen. Due to the high estrogen concentration, in the absence of high progesterone, the cow will start to show signs of estrus. At a certain threshold level,

estrogens also trigger the hypothalamus to produce gonadotropin-releasing hormone, which in turn leads to a preovulatory surge release of luteinizing hormone, resulting in ovulation, rupture of the dominant follicle and release of the egg cell, the oocyte. Secretion of follicle-stimulating hormone induces the development of a new follicular wave in the subsequent estrous cycle (Crowe, 2008; Forde et al., 2011).

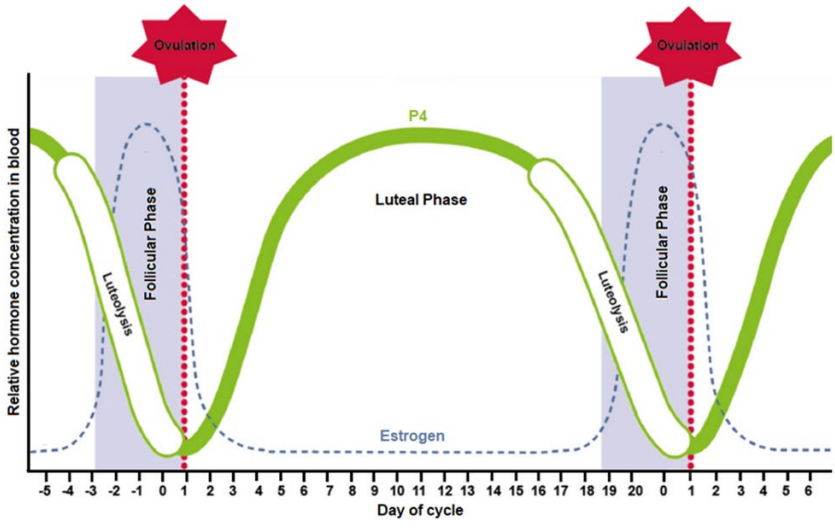

Figure 2. Schematic illustration of the estrous cycle in dairy cattle, showing the relationship between estrogen and progesterone (P4) during the follicular and luteal phases. Adapted from Ali et al. (2022).

In this new cycle, the newly ruptured follicle develops into a corpus luteum, which begins to produce P4. Around approximately 17 days into the estrous cycle, if the oocyte has not been fertilized or there is no maternal recognition signaling by the conceptus through the actions of interferon tau, the uterus will by default release prostaglandins. These cause the corpus luteum to regress and luteolyze, thus decreasing secretion of P4. Low P4 concentrations allow the dominant follicle of the last follicular wave to develop until ovulation. However, if the cow becomes pregnant, the corpus luteum is rescued from luteolysis and the high P4 level is maintained (Crowe, 2008; Forde et al., 2011).

Shortly after fertilization, the zygote begins cell division and is transported toward the uterus. Approximately 4-6 days after fertilization, the zygote has formed into a compact sphere of cells called a morula. The cells

which constitute the morula continue to multiply and by day 7 have developed into a differentiated blastocyst with two cell types: the inner cell mass (the embryo proper) and the trophoblast cells, which develop into the fetal part of the placenta. On day 8-10, the blastocyst hatches from the zona pellucida and the trophoblast starts to elongate. Several morphological changes occur during elongation, with transition from a spherical blastocyst to a filamentous conceptus (Lonergan et al., 2016; Spencer et al., 2016; Tinning et al., 2023). Meanwhile, maternal recognition of pregnancy is initiated by interferon tau, produced by the trophoblast cells, blocking the synthesis of oxytocin receptors in the uterus. This in turn prevents the release of prostaglandins which would otherwise luteolyze the corpus luteum (Crowe, 2008; Forde et al., 2011; Tinning et al., 2023). As elongation progresses, the corpus luteum continues to release P4, which suppresses estrus cyclicity while also supporting embryo development and survival (Diskin et al., 2011; Bruinjé et al., 2017; Blavy et al., 2018) via its actions on uterine function (Diskin et al., 2011). Once elongated, at approximately day 19-20 of gestation, implantation and placentation begins. Apposition ensues with cell-to-cell contact between the trophoblast and uterine epithelial cells, which is followed by firm adhesion and attachment of the conceptus to the endometrial epithelium (Østrup et al., 2011; Lonergan et al., 2016; Spencer et al., 2016), forming the feto-maternal interface.

As differentiation continues, binucleate cells migrate from the fetal placenta into the uterine epithelium, after which these cells start producing pregnancy-associated glycoproteins (PAG). The PAG are stored in granules in the cells and are secreted through exocytosis to reach the maternal blood circulation during pregnancy (Green et al., 2000). They are secreted into the mother's blood stream, and can be detected in plasma and milk from approximately the third week of gestation (Lawson et al., 2014; Ricci et al., 2015).

The genetic effect of PAG levels in milk is moderate, ranging between 27% and 37% of the direct and maternal phenotypic variance (Santos et al., 2018). The physiological function of PAG is still uncertain, but their spatio-temporal gene expression and secretion patterns (Green et al., 2000; Garbayo et al., 2008) suggest that they are involved in key moments in gestation, such as placental formation, embryonic growth and development (Patel et al., 2004; Mercadante et al., 2016), pregnancy maintenance (Santos et al., 2018), and preparing the uterine environment for parturition (Patel et al., 2004). In

the event of embryonic or fetal loss, production and secretion of PAG are disturbed by the regressing placenta. The concentration of PAG then continuously declines and returns to non-pregnant levels (Ricci et al., 2015).

1.2.2 Pregnancy diagnosis

Accurate and early pregnancy diagnosis is a vital part of reproductive management in dairy herds. There are currently two types of pregnancy diagnosis available to infer the reproductive status of the cow, manual and chemical. Manual methods use rectal palpation or transrectal ultrasonography to identify pregnant animals (Whitlock & Maxwell, 2008; Lawson et al., 2014). Accordingly, these methods are more labor-intensive and time-consuming and require additional handling of animals compared with chemical diagnostic methods, where sampling is performed concurrently with milking. Further, these manual methods are subjective and require access to skilled technicians or veterinarians to perform the diagnosis.

Chemical diagnosis is more objective and can either be non-pregnancy-specific or pregnancy-specific. Non-pregnancy-specific tests rely on indirect measurements of gestation for the diagnosis, for instance fluctuations in P4 concentration during the estrous cycle (Lawson et al., 2014). In contrast, pregnancy-specific diagnosis uses markers that are directly produced by the developing pregnancy, such as PAG, to infer pregnancy status (Zoli et al., 1992; Lawson et al., 2014; Ricci et al., 2015).

In addition to pregnancy diagnosis, estrus detection is important in order to follow up on cows that have returned to estrus. This is performed at approximately three weeks after insemination, when the next estrus is expected, which is earlier than other manual pregnancy diagnosis methods and PAG analysis can be applied. Identifying non-pregnant animals is important for farm reproductive efficiency in order to minimize number of days open and re-inseminate these animals as soon as possible.

1.2.3 Pregnancy loss

Numerous definitions of pregnancy loss can be found in the literature. In an attempt to standardize the categorization of pregnancy loss, the Committee on Bovine Reproductive Nomenclature (1972) suggested the following classifications: i) embryonic loss is pregnancy loss occurring from 1 day until approximately 41 days post-AI, which includes conception up until the end

stage of embryonic differentiation, and ii) fetal loss is loss from approximately 42 days post-AI until expected calving. Embryonic losses are further subdivided into early or late embryonic losses, where pregnancy loss during the first 24 days post-AI is considered early embryonic loss, and pregnancy loss between 25 and 41-50 days is considered late embryonic loss (e.g., Santos et al., 2004). Other definitions of fetal loss in the literature consider fetal loss as losses occurring until 260 days post-AI, while losses after this period are regarded as premature deliveries (e.g., Miller, 1982).

Fertilization rates after insemination are high in dairy cattle, indicating that the losses in production are mainly due to embryonic and fetal mortality (Santos et al., 2004; Diskin et al., 2011; Nyman et al., 2018). Fertilization rates are approximately similar in non-lactating and lactating cows (Santos et al., 2004), but greater pregnancy losses have been reported in high-producing cows (Santos et al., 2004; Diskin & Morris, 2008) suggesting a detrimental effect of lactation status on embryo survival.

By 5-6 days post-AI, only 65% of fertilized oocytes develop into viable embryos, which constitutes approximately 50% of all embryos (Santos et al., 2004). This period coincides with formation of the morula, followed by the first critical step of cell differentiation into a blastocyst at day 7, where a great proportion of embryos are lost. Further losses are expected during the second and third weeks post-AI, corresponding to the timing of blastocyst hatching, conceptus elongation, pregnancy recognition and implantation (Lonergan et al., 2016; Spencer et al., 2016; Tinning et al., 2023). Late embryonic loss in dairy cows is reported to be around 10-15% and fetal loss comprises about 5-10% (e.g., Bruinjé et al., 2017; Nyman et al., 2018). An effect of parity has also been observed, with pregnancy losses increasing with age (Santos et al., 2004; Nyman et al., 2018).

Instead of using pregnancy loss traits, non-return rate is a widely used term to indicate a female's ability to become pregnant after insemination. However, non-return rate reflects whether or not a cow has been identified in estrus and re-serviced within a certain time period after breeding (Berry et al., 2014) and not actual pregnancy loss during gestation. Accordingly, the non-return rate is often overestimated.

Apart from genetic ability of the cow to maintain normal reproductive function, certain environmental factors impact fertility considerably. For instance, exposure to thermal stress impairs steroidogenesis and oocyte viability, and reduces oocyte quality and fertilization rate. Metabolic status

also affects embryonic and fetal survival, as negative energy balance and loss of body condition compromise the quality and development of the oocytes maturing during the follicular waves (Santos et al., 2004; Crowe, 2008; Ritter et al., 2019). In addition to this, reproductive performance is affected by various diseases (Whitlock & Maxwell, 2008; Ritter et al., 2019), causing lower conception rate and delayed conception (Crowe, 2008; Ritter et al., 2019). For instance, inflammatory diseases can affect pregnancy outcome by reducing fertilization, impairing development of the morula, and causing changes in development of the conceptus during elongation and secretion of interferon tau in the uterus (Ribeiro et al., 2016). Reproductive performance is also influenced by genotype-by-environment interactions (e.g., Shi et al., 2021).

1.3 Progesterone recording using Herd Navigator™

Herd Navigator™ (DeLaval Int., Tumba, Sweden) is an automated management system developed for monitoring energy balance, mastitis and fertility in dairy cows that enables large-scale recording in herds. The fertility module automatically samples the milk at robot milking and analyzes the P4 concentration in the milk (Figure 3), which provides an overview of the ovarian activity owing to the strong correlation between blood and milk P4 concentrations (Roelofs et al., 2006). The results are available to view directly, which is why Herd Navigator™ is commonly referred to as an in-line milk trait recording system.

Herd Navigator™ can minimize the influence of management on fertility through alarms and action points sent out by the system, which are calculated by the system's biomodel (Friggens & Chagunda, 2005). The default sampling window is standardized at 20-240 days in milk (DIM) in order for the biomodel to assess the current reproductive state of the cow. Once the next heat is detected, which is noted by the system as decreasing P4 levels reaching below threshold values of 5 ng/mL, insemination alerts are sent out to the herd manager to notify them of cows that should be inseminated. Following insemination, the system continues sampling on approximately day 5, 9 and 14 to evaluate if the cow has become pregnant or has developed a cyst. After that, the system continues frequent sampling after 18 days into the estrous cycle, in order to find a potential next heat. The system monitors

the cow until approximately 60 days post-AI for pregnancy maintenance or potential pregnancy loss (Bruinjé et al., 2019).

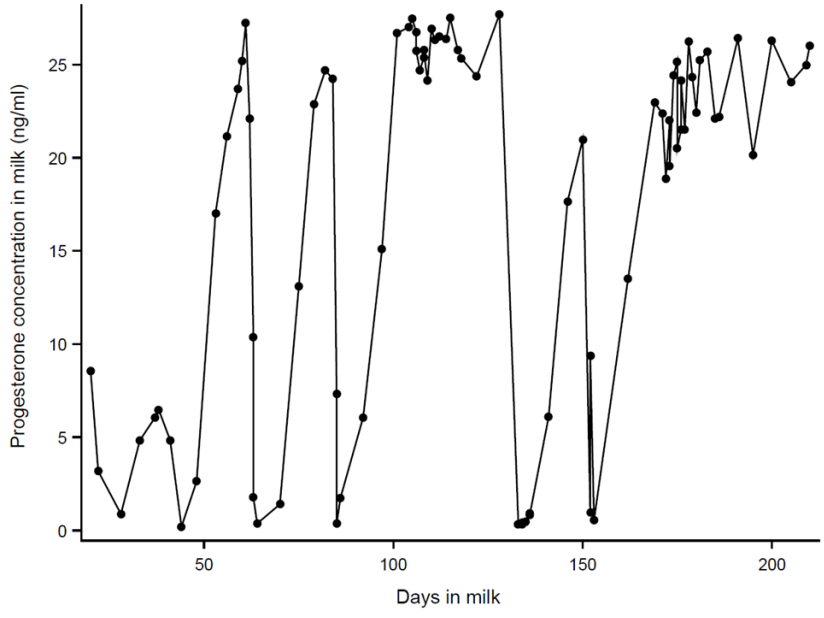

Figure 3. Illustration of milk progesterone profile as monitored by the Herd Navigator™ system, with pregnancy loss at approximately 130 days in milk indicated by a drop in progesterone concentration and the cow returning to ovarian activity.

1.4 Recording milk pregnancy-associated glycoproteins

An enzyme-linked immunosorbent assay developed for detection of PAG has been incorporated into the Swedish routine milk recording system for dairy cattle, making it a convenient early indicator and monitoring system for pregnancy status in dairy herds on a large scale. The PAG gene family comprises 20 transcribed genes and some variants (Green et al., 2000), but the assay only recognizes a few of these (Ricci et al., 2015). The PAG analysis has been offered by the Swedish milk recording scheme since 2014, and approximately half of milk recording herds in Sweden subscribe to the service today.

The PAG profile in pregnant cows has three phases: the first phase is characterized by an initial increase in PAG during the embryonic stage, followed by decrease to a minimum at around 46 to 72 days post-AI, and

then a rebound from nadir where the PAG concentration continues to increase throughout the final stage of gestation (Lawson et al., 2014; Ricci et al., 2015) (Figure 4). The PAG concentration increases with gestational stage, i.e., the longer the cow has been pregnant, the higher the PAG level in milk samples (Green et al., 2000; Garbayo et al., 2008; Santos et al., 2018).

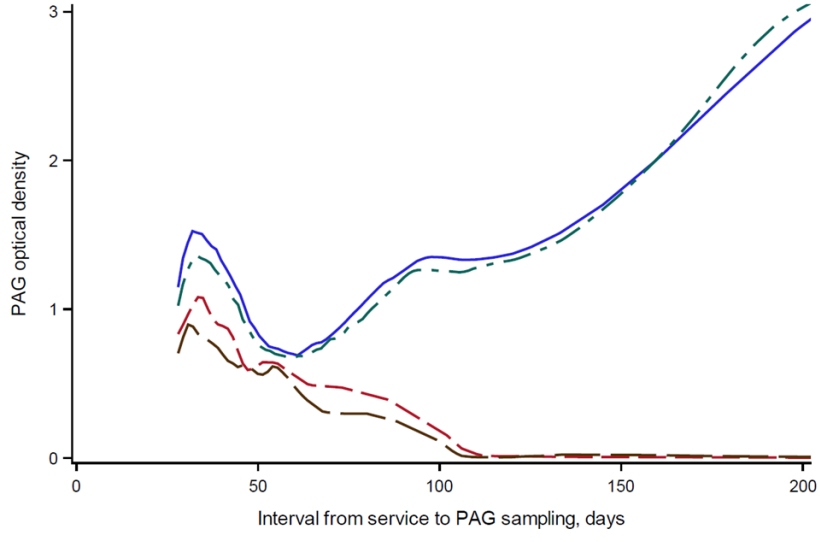

Figure 4. Optical density of pregnancy-associated glycoproteins (PAG) in milk in pregnant Swedish Red (blue line), pregnant Swedish Holstein (green dashed line), and in Swedish Red cows with pregnancy loss (red dashed line), and Swedish Holstein cows with pregnancy loss (brown dashed line).

1.5 Single-step genome-wide association studies

During the past decade, there has been growing interest in genome-wide association studies (GWAS) to find associations between SNP and economically important traits in dairy cattle (Wang et al., 2012). The GWAS methodology has been developed further into a single-step best-linear unbiased prediction approach (ssGWAS), where pedigree, phenotypes and genotypes are combined in a single evaluation. Using ssGWAS, data from both genotyped and non-genotyped animals are considered simultaneously in the evaluation by replacing the relationship matrix based on pedigree (A) with an augmented matrix (H) which also includes genomically derived relationships (G) (Misztal et al., 2009; Aguilar et al., 2010; Christensen &

Lund, 2010; Wang et al., 2012). Thus, enabling the inclusion of non-genotyped animals has increased the power and precision of the evaluation without additional cost of genotyping. Including phenotypes from related animals using traditional pedigree relationships with genotyped animals allows genomic estimated breeding values to be converted into marker effects and weights, which are applied in an iterative approach to update solutions (Wang et al., 2012).

Identifying genomic regions and candidate genes related to reproductive performance is important in order to better understand the biological mechanisms and pathways underlying the phenotypic expression of fertility (Zolini et al., 2020). The genomic evaluation of fertility could be advanced by taking these regions and candidate genes into consideration when defining selection strategies in breeding programs.

1.6 Genetic defects affecting embryo and fetal survival

Prior to the genomic era, genetic defects were mainly observed through perinatal mortality and stillbirth. They had to be confirmed in breeding trials due to issues with observing the phenotype clearly *in utero* and distinguishing these losses from pregnancy losses due to causes other than genetic defects (VanRaden et al., 2011; Fritz et al., 2013). The increase in genotyping during the past decade has enabled the development of high-density SNP chips which allow for identification of possible genetic defects by deficit of homozygous individuals for the particular haplotype and associated deleterious effects on fertility. This has facilitated determination of underlying causative variants and assigning carrier status of lethal recessive genetic defects in genotyped animals (VanRaden et al., 2011; Fritz et al., 2013; Cole et al., 2016), several of which affect early pregnancy and could previously not be discriminated from failed inseminations (Cole, 2015). Ten recessive genetic defects associated with pregnancy loss are currently included in the SNP chip used for genotyping by NAV of Nordic Red Dairy Cattle (RDC, including Swedish Red, Danish Red and Finnish Ayrshire) and SH.

Lethal recessive genetic defects can increase in the population through genetic drift, high linkage with favorable alleles, and through inferring positive (direct or indirect) effects to heterozygote carriers (Jenko et al., 2019). However, the carrier status of these defects could be used in selection

to avoid at-risk matings (i.e., carrier male mated with carrier female) and reduce carrier frequencies in the population (VanRaden et al., 2011; Cole et al., 2016; Bengtsson et al., 2022).

1.7 Consequences of pregnancy loss

Reproductive performance is an important economic trait in dairy production, generating approximately one annual calf and lactation, but a high incidence of pregnancy loss during gestation compromises herd productivity (Diskin et al., 2011; Nyman et al., 2018). This influences production efficiency by delaying the next lactation through increased NINS per successful pregnancy, thus increasing the CIN. The economic impact of poor fertility is further affected by higher labor and veterinary costs, and increased risk of involuntary culling (de Vries, 2006; Höglund et al., 2015).

While the majority of pregnancy losses occur during the early embryonic period (i.e., first 24 days post-AI), losses in later gestation generate more serious costs because of extension of the service period and a delay in the next lactation (de Vries, 2006; Cole et al., 2016). Accordingly, the economic value of fertility is mostly attributed to changes in CIN and the cost of AI (Nordic Cattle Genetic Evaluation, 2022).

Extension of the service period and delayed CIN associated with pregnancy loss also affect herd dynamics, e.g., by changing stocking density and allocating animals to different age groups than would normally be expected under the reproductive management strategy. This also influences herd health status and increases the workload in the herd, further affecting the economic outcome of production. In addition, involuntary culling should be taken into consideration as impaired fertility is one of the major reasons for culling in Swedish dairy cattle, influencing the longevity of the cow (Växa, 2023). Involuntary culling will further change the herd dynamics, increasing the need for access to replacement heifers. Associating an economic value to pregnancy loss in production can assist producers when setting up their breeding plans.

Pregnancy loss can also have consequences at the individual level. While early losses are not noticed by the animal, later losses may cause reduced animal welfare and involuntary culling. This in turn is an ethical problem which influences the acceptance of the whole dairy sector, as societal values are evolving (e.g., Ritter et al., 2019).

Lastly, suboptimal fertility and consequences of this increase the environmental impact associated with dairy production (Tinning et al., 2023). Reducing pregnancy losses and optimizing on-farm reproductive management would therefore reduce the carbon footprint of dairy farming.

2. Aim of the thesis

The overall aim of this thesis was to examine potential use of new phenotypes of pregnancy loss in improving genetic progress in dairy cattle reproduction. Specific objectives were to:

- Assess the extent of pregnancy loss and associated genetic parameters in dairy cattle, using in-line milk progesterone records (Paper I),
- Evaluate the quality of pregnancy-associated glycoprotein analysis, examine factors influencing glycoprotein concentration in milk and assess the extent and genetic variation in pregnancy loss traits (Paper II),
- Investigate the association of single-nucleotide polymorphism with pregnancy loss traits based on pregnancy-associated glycoproteins using a single-step genome analysis (Paper III),
- Estimate the extent of genetic defects and analyze the effect of genetic defects on pregnancy maintenance in Red Dairy Cattle and Swedish Holstein (Paper IV).

3. Summary of Papers I-IV

The four studies described in Papers I-IV investigated the extent of pregnancy loss, sought to identify candidate genomic regions associated with pregnancy loss traits, and examined the occurrence of genetic defects and their implications for pregnancy loss traits in Swedish dairy cattle. The analyses were based on data from commercial herds collected in the Swedish cow database managed by Växa (Stockholm, Sweden). Paper I also analyzed P4 records from 14 Herd Navigator™ herds in Sweden, Paper IV used information about the carrier status of genetic defects, and Papers III and IV used genotypic information collected from the Nordic Dairy Cattle Evaluation (NAV).

Table 1. Summary of data analyzed in Papers I-IV

	Cows/Females[1]	Lactations	Inseminations
Paper I			
SR and SH[2]	3,304	5,238	10,219
SR	1,457	2,386	4,399
SH	1,847	2,852	5,820
Paper II			
SR and SH	124,076	214,134	264,009
SR	41,889	73,340	88,748
SH	82,187	140,794	175,261
Paper III			
RDC and SH	167,550	311,608	388,873
RDC	64,662	121,481	148,993
SH	102,888	190,127	239,880
Paper IV			
RDC and SH	50,450	N/A	158,795
RDC	28,432	N/A	97,551
SH	22,018	N/A	61,244

[1]Cows in Papers I-III, females in Paper IV as that study included both heifers and cows.
[2]SR = Swedish Red cattle; SH = Swedish Holstein cattle; RDC = Red Dairy Cattle.
N/A – Not applicable.

3.1 Pregnancy loss traits studied in Papers I-IV

In Paper I, pregnancy loss was defined based on the P4 profile. Sampling routines in the Herd Navigator™ system allowed early embryonic loss to be defined as pregnancy loss occurring from one day until 24 days post-AI and late embryonic loss as pregnancy loss at 25 to 41 days post-AI. In Papers II and III, however, pregnancy loss was defined based on PAG analyses, which are performed concurrently with monthly test milking. Due to the PAG analysis starting at the earliest 28 days post-AI, embryonic loss was categorized as pregnancy loss from 28 days post-AI until 41 days post-AI. In Papers I-III, fetal loss was defined as pregnancy loss occurring between 42 days post-AI until expected calving. Total pregnancy loss was defined as loss occurring from one day post-AI until expected calving in Paper I, and as loss occurring from 28 days post-AI until expected calving in Papers II and III. In Paper IV, pregnancy loss was defined as failure to maintain pregnancy

from one day post-AI until expected calving, in order to study the full gestation period.

3.2 Extent of pregnancy loss based on P4 (Paper I)

The extent of pregnancy loss and associated genetic parameters were estimated using in-line milk P4 records. The P4 concentration in milk was also used to predict the pregnancy status on four occasions after insemination, in pregnant cows and cows with pregnancy loss.

In-line milk P4 concentration (ng/mL) and milk yield records were obtained for 14 Swedish herds during 2015 to 2019. The Herd Navigator™ system automatically samples and analyzes milk P4 at frequencies specified by the system's biomodel, which is based on calculations by Friggens and Chagunda (2005). The P4 data were managed as follows to standardize the material in preparation for analysis: (i) the P4 data were linearly interpolated to estimate the beginning and end of each estrous cycle, where a P4 concentration above 5 ng/mL was used to define luteal activity; (ii) at least two consecutive P4 records above the threshold for luteal activity, a luteal phase length of at least four days and an interovulatory interval greater than four days were required in each estrous cycle; (iii) the cow had to commence luteal activity by 60 DIM; (iv) a minimum of ten P4 samples were required per lactation to account for cows without a full sampling series; and (v) the first sample had to be taken by 25 DIM and the last sample after 60 DIM to constitute a full sampling series. Each insemination was aligned with the P4 records, and only one insemination was accepted per cycle. In total, the data covered 330,071 P4 samples in 10,219 inseminations on 1,457 Swedish Red and 1,847 Swedish Holstein cows (Table 1). Pedigree, insemination, calving, culling and disease data were also extracted from the Swedish cow database managed by Växa (Stockholm, Sweden) to confirm pregnancy status during gestation. Apart from pregnancy loss traits, five classical fertility traits were also analyzed: CFS, CLS, FLS, CIN and NINS.

3.2.1 Main findings

Extensive early embryonic loss was detected, of approximately 45% in both SR and SH, which was expected based on previous studies (e.g., Santos et al., 2004; Nyman et al., 2018). Swedish Red cows were superior to SH cows in late embryonic, fetal, and total pregnancy loss, i.e., SR had better

pregnancy maintenance (Table 2). Most notably, late embryonic loss was more than twice as high in SH compared with SR. Primiparous cows had lower pregnancy loss compared with multiparous cows. Very few fetal losses were reported in primiparous cows compared with second and third parity cows. Total pregnancy loss also increased with parity.

Table 2. Least squares mean differences (percent, A-B) in pregnancy loss traits based on in-line progesterone recording in Swedish Red (SR) and Swedish Holstein (SH) cows in Paper I

A	B	EEL[1]	LEL	FL	TPL
SR	SH	-1.4	-7.2*	-5.3*	-6.2*
Parity 1	Parity 2	-0.1	-1.8	-6.7*	-3.7*
Parity 1	Parity ≥3	-0.6	-1.8	-8.8*	-4.9*
Parity 2	Parity ≥3	-0.5	0.0	-2.1	-1.2

[1]EEL = Early embryonic loss, 1 to 24 days post-artificial insemination (AI); LEL = late embryonic loss, 25 to 41 days post-AI; FL = fetal loss, 42 days post-AI until expected calving; TPL = total pregnancy loss, 1 day post-AI until expected calving. Asterisks indicate a significant level of $p \leq 0.05$.

Pregnancy outcome was highly dependent on the P4 concentration at day ten, 20, 30, 40 and 50 during gestation. Pregnant cows that later suffered pregnancy loss had significantly lower P4 concentrations than pregnant cows which successfully maintained pregnancy from embryonic stage onwards. This implies that a stable supply of P4 is important during gestation in order to support the development of the embryo and fetus.

Similarly to classical fertility traits, low heritabilities were estimated for all pregnancy loss traits, with values ranging from 0.00 to 0.07. Furthermore, early and late embryonic loss were moderately genetically correlated with milk yield in Paper I (r_g = 0.52 and 0.39, respectively), which was expected as high-yielding dairy cattle require higher NINS per successful conception with subsequent calving. There were also moderate to strong positive genetic correlations between pregnancy loss traits and classical fertility traits, which indicate that cows with impaired fertility have difficulties conceiving and in supporting early embryonic development and survival.

3.3 Extent of pregnancy loss based on PAG (Paper II)

In Paper II, the PAG records collected in the monthly milk recording scheme 2014-2020 for 1119 Swedish dairy herds were extracted from the national

cow database to examine pregnancy loss. A total of 374,206 PAG observations from 214,134 lactations in 41,889 SR and 82,187 SH cows were analyzed. Herds subscribing to Växa's PAG analysis in Sweden are offered four strategies: a single analysis from 28 days post-AI; two analyses, one after 28 days post-AI, and if positive, confirmed by a second analysis sometime from 60 days post-AI (recommended strategy); one analysis in preparation for drying off; and an additional individual analysis at some point during gestation (Figure 4). Approximately 36% of all inseminations were excluded from the analysis in Paper II, as these were never monitored in connection with PAG analysis. Data on manual pregnancy diagnosis, repeated inseminations, calving and culling were also extracted from the Swedish cow database to evaluate the pregnancy status of individual cows in each gestation. As in Paper I, five classical fertility traits (CFS, CLS, FLS, CIN, and NINS) were also analyzed.

3.3.1 Main findings

The risk of pregnancy loss was strong early in gestation and tapered off as gestation progressed (Figure 5). The probability of the growing conceptus surviving to about 70 days or less was nearly 70%. After 70 days, the risk of pregnancy loss accumulated more slowly, which implies that fewer fetal losses will occur.

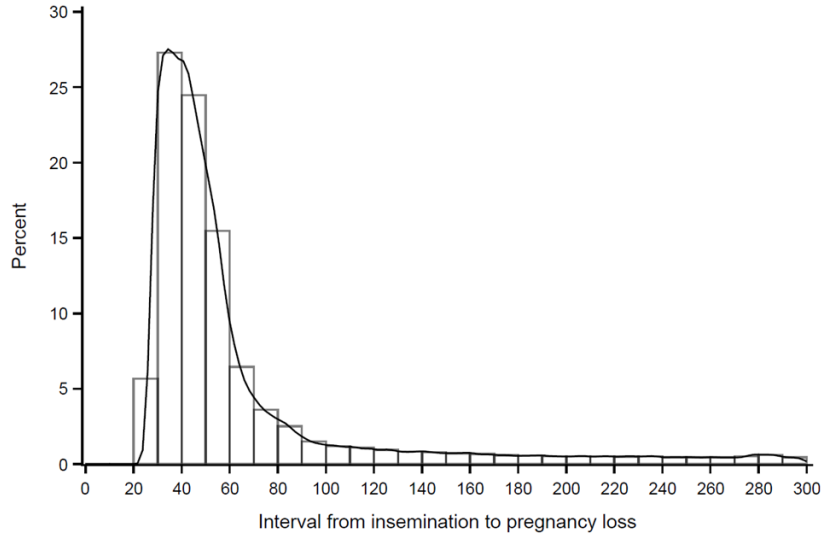

Figure 5. Probability density function of total pregnancy loss based on pregnancy-associated glycoprotein analysis after insemination in Swedish dairy cattle in Paper II.

Estimates of pregnancy loss traits constructed from PAG recordings differed substantially from those based on P4. Normally, embryonic losses are expected to make up about 40-50% of losses and fetal losses another 5-10%. However, in Paper II, embryonic losses were estimated to be 15.7-20.2% while fetal losses were 29.2-38.5%. These differences are probably due to the delayed analysis of PAG compared with P4, which is determined continuously during gestation. Similarly to Paper I, pregnancy loss was significantly more frequent ($p < 0.0001$) in SH than in SR cows (Table 3), regardless of time period. Again, pregnancy loss increased significantly with parity ($p < 0.0001$).

Table 3. Least squares mean differences (percent, A-B) in pregnancy loss traits based on pregnancy-associated glycoprotein data on Swedish Red (SR) and Swedish Holstein (SH) cows in Paper II

A	B	EL[1]	FL	TPL
SR	SH	-1.2*	-2.3*	-3.2*
Parity 1	Parity 2	-2.6*	-4.9*	-3.4*
Parity 1	Parity ≥3	-4.5*	-9.3*	-4.8*
Parity 2	Parity ≥3	-1.9*	-4.4*	-1.4*

[1]EL = Embryonic loss, 28 to 41 days post-AI; FL = fetal loss, 42 days post-AI until expected calving; TPL = total pregnancy loss, 28 days post-AI until expected calving. Asterisks indicate a significance level of $p \leq 0.05$.

Cows that later suffered pregnancy loss had reduced PAG levels in milk at test-day, confirming that PAG is a useful biomarker for placental function and a good management tool for prediction of pregnancy maintenance after insemination. The PAG concentration also increased with gestational stage, i.e., the longer the cow had been pregnant, the higher the PAG level in the milk sample. Furthermore, the PAG concentration was higher in younger animals than in multiparous cows, while SR cows had higher PAG levels in milk samples than SH cows. The PAG concentration was also influenced by calf variables such as calf survival and calf sex (only SR), and number of calves with higher PAG in twin births (both SR and SH). In addition, higher milk yield at monthly test-day recording was associated with lower PAG level.

An ideal pregnancy test should have high sensitivity (i.e., correctly identify pregnant animals), high specificity (i.e., correctly identify non-pregnant animals) and high accuracy, and should be simple and inexpensive to use. The high negative predictive value reported for the assay (ranging between 81 and 100% in various studies) indicates that PAG analysis is efficient in finding non-pregnant cows that should be returned to service. Similarly, reported positive predictive values are high (79-91%), indicating that a few cows are still at risk of losing their pregnancy later in term. However, while the PAG analysis performed well for the test parameters, a major drawback of the method is the limited analysis during the embryonic stage, as the start of sampling is at 28 days post-AI at the earliest (Figure 4). Analysis concurrently with the milk recording scheme could further increase the interval from insemination to first PAG analysis for some cows.

Therefore, embryonic losses were underestimated, while fetal losses were overestimated, in Paper II.

As seen in Paper I, the heritability estimates for pregnancy loss traits based on PAG data in Paper II were low. This could be due to the binary nature of the pregnancy loss traits and large non-genetic effects. Both embryonic and fetal loss had a strong positive genetic correlation with CLS, FLS, CIN and NINS.

3.4 Single-step genome-wide association study of pregnancy loss based on PAG (Paper III)

In Paper III, ssGWAS was performed using the BLUPF90 software family (Masuda, 2018; Misztal et al., 2022) to identify significant SNPs associated with the three pregnancy loss traits (embryonic, fetal and total pregnancy loss) in RDC and SH cows. An additional two years' worth of PAG recordings from 2020 to 2022 were collected from the national cow database to evaluate pregnancy status, comprising a total of 643,277 PAG recordings from 64,662 RDC and 102,888 SH cows. After quality control, a total of 40,906 and 40,506 SNPs were used in the analysis for RDC and SH, respectively. The ssGWAS results were plotted using the qqman package in R (Turner, 2018). Markers with -log(p-value) ≥ 5 or located within 250-kb flanking regions of the SNP position were considered candidate genomic regions associated with the pregnancy loss traits. Gene information was extracted from the Ensembl Genome Browser using the bovine genome assembly (*Bos taurus* ARS-UCD 1.2) (Martin et al., 2023). Information about gene function was extracted from the Universal Protein Resource database (The UniProt Consortium, 2023a).

3.4.1 Main findings

Only one SNP associated with a pregnancy loss trait reached the Bonferroni level of significance (-log (p-value) ≥ 5.91, Figure 6b). The SNP in question was associated with fetal loss in SH and was detected on BTA 5 within the *TBC1D22A* gene, which is important in protein binding, intracellular protein transport and activation of GTPase activity (The UniProt Consortium, 2023b). Another seven SNPs on BTA 4, 10, 12, 14, 16, and 24 reached the suggestive significance level (-log (p-value) ≥ 5, Figure 6a-b, 7a-b). Among these, no SNP was associated with embryonic loss in RDC or with total

pregnancy loss in SH. Furthermore, no candidate gene was identified within flanking regions of the "ARS-BTGL-NGS-37757" SNP in SH. Of the total 19 candidate genes identified, 15 were protein coding genes, three were RNA genes and one was a pseudogene. A majority of the protein coding genes identified are involved in physiological processes such as protein activity, activation of GTPase activity, proliferation, apoptosis, immune response, neurogenesis, organogenesis, and regulating the hypothalamus-pituitary-thyroid axis, synapse function, cell cycle, DNA repair and transcription (Table 4).

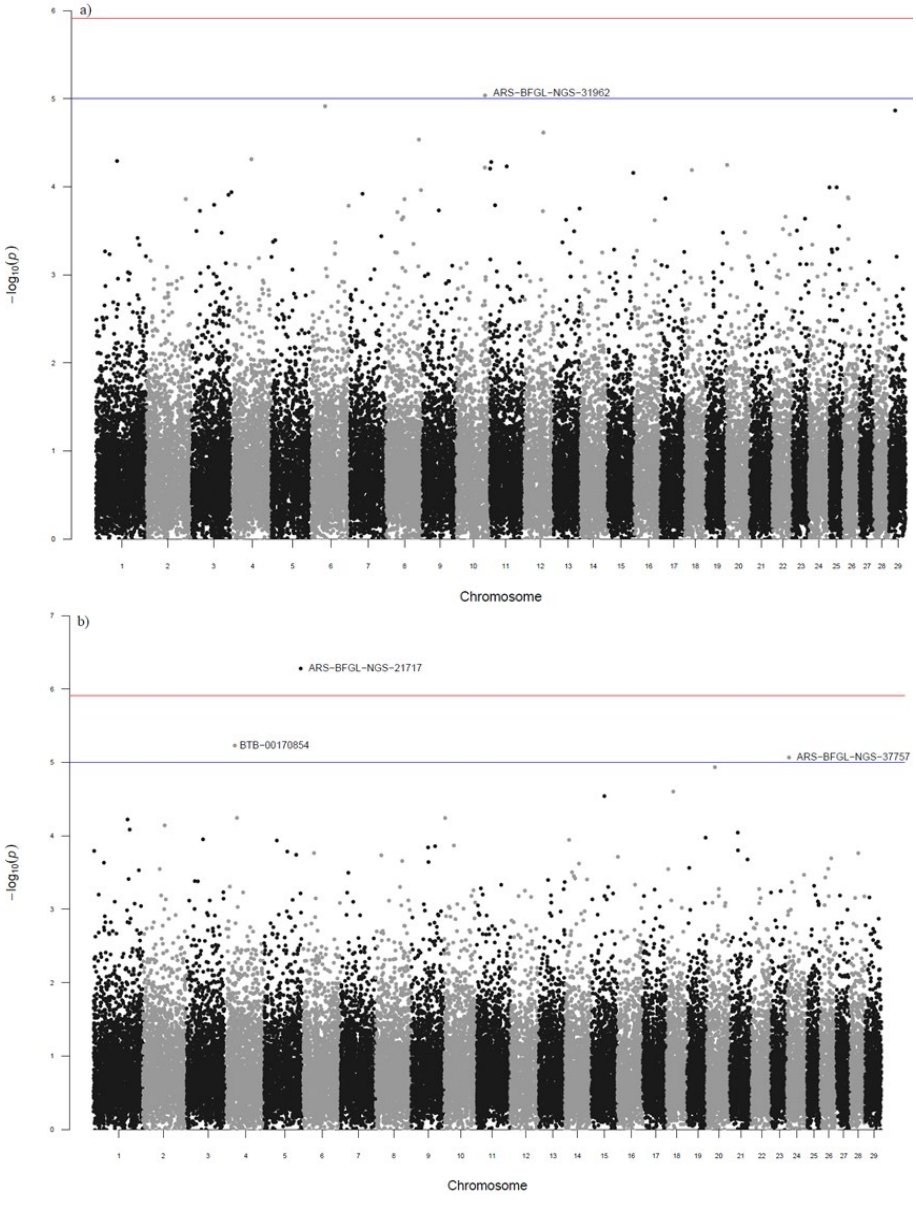

Figure 6. Manhattan plots of genome-wide association analysis results of (a) embryo loss and (b) fetal loss in Swedish Holstein. The red horizontal line displays the Bonferroni level of significance (-log (*p*-value) ≥ 5.91) and the blue horizontal line is the suggestive associated line (-log (*p*-value) ≥ 5).

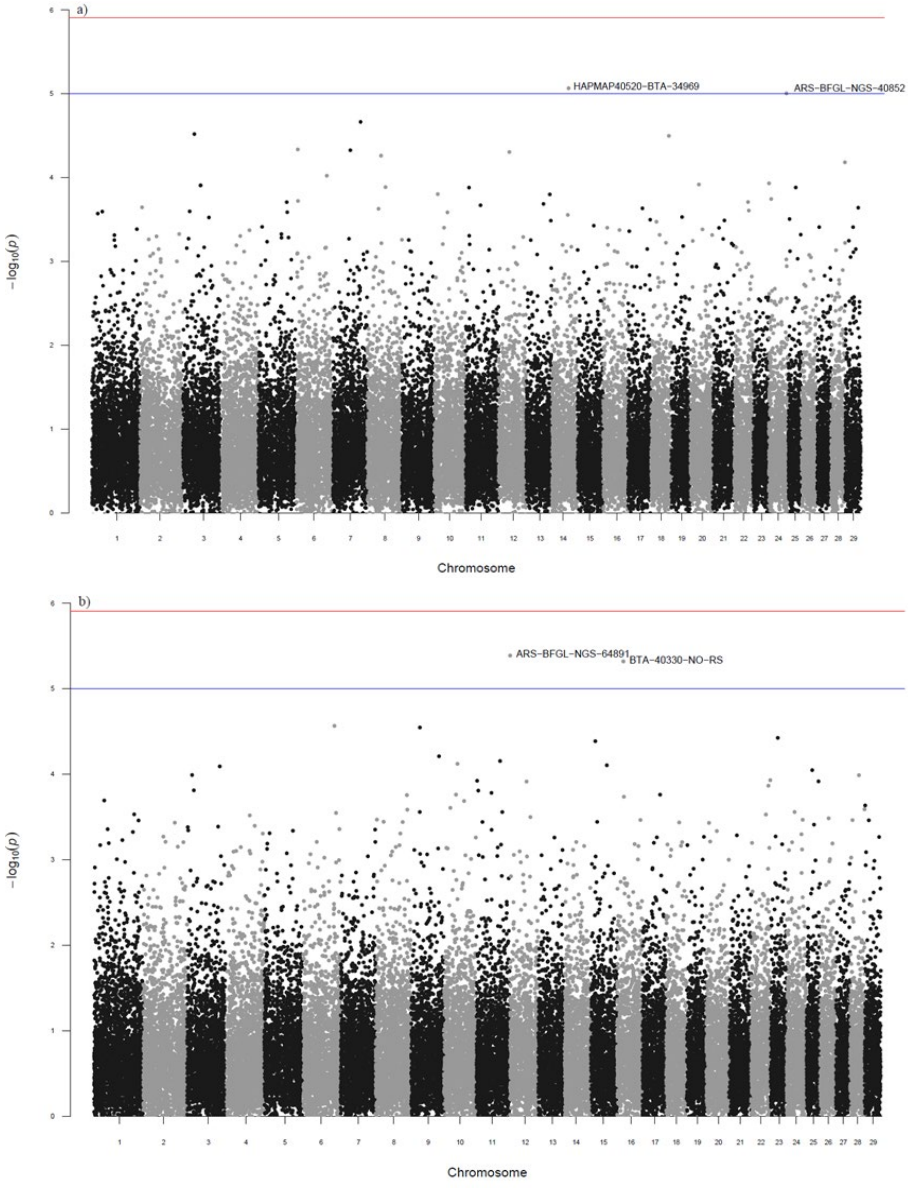

Figure 7. Manhattan plots of genome-wide association analysis results of (a) fetal loss and (b) total pregnancy loss in Red Dairy Cattle. The red horizontal line displays the Bonferroni level of significance (-log (*p*-value) ≥ 5.91) and the blue horizontal line is the suggestive associated line (-log (*p*-value) ≥ 5).

Table 4. Possible candidate genes and functions based on ssGWAS analysis in Red Dairy Cattle and Swedish Holstein

BTA[1]	SNP position (Mbp)	Candidate genes	Possible association with pregnancy loss trait
4	27.02	*HDAC9*	Histone deacetylation
5	117.10	*TBC1D22A*	Protein binding, intracellular protein transport, activation of GTPase activity
5	117.10	*CERK*	Proliferation, apoptosis, phagocytosis, immune response
5	117.10	*GRAMD4*	Apoptosis, immune response
5	117.23	*ENSBTAG00000044449*	Immune response
10	89.62	*NRXN3*	Regulating synaptic properties
12	0.74	*ENSBTAG00000035926*	GTP binding protein, GTPase activity
14	55.55	*TMEM74*	Transmembrane transporter binding protein, macroautophagy, autophagy
14	55.55	*TRHR*	Encoding the thyrotropin-releasing hormone receptor
16	20.08	*ESRRG*	Energy metabolism, placental formation
24	57.91	*ZNF532*	DNA and ion binding activity, transcriptional regulation
24	57.91	*OACYL*	Acyltransferase activity
24	57.91	*SEC11C*	Signal peptide processing
24	57.91	*MALT1*	Immune response
24	57.91	*ALPK2*	Regulating apoptosis, gene expression, muscle development, stem cell differentiation

[1]*Bos taurus* autosome.

3.5 Pregnancy loss due to genetic defects (Paper IV)

Information on carrier status of ten lethal recessive genetic defects was obtained from NAV in Paper IV in order to evaluate the effect of carrier status on pregnancy maintenance (Table 5). If pregnancy losses due to genetic defects are substantial, carrier status could be used in the breeding program to optimize mating plans and avoid at-risk matings (Bengtsson et

al., 2022). NAV uses the Illumina 50k chip (Illumina Inc.) to analyze genetic defects and FImpute software to impute genotypes of animals with lower-density chips to 50k. The genetic defects studied were: Ayrshire Haplotype 1, Ayrshire Haplotype 2, *Bos taurus* autosome 12 (BTA12), *Bos taurus* autosome 23 and Brown Swiss Haplotype 2 in RDC, and Holstein Haplotype 1, 3 (HH3), 4, 6 and 7 in SH. Data from 158,795 inseminations in 28,432 RDC and 22,018 SH were analyzed. The data permitted separate analyses of BTA12 and HH3, but carrier frequencies of the remaining defects were too low and at-risk matings too few to enable further analysis.

Table 5. Recessive haplotypes associated with pregnancy loss in Red Dairy Cattle (RDC) and Swedish Holstein (SH) that are currently tracked in genetic evaluation by Nordic Cattle Genetic Evaluation (NAV)

Breed	Haplotype	Affected genes	Chromosome	Position
RDC	Ayrshire Haplotype 1	*UBE3B*	17	65,921,497
RDC	Ayrshire Haplotype 2	*RPAP2*	3	51,267,548
RDC	*Bos taurus* autosome 12	*RNASEH2B*	12	20,346,401-20,423,092
RDC	*Bos taurus* autosome 23	*BTBD9, DNAH8, GLO1*	23	12,291,761-12,817,087
RDC	Brown Swiss Haplotype 2	*TUBD1*	19	11,063,520
SH	Holstein Haplotype 1	*APAF1*	5	63,150,400
SH	Holstein Haplotype 3	*SMC2*	8	95,410,507
SH	Holstein Haplotype 4	*GART*	1	1,277,227
SH	Holstein Haplotype 6	*SDE2*	16	29,005,214-29,020,714
SH	Holstein Haplotype 7	*CENPU*	27	15,119,556-15,165,355

Conception rate was also analyzed. It was defined according to the NAV trait definition used in the genetic evaluation of fertility (Muuttoranta et al., 2019; Nordic Cattle Genetic Evaluation, 2022), where each insemination was assigned a phenotypic value of failure to conceive (0) or successful conception (1). The pregnancy status was evaluated based on subsequent inseminations, pregnancy diagnosis (manual and PAG analysis) and data on calving, sales of animals during the service period and culling to assess the pregnancy outcome.

3.5.1 Main findings

Few at-risk matings were observed in Paper IV, which indicates that Swedish milk producers and breeding companies are clearly aware of the severe consequences of genetic defects for dairy reproduction and are actively working to avoid at-risk matings. In cases where at-risk matings had been carried out, pregnancy loss was more frequent in both RDC and SH, with up to 14.9% more losses than in not-at-risk matings (Table 6). The majority of pregnancy losses in relation to genetic defects were reported within the first three months post-AI. Conception rate was also significantly lower in at-risk matings, between 0.11 and 0.14 units lower in RDC and SH, respectively, compared with not-at-risk-matings.

Table 6. Differences in least squares mean of conception rate (CR) and pregnancy loss (PL, percent) between at-risk and not-at-risk matings in Red Dairy Cattle females carrying *Bos taurus* autosome 12 and Swedish Holstein females carrying Holstein Haplotype 3

At-risk matings	Not-at-risk matings	CR	PL
Bos taurus autosome 12		-0.11*	14.1*
Holstein Haplotype 3		-0.14*	14.9*

Asterisks indicate a significance level of $p \leq 0.05$.

4. Economic value of pregnancy loss

An economic value for pregnancy maintenance was calculated for each insemination event in the data available from Paper III. The value of pregnancy loss (either embryonic or fetal loss during gestation) was calculated based on the cost of AI, cost of delayed CIN and cost of involuntary culling. All economic entries were recalculated based on 2023 inflation, with an assumed exchange rate of 1 SEK to €0.085. All successful inseminations were allocated a cost of 0.

The cost per semen dose was assumed to be €23.4 for both RDC and SH (Viking Genetics, 2023). Data on semen type were not available for the calculations and thus all semen was assumed to be conventional semen. Furthermore, while sexed semen is available in Sweden through Viking Genetics, its use is limited in Swedish herds and it constitutes approximately 10.8% of inseminations (Växa, 2022). Labor related to one AI performed by herd personnel, including heat detection and performing AI, was assumed to be 0.46 hours (0.25 hours + proportion of owner inseminations × 0.25 hours) (Sørensen et al., 2018). The proportion of AI performed by herd personnel was 85% based on the available data. Cost of labor was assumed to be €9.3 per hour (SCB, 2023) and the total cost of performing one AI was calculated to be €27.7. The cost of AI performed by technicians was assumed to be €42.0 (Sørensen et al., 2018).

The cost of delayed CIN consists of two parts: the cost of failed insemination and the cost of delay to the next possible window for insemination. The cost of failed insemination was calculated as the number of days from insemination to the day on which the cow was confirmed non-pregnant by PAG sample. This was multiplied by €-1.4, which is the daily cost of keeping an empty cow in the herd according to Oskarsson and Engelbrekts (2015). The cost of delay to the next possible window for

insemination was calculated as the number of days the cow is empty from a previously failed insemination (i.e., confirmed non-pregnant by PAG analysis) up to the day of next insemination, multiplied by €-1.4 per day (Oskarsson & Engelbrekts, 2015).

The cost of involuntary culling due to fertility-related causes was calculated according to Oskarsson and Engelbrekts (2015), where the cost was estimated as the difference between the value of a heifer ready to calve and the slaughter revenue from a culled cow. An assumed slaughter weight of 317 kg and slaughter revenue of €3.0 per kg were used. This cost was multiplied by 0.17 to correspond to the reported 17% culling percentage due to fertility-related causes (Växa, 2023). The cost of keeping the cow in the herd until culling was calculated as the number of days from the cow was confirmed non-pregnant until the day of slaughter, and was multiplied by €-1.4 per day (Oskarsson & Engelbrekts, 2015).

The economic value of pregnancy maintenance was estimated using mixed linear models in SAS. Model 1 was used to estimate the overall economic value of pregnancy status during the embryonic and fetal stage, while model 2 was used to estimate the economic loss given that embryonic or fetal loss occurred:

$$y_{ijk} = \mu + PS_i + hys_j + c_k + e_{ijk} \quad [1]$$

$$y_{jklm} = \mu + B_l + P_m + b_1*MY + hys_j + c_k + e_{jklm} \quad [2]$$

where y_{ijk} is the economic value for a given insemination, successful or not; y_{jklm} is the cost attributed to unsuccessful inseminations; μ is the overall mean; PS_i is the fixed effect of *i*th pregnancy status (pregnant or non-pregnant); B_l is the fixed effect of the *l*th breed (RDC or SH); P_m is the fixed effect of the *m*th parity (lactation group 1, 2, ≥3); b_1*MY is the fixed linear regression on 305-d MY with coefficient b_1; hys_j is the random effect of herd by insemination year and season (with 1055 herds, eight years (2014-2022) and four seasons (Dec-Feb, Mar-May, Jun-Aug, Sep-Nov) and ~ $N(0, I\sigma_{hys}^2)$, where I is an identity matrix and σ_{hys}^2 is the random herd-year-season variance); c_k is the random effect of cow *k* ($c_k \sim N(0, I\sigma_c^2)$, where σ_c^2 is the variance of the cow); and e is a random error term ($e \sim N(0, I\sigma_e^2)$, where σ_e^2 is residual variance). Carrier status of genetic defects was not included in the model due to the low incidence and few at-risk matings performed during the study period.

Fetal loss was associated with a higher economic cost (€198) than embryonic loss (€132). There were no differences between RDC and SH in

the cost of embryonic or fetal loss ($p = 0.9055$ and 0.4835, respectively). The cost of embryonic loss increased with parity ($p \leq 0.0001$). Similarly, fetal loss was more expensive in multiparous cows compared with primiparous cows, costing up to €11.1 more in older cows. Higher milk yield was associated with a lower cost of both embryo and fetal loss (€0.4 and €0.3 per 100 kg, respectively).

5. General discussion

High frequency of pregnancy loss is a major concern in the dairy industry, as it extends the planned CIN, affecting herd profitability. It is therefore important to determine genetic variation in pregnancy loss traits, keep apprised of genetic defects that adversely affect embryo and fetal survival and find candidate genes for pregnancy loss to genetically improve the ability to maintain pregnancy to full term. This could increase favorable pregnancy outcomes and thereby improve production efficiency.

5.1 Automated recordings for improved phenotyping of fertility traits

The majority of fertility traits are currently defined based on indirect measures estimated from calving and insemination data (Nordic Cattle Genetic Evaluation, 2022). These traits can be biased due to e.g., management decisions and recording errors (Tenghe et al., 2015). The classical fertility traits generally have low heritability, making genetic progress slow (Berry et al., 2014; Muuttoranta et al., 2019). A means of achieving genetic improvement in fertility is by using traits that are closer to the physiological nature of the cow, traits that have higher heritability and/or traits that have a higher correlation to the true breeding goal traits (Tenghe et al., 2015).

There has also been an increase in the use of electronic equipment and automation in dairy production in recent years, which could be beneficial in creating new phenotypes for genetic and genomic evaluation of fertility. For instance, one way to improve phenotyping could be to use endocrine measurements of fertility, which are direct indicators of ovarian activity and conceptus development. This has led to the development of chemical

pregnancy diagnosis in the form of P4 and PAG analysis. However, in the past, sampling and analysis of P4 and PAG were performed manually, which strongly restricted the number of animals that could be sampled. Manual sampling is also labor-intensive, increasing the cost associated with production. Therefore, recordings from automatic milking systems or test-day milk recording have potential, as they can be scaled up without the increased labor requirements commonly associated with manual sampling methodology. Recording practices that have been incorporated into milking systems and management routines, such as test-day milk recording schemes, also avoid excess handling of animals.

5.2 Differences and similarities between automated recording of P4 and PAG

The most obvious similarity between P4 and PAG analyses is that they are both chemical pregnancy diagnoses based on analysis of milk samples. This is far more efficient and accurate than manual pregnancy diagnosis, as it is non-invasive, objective and does not require additional handling of animals (Bruinjé & Ambrose, 2019). This is part of the reason why using PAG analysis has become common practice in many dairy herds in Sweden today. Both P4 and PAG analyses are also useful in identifying non-pregnant animals early, allowing them to be returned to service, which is important for reproductive efficiency and herd profitability. Management decisions and compliance issues can, however, still affect both systems, such as whether and when to perform manual estrus detection and pregnancy diagnosis, when to inseminate and allowing for preferential treatment of certain groups of animals.

The cost of observing the reproductive status of the cow is one of the most important differences between the two analyses. The P4 recording with Herd Navigator™ involves a higher cost than PAG because the farmer pays for an additional unit for the milking system as well as the dry sticks required for each analysis. In contrast to this, PAG is another analysis performed on the milk sample sent for monthly milk recording. Accordingly, PAG analysis is available to all herds in the milk recording scheme who participate in test-day milking, while the automatic P4 recording system studied in this thesis demands a special in-line system. Since the recordings are originally used for management purposes, using them to improve breeding as well would

bring an added value without the full cost should the recordings be performed simply for use in genetic evaluation. The PAG analysis is also available to those herds that do not participate in the milk recording scheme, but they have to rely on manual, individual sampling and analysis.

Data resolution is much higher with P4, where the endocrine measurements provide an overview of ovarian activity, thus allowing for real-time monitoring of the estrous cycle and gestation (Blavy et al., 2018; Bruinjé & Ambrose, 2019). In contrast, PAG analysis is based on a few observations per insemination with the current analysis regime. It is also important to point out that PAG is a direct measure of placental function, while P4 is an indirect marker of pregnancy status as cows can have high P4 concentrations without being pregnant, e.g., in the case of normal or abnormal cyclicity or endometritis.

Multiple studies have investigated various features of the P4 profile in dairy cattle, but many of these have used manual sampling to obtain P4 data, limiting data collection in terms of both sampling frequency and number of animals sampled (e.g., Petersson et al., 2006; Nyman et al., 2018). With an in-line system, it is possible to achieve relatively larger datasets, but recording of pregnancy loss traits was still restricted in this thesis since few herds in Sweden have the specific in-line milking system that automatically samples P4. This is predominantly due to the high cost of the system. In particular, this affected estimation of the heritability of fetal loss, where too few data were available for accurate estimation. DeLaval, the manufacturer of Herd Navigator™, has continued to develop its milking systems and is currently marketing a new system, the DeLaval VMS V310 RePro, which contains a reproductive module similar to that in Herd Navigator™ but is less expensive. It might be installed in more herds, enabling progesterone recording for a broader group of animals.

The most crucial time to observe the reproductive status of cows is during the early embryonic period, because most losses occur during this stage of development (Santos et al., 2004; Nyman et al., 2018). Diagnosing pregnancy loss at an early stage makes it possible to identify non-pregnant cows and return them to service as soon as possible. As observed in Paper I, approximately 45% of all embryos are lost by 24 days post-AI. In contrast to P4 recording, which may identify a non-pregnant cow in order to have her re-inseminated already at the first possible estrus after an unsuccessful insemination, the sampling for PAG analysis is not informative until 28 days

post-AI at the earliest. This means that PAG analysis in its present form cannot be used to predict early embryonic loss, which accounts for the majority of pregnancy losses during gestation. Therefore, embryonic losses will be underestimated and fetal losses are overestimated in Paper II. Subsequently, at sampling after 28 days post-AI, the majority of cows that are diagnosed non-pregnant have been so for a couple of weeks since the early losses primarily happen prior to the maternal recognition of pregnancy at 14 to 19 days post-AI (Santos et al., 2004; Whitlock and Maxwell, 2008; Diskin et al., 2011). It is also important to note that under traditional reproductive management early embryonic loss occurring after maternal recognition of pregnancy will most likely be classified as prolonged estrous cycle (between 25 to 35 days since the last estrus), rather than being considered an embryonic loss, because the corpus luteum can persist. However, when using Herd Navigator™ these events can be detected and the system issues an "Early Embryonic Loss" or "Abortion" alarm to inform the user that the cow was pregnant, but lost the embryo.

A drawback of both P4 and PAG analysis is that the concentrations may remain high for some time after the embryo or fetus has died. There may be continued P4 production from the corpus luteum before it is fully luteolyzed (e.g., Wiltbank et al., 2023), and the long half-life of the PAG effectively means that it takes 7-14 days for the concentration to break down and decrease in circulation (Ricci et al., 2015). Thus the cow can still test positive during this period (Lawson et al., 2014). In the case of PAG, this also depends on when in gestation the pregnancy loss occurred, as PAG concentrations increase throughout gestation (Figure 4) and therefore take longer to clear from the system the further along the pregnancy has developed (Green et al., 2000; Garbayo et al., 2008; Santos et al., 2018). Furthermore, because sampling for PAG analysis is performed concurrently with milk recording, testing is done once per month within the herd. If the cow is out of synchronization with the test-day of the herd, i.e., was inseminated sooner than 28 days previously, no sample will be taken until the next month's test-day. Therefore, PAG sampling in the milk recording scheme has a longer analysis interval from the first analysis post-AI (50 ± 24.3 days) than 28 days. This effectively means that a potential pregnancy loss may not be detected until several weeks later, thus contributing to the high estimate of fetal loss in Paper II. In addition, gestation and pregnancy loss were confirmed using insemination data in this thesis, rather than using

estrus detection in the herds, further delaying confirmation of pregnancy or lack thereof. It would be possible to overcome these problems by having additional PAG analysis outside the monthly test-day milk recording after 28 days post-AI, but before the next scheduled milk recording test-day. This also highlights the need for other diagnostic tools, such as estrus detection and manual or other chemical (e.g., P4) pregnancy diagnosis techniques. Ultimately, reproductive management strategies have to be optimized for the individual herd, to suit the specific herd dynamic while taking cost of production into account.

Lastly, the P4 and PAG data used in this thesis are both based on analysis of milk samples, which means that neither system can be used for pregnancy diagnosis in heifers. The alternative would be analysis of blood samples, however, since this require additional handling of animals anyway, it is still common to perform manual pregnancy diagnosis for this group of animals.

5.3 Genomic regions associated with pregnancy loss

Identifying genes associated with pregnancy loss traits could lead to a better understanding of the processes and pathways important for embryonic and fetal development and survival (Zolini et al., 2020). The lack of strong associations for the pregnancy loss traits studied in Paper III implies that these traits are controlled by many genes, each with small effects, and by environmental factors. Even though Paper III used maternal genotype in the model and not that of the conceptus, several candidate genes that have been shown to be important for embryo and fetal development and survival were identified. These were related to diverse biological processes and pathways. Including these in the breeding program could advance genetic gain of pregnancy maintenance (Diskin et al., 2011; Mesbah-Uddin et al., 2022).

All except two of the genes identified in Paper III were associated with fetal loss based on the PAG records. Previous studies have identified these 13 genes as important at embryonic level, which is when the majority of pregnancy losses occur, but the results in Paper III indicate that these candidate genes could also be relevant for conceptus development and survival during later gestation. However, some of these genes could have been associated with fetal loss in Paper III because sampling for the PAG analysis resulted in overestimation of fetal loss and some associations found in this category should belong to embryonic losses.

The only SNP associated with embryonic loss was neurexin-3 (*NRXN3*) in SH. It encodes a neuronal cell surface protein mainly found in presynaptic membranes (Puschel & Betz, 1995; Bang & Owczarek, 2013), and is important in regulating synapse formation, differentiation, maturation and function (Zhang et al., 2022). It may also be involved in cell recognition, cell adhesion and mediating intracellular signaling (The UniProt Consortium, 2023c).

The SNP at BTA16 in RDC affecting total pregnancy loss is of particular interest for cattle fertility, as it is located within the estrogen related receptor gamma (*ESRRG*) gene. *ESRRG* could be involved in pregnancy maintenance through changes in energy metabolism, as it exerts direct control over gene expression of mitochondrial biogenesis and function (Giguère, 2008; Hock & Kralli et al., 2009; Wang et al., 2015), lipid and glucose metabolism (Giguère, 2008; Poidatz et al., 2012; Takada et al., 2018), and oxidative phosphorylation (Poidatz et al., 2012) in highly energy-demanding tissues. The *ESRRG* expression increases during pregnancy, as the placenta has elevated metabolic demand. Limited expression of *ESRRG* has been found to compromise energy metabolism in the placenta, which might have a negative influence on trophoblastic cell differentiation, and thus affect implantation and placentation (Poidatz et al., 2012; Takada et al., 2018).

5.4 Changes in fertility and production traits due to genetic defects

A decrease in BTA12 carrier frequency in males was observed in Paper IV, from 32.2% in 2014 to 12.8% in 2020. Consequently, carrier frequency has more than halved in seven years, which might be due to increased genotyping and to re-genotyping of older bulls with newer SNP chips, enabling their continued use in breeding (Diskin et al., 2011). Meanwhile, carrier frequency in females increased from 0% in 2014 to 15.4% in 2020, which is possibly due to increased genotyping during the past few years compared with a lack of genotyping of older cows born in the beginning of the study period. In contrast, carrier frequency of HH3 in SH was lower in both males and females, and was similar to values reported in previous studies (Fritz et al., 2013; Cole et al., 2016).

It is possible that the BTA12 lethal defect is segregating in the RDC population because of a strong positive effect on milk, protein and fat yields

in carriers (Kadri et al., 2014), but it is unclear if HH3 influences production traits. For instance, HH3 did not influence milk production in Paper IV. This is in contrast to findings by Cole et al. (2016), who observed lower milk and protein yield in HH3 carriers compared with non-carriers.

Furthermore, several sources indicate that carrier status of genetic defects influences fertility. Previous studies report lower cow and heifer CR, daughter pregnancy rate (Cole et al., 2016), calving rate (Fritz et al., 2013) and non-return rate (Segelke et al., 2016; Wu et al., 2019; Wu et al., 2020) in carriers compared with non-carriers. This results in a delay in the next lactation, owing to more inseminations being needed per successful pregnancy, and undesired extended CIN. In Paper IV, carriers of BTA12 also had a longer FLS (6 days, $p \leq 0.0001$), but there was no difference in FLS in HH3 carriers in SH.

While the negative effects of genetic defects such as the HH3 and BTA12 lethal haplotypes are disconcerting and can influence herd profitability, it is encouraging that few at-risk matings have been performed in the Nordic countries during the past few years. This suggests that producers and breeding companies are clearly aware of the disadvantages of at-risk matings and are working to optimize breeding plans to avoid matings between carriers. Assigning carrier status on a regular basis when genotyping animals has probably also facilitated continued use of popular carrier bulls that have a high genetic level in other desirable traits (Cole et al., 2016; Bengtsson et al., 2022) or have valuable pedigrees, rather than excluding these completely from use (Bengtsson et al., 2022). Furthermore, it is important to continue the development of diagnostic SNP tests for genetic defects that cause embryonic and fetal mortality, and to add these to the SNP chips used for genotyping in order to manage defects in the population and associated decrease in fertility (McClure et al., 2014).

5.5 Economic ramifications of pregnancy loss

The economic cost of pregnancy loss has been reported previously to range between $0 and $2333 (€0-2168) using various models (de Vries, 2006; Lee & Kim, 2007; Inchaisri et al., 2010). For instance, de Vries (2006) estimated an average cost of $555 for fetal loss, while Inchaisri et al. (2010) reported a net economic loss of €231 per cow per year in poor-fertility cows compared with high-fertility cows. According to de Vries (2006), the cost of pregnancy

loss is dependent on milk yield, lactation number and stage of lactation at conception. Similarly to these previous findings (de Vries, 2006), the cost related to pregnancy loss was found to increase with gestation length in this thesis, i.e., losses in later gestation are more expensive due to extended service period and CIN. Extra feeding cost due to the longer CIN is a major contributor to the economic loss in the herds, making up 45-52% of the cost of pregnancy loss (de Vries, 2006; Lee & Kim, 2007). Unfortunately, data on feed rations used and related costs were not available for the economic calculations in this thesis.

The cost of reproduction losses increases with parity, according to both the economic calculations in this thesis and the literature (de Vries, 2006; Inchaisri et al., 2010). In addition, de Vries (2006) concluded that pregnancy loss is more expensive for high-lactating cows except when the pregnancy loss happens early in the first lactation. This is in contrast to results obtained using the second economic model in this thesis, where higher milk yield was associated with a lower cost.

While the annual economic loss attributed to Holstein Haplotype 3 has been estimated at around $1.38 million in the USA (Cole et al., 2016), the effect of carriers of genetic defects was not included in the model for economic value of pregnancy in this thesis due to the low carrier frequency and few at-risk matings occurring in the Nordic population, meaning that it is unlikely that the economic loss due to genetic defects is large. The economic impact is further limited because pregnancy loss due to homozygosity of HH3 or BTA12 occurred in early gestation in Paper IV, as opposed to later in gestation which would delay the next lactation further and increase the risk of involuntary culling.

6. Conclusions

- The extent of pregnancy loss in dairy cattle was considerable, ranging from 30% to 60%
- In general, Swedish Red cows had a lower incidence of late embryonic loss, fetal loss and total pregnancy loss than Swedish Holstein cows.
- Fetal loss and total pregnancy loss increased with age of the cow.
- Non-pregnant cows had lower P4 and PAG concentrations in milk than pregnant cows, indicating the importance of continuously high P4 and PAG levels during gestation to support the developing embryo and fetus.
- Low heritabilities and moderate to strong genetic correlations to classical fertility traits were observed, indicating that the potential usefulness of P4- and PAG-derived pregnancy loss traits in their present form in selection is probably limited. However, embryonic loss based on P4 showed an antagonistic relationship with milk production, which could indicate a declining trend in pregnancy maintenance if it is not considered in genetic evaluation.
- ssGWAS identified 19 candidate genes associated with pregnancy loss traits. Several of these genes are reported to influence activation of GTPase activity, proliferation, apoptosis, immune response, neurogenesis, organogenesis, and regulation of synapse function, cell cycle, DNA repair and transcription.
- At-risk matings adversely affected fertility, and caused up to 15% higher mortality compared with non-carrier matings.

7. Practical implications and future perspectives

Using P4 and PAG data to define pregnancy loss traits in dairy cattle offers interesting opportunities because of their biological origin and function during gestation. They are thus a more direct reflection of the cow's reproductive physiology in terms of pregnancy maintenance than the classical fertility traits currently used in the Nordic breeding program and could offer a more accurate trait definition of fertility. However, these traits showed low heritability estimates, similar to those for classical fertility traits, meaning that a large amount of phenotypes would be required in genetic and/or genomic evaluation to ensure a desired genetic gain.

For some pregnancy loss traits in Paper I the heritability estimates were zero, indicating that the data material was not large enough for estimation. Larger studies are required for better predictive estimates of these novel traits. It is therefore important that data from automatic recording systems are made available to registration and breeding organizations and included in the cow database. The data could be collected by the breeding associations in the same way as the data currently used for genetic evaluation. However, it would be most beneficial to use endocrine fertility traits defined from P4 in milk in a genomic selection scheme, because few herds currently use the Herd Navigator™ system. Cows from these herds could then form the reference population for trait recording and genotyping.

Continued technological development is also leading to the emergence of new milking systems, e.g., the DeLaval VMS V310 RePro. This is a simpler and less expensive system than Herd Navigator™, and it might be used in more herds to record progesterone on a larger group of animals. However, further studies are required into how best to exploit modern biosensor technologies for defining novel traits that could lead to genetic

improvements in dairy cattle fertility. Papers I and II merely represent the first steps in determining how to construct automatically recorded endocrine traits, their attributed heritabilities and how they relate to other traits used in the current breeding program. More specifically, further research is needed into optimized recording strategies, to analyze the prediction accuracy of (genomic) breeding values and assess whether and how these pregnancy loss traits could actually benefit selection for fertility as a complement to the classical traits currently in use in the Nordic breeding program.

Previous studies report low calving rates despite high fertilization rates in cattle, indicating that more effort must be made to exploit the genetic ability to improve pregnancy outcome. Pregnancy-associated glycoproteins show potential to describe pregnancy maintenance in dairy cattle compared with conventional measures, as suggested in Paper II, since they are a direct marker of pregnancy as opposed to relying on calving and insemination events or P4 data, which are non-pregnancy-specific. Utilizing PAG data could therefore advance breeding strategies for improved fertility. The PAG data are already being applied in the Nordic genetic evaluation, as one of the components used in defining CR, which specifies the cow's ability to become pregnant when inseminated, and could be extended to take pregnancy maintenance into consideration.

The emergence of genomics and advances in genotyping technologies have led to decreasing SNP chip assay costs, which has enabled the large-scale genotyping necessary for the implementation of genomic selection. This allows for an earlier selection process and increased genetic gain, with selection candidates being allocated their genomic estimated breeding values shortly after birth, drastically reducing the generation interval. This is likely to continue as genotyping costs decrease and prediction accuracies increase.

Identifying genomic regions and candidate genes affecting pregnancy loss traits in dairy cattle also provides further insights into underlying biological mechanisms important to fertility. Using these candidate regions in selection might advance genetic gain in fertility, especially as marker-assisted selection is most beneficial for traits that require a long time to generate phenotypes, such as reproductive performance.

It is challenging to remove lethal recessive genetic defects from a population, but it is possible to decrease the frequency of such defects using selection. Using carrier status in selection to avoiding matching carriers of genetic defects also allows continued use of animals with high genetic merit

or animals with important pedigrees. Likewise, it is important to enable continued screening of genetic defects to find novel lethal recessive defects that may be segregating in the Nordic dairy cattle population and to formulate appropriate counter-measures, such as establishing the carrier status of breeding animals and avoiding at-risk matings.

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Popular science summary

Pregnancy loss is a major concern in dairy production, as more inseminations are required for each successful pregnancy, the interval between calvings is increased and the risk of involuntary culling rises, all of which have negative effects on herd profitability. Despite a fertilization rate after insemination of around 90% in dairy cattle, only 30-50% of all inseminations result in a calving, which indicates that extensive embryonic and fetal losses occur during early gestation. However, little is known about the genetic background of pregnancy loss. Results presented in this thesis could be valuable in determining the genetic variation in pregnancy loss and its potential usefulness as a trait to be considered in breeding programs to improve dairy cattle fertility and reduce pregnancy losses. Genetic improvement could give a permanent increase in the total reproductive performance of the herd without extensive continuous management and administrative interventions, and enable an increased lifespan for the cows, leading to better profitability for Swedish dairy herds.

Information from calvings and inseminations is currently used in breeding for fertility. These parameters are largely determined by management factors and are therefore not as reliable as physiological measures. Previous studies have shown that hormone values, so-called endocrine measures of fertility, have higher heritability than classical fertility measures, which may be explained by the fact that they more directly reflect the cow's reproductive physiological background. However, the need for manual sampling and analysis of endocrine measures has limited the possibilities of using these measures on a large scale. On the other hand, recordings by automatic systems have increased in milk production and could be used in breeding for improved fertility. Therefore in this thesis, pregnancy loss traits were assessed based on automatically collected

progesterone measurements and recordings of pregnancy-associated glycoproteins (PAG).

Early embryonic losses were considerable (approximately 45%), based on automatically recorded progesterone levels in milk. Late embryonic losses were estimated to be 6.1-13.3% and fetal losses to be 4.5-13.3%. The novel fertility traits were found to have low heritability, and were moderately to strongly correlated with classical fertility traits.

Pregnancy loss traits based on PAG analysis following monthly test-day milking in the milk recording scheme were also investigated. This analysis is performed on a large scale in Sweden, where more than half of all dairy herds in the milk recording scheme subscribe to the service in order to distinguish between pregnant and non-pregnant cows in the herds. Pregnancy losses estimated using the PAG assay indicated lower embryonic losses (17.5-20.2%) and higher fetal losses (29.2-38.5%) compared with estimates based on progesterone measurements, because sampling can be done at 28 days after insemination at the earliest and is limited to monthly sampling. Similarly to the progesterone-based traits, heritability estimates were low for the pregnancy loss traits. The genetic correlations between embryonic and fetal losses and classical fertility traits were generally high, suggesting that an improvement in terms of e.g., shortening the interval from first to last service and lowering the number of inseminations per successful pregnancy will also give an improvement in pregnancy maintenance.

Pregnancy traits based on PAG analysis were used in genomic analysis to identify candidate genes associated with pregnancy loss. Nineteen candidate genes were identified, most of which are known to be involved in physiological processes such as protein activity, activation of GTPase activity, proliferation, apoptosis, immune response, neurogenesis, organogenesis, and regulation of synapse function, the cell cycle, DNA repair and transcription. These candidate genes may provide a better understanding of the underlying biological mechanisms of pregnancy loss than fertility traits based on conventional measures, such as dates for calving and insemination, and could help increase genetic progress in fertility if used in selection.

Lastly, ten recessive genetic defects affecting embryonic and fetal survival in dairy cows were analyzed. These mutations are lethal if inherited from both parents, but there were few at-risk matings, i.e., inseminations where both the insemination bull and the heifer or cow carry a genetic defect.

This indicates that herd owners and breeding organizations are clearly aware of the serious consequences of these genetic defects for dairy reproduction and are actively working to avoid at-risk matings. In cases where at-risk matings had been carried out, those heifers and cows (of both the Swedish Red and Swedish Holstein breeds) suffered more often from pregnancy losses, with around 15% more pregnancy losses compared with non-carriers. The conception rate was also significantly lower for at-risk matings (between 0.11 and 0.14 units lower in Swedish Red and Swedish Holstein cows) compared with matings between non-carriers. Therefore, by taking carrier status into consideration during insemination, it is possible to improve the fertility and financial viability of the dairy herd.

Populärvetenskaplig sammanfattning

Dräktighetsförluster är ett stort problem inom mjölkproduktionen eftersom det kräver fler inseminationer för varje framgångsrik dräktighet, förlänger intervallet mellan kalvningar, och ökar risken för tidig utslagning vilket påverkar besättningens lönsamhet negativt. Trots att 90 % av korna blir befruktade efter insemination så resulterar endast 30-50 % av alla insemineringar i en kalvning, vilket tyder på omfattande embryo- och fosterförluster under tidig dräktighet. Trots detta vet vi lite om den genetiska bakgrunden till dräktighetsförluster. Resultaten som presenteras i denna avhandling kan vara värdefulla för att fastställa den genetiska variationen i dräktighetsförluster och dessa egenskapers potentiella användbarhet i avelsprogram för att förbättra mjölkkors fruktsamhet och minska dräktighetsförluster. Genetisk förbättring skulle kunna ge en permanent ökning av den totala fruktsamheten i besättningen utan omfattande skötselinsatser, samt möjliggöra en ökad livslängd för korna vilket leder till bättre lönsamhet i svenska mjölkkobesättningar.

Idag används information från kalvningar och insemineringar i aveln för fruktsamhet. Dessa data är i stor utsträckning bestämda av skötselfaktorer och är därför inte lika säkra jämfört med fysiologiska mått. Tidigare studier har visat att hormonvärden, så kallade endokrina mått på fruktsamheten, har högre arvbarhet än klassiska fruktsamhetsmått vilket skulle kunna förklaras av att de mer direkt återspeglar kons reproduktiva fysiologiska bakgrund. Manuell provtagning och analys av endokrina mått har dock begränsat möjligheterna att använda dessa mått i stor skala. Å andra sidan har registreringar från automatiska system ökat inom mjölkproduktionen och skulle kunnas utnyttjas inom aveln för förbättrad fruktsamhet. Dräktighetsegenskaper definierades därför i denna avhandling utifrån

automatiskt insamlade progesteronmätningar och registreringar av dräktighetsspecifika glykoproteiner (PAG).

Omfattande tidiga embryonala förluster (cirka 45 %) konstaterades baserat på automatiskt registrerade progesteronnivåer i mjölk. Sena embryonala förluster beräknades vara 6,1-13,3 % och fosterförluster 4,5-13,3 %. De nya fruktsamhetsegenskaperna visade sig ha låg ärftlighet och var måttligt till starkt korrelerade med klassiska fertilitetsegenskaper.

Egenskaper för dräktighetsförluster baserade på PAG-analyser tagna under månadsvis provmjölkning i Kokontrollen undersöktes också. Denna analys görs i stor skala i Sverige där mer än hälften av alla mjölkbesättningar i Kokontrollen abonnerar på tjänsten för att särskilja på dräktiga och icke-dräktiga kor. Dräktighetsförluster skattade med hjälp av PAG-analysen indikerade lägre embryonala förluster (17,5-20,2 %) och högre fosterförluster (29,2-38,5 %) jämfört med progesteronmätningar eftersom provtagningen kan som tidigast göras 28 dagar efter inseminering och är begränsad till månadsvisa provmjölkningstillfällen. Arvbarhetsskattningarna var låga för dräktighetsförlustsegenskaperna, precis som för de progesteronbaserade egenskaperna. De genetiska korrelationerna mellan embryo- och fosterförluster och klassiska fertilitetsegenskaper var generellt sett höga vilket tyder på att en förbättring i form av t.ex. förkortning av intervallet från första till sista inseminationen och minskat antal inseminationer per lyckad dräktighet även kommer ge ett förbättrat dräktighetsresultat.

Dräktighetsegenskaper baserade på PAG-analysen användes i den genomiska analysen för att identifiera kandidatgener kopplade till dräktighetsförluster. Nitton kandidatgener gick att utskilja, som är kända för att vara involverade i fysiologiska processer så som proteinaktivitet, aktivering av GTPas aktivitet, celltillväxt, celldöd, immunrespons, bildandet av nya neuroner, utvecklandet av organ, och reglering av synapsfunktion, cellcykeln, DNA-reparation och transkription. Kandidatgenerna kan ge en bättre förståelse av de underliggande biologiska mekanismerna för dräktighetsförlust än fruktsamhetsegenskaper baserat på konventionella mått såsom datum för kalvning och insemination, och användas för att öka genetiska framsteget i fertilitet om de används i selektion.

Slutligen analyserades tio recessiva genetiska defekter som påverkar embryo- och fosteröverlevnad hos mjölkkor. Dessa mutationer är dödliga om de nedärvs från båda föräldrarna, men i studien fanns få bärarparningar, dvs.

inseminingar där både inseminationstjuren och kvigan eller kon bär på en genetisk defekt. Detta tyder på att besättningarna och avelsorganisationerna är medvetna om de allvarliga konsekvenserna av dessa genetiska defekterna för mjölkkors reproduktion och arbetar aktivt för att undvika bärarparningar. I de fall då bärarparningar hade genomförts så drabbades dessa kvigor och kor (av både svenska röda kor och holstein) oftare av dräktighetsförluster, med nära 15 % fler dräktighetsförluster jämfört med ickebärare. Dräktighetsprocenten blev också betydligt lägre för bärarparningar (mellan 0.11 och 0.14 enheter lägre hos svenska röda kor och holstein) jämfört med parningar mellan ickebärare. Genom att ta hänsyn till bärarstatus vid inseminering är det därför möjligt att förbättra mjölkbesättningens fertilitet och ekonomiska lönsamhet.

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Genetic parameters for reproductive losses estimated from in-line milk progesterone profiles in Swedish dairy cattle

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ABSTRACT

This study assessed the extent of reproductive losses and associated genetic parameters in dairy cattle, using in-line milk progesterone records for 14 Swedish herds collected by DeLaval's Herd Navigator. A total of 330,071 progesterone samples were linked to 10,219 inseminations (AI) from 5,238 lactations in 1,457 Swedish Red and 1,847 Swedish Holstein cows. Pregnancy loss traits were defined as early embryonic loss (1–24 d after AI), late embryonic loss (25–41 d after AI), fetal loss (42 d after AI until calving), and total pregnancy loss (from d 1 after AI until calving). The following classical fertility traits were also analyzed: interval from calving to first service, interval from calving to last service, interval between first and last service, calving interval, and number of inseminations per service period. Least squares means with standard error (LSM ± SE), heritabilities, and genetic correlations were estimated in a mixed linear model. Fixed effects included breed, parity (1, 2, ≥3), estrus cycle number when the AI took place, and a linear regression on 305-d milk yield. Herd by year and season of AI, cow, and permanent environmental effect were considered random effects. Extensive (approximately 45%) early embryonic loss was found, but with no difference between the breeds. Swedish Red was superior to Swedish Holstein in the remaining pregnancy loss traits with, respectively: late embryonic loss of 6.1 ± 1.2% compared with 13.3 ± 1.1%, fetal loss of 7.0 ± 1.2% compared with 12.3 ± 1.2%, and total pregnancy loss of 54.4 ± 1.4% compared with 60.6 ± 1.4%. Swedish Red also had shorter calving to first service and calving to last service than Swedish Holstein. Estimated heritability was 0.03, 0.06, and 0.02 for early embryonic, late embryonic, and total pregnancy loss, respectively. Milk yield was moderately genetically correlated with both early and late embryonic loss (0.52 and 0.39, respectively). The pregnancy loss traits were also correlated with several classical fertility traits (−0.46 to 0.92). In conclusion, Swedish Red cows had lower reproductive loss during late embryonic stage, fetal stage, and in total, and better fertility than Swedish Holstein cows. The heritability estimates for pregnancy loss traits were of the same order of magnitude as previously reported for classical fertility traits. These findings could be valuable in work to determine genetic variation in reproductive loss and its potential usefulness as an alternative fertility trait to be considered in genetic or genomic evaluations.
Key words: progesterone, pregnancy loss, heritability, genetic correlation

INTRODUCTION

Low fertility in dairy cattle manifests itself as a long AI period requiring additional inseminations per successful pregnancy, indicating substantial reproductive losses and resulting in a long calving interval (Tenghe et al., 2015; Nyman et al., 2018). Consequently, impaired fertility is one of the main reasons for culling in Swedish dairy herds, accounting for approximately 18% of cows culled in 2019 (Växa Sverige, 2020). This has major economic consequences for the herd owner (Santos et al., 2004; Diskin et al., 2012).

Different approaches have been suggested to predict reproductive loss in cattle. Ultrasonography, palpation, and analysis of substances associated with pregnancy found in blood and milk (e.g., progesterone, **P4**) are predominantly used today (Bruinjé and Ambrose, 2019; Ealy and Seekford, 2019). A more precise diagnosis of reproductive status can be obtained with higher sampling frequency (Blavy et al., 2018; Bruinjé and Ambrose, 2019). Milk samples are preferable, because the methodology is noninvasive and does not require additional handling of live animals, which is laborious and time-consuming (Bruinjé and Ambrose, 2019).

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The Herd Navigator system (**HN**; DeLaval International, Tumba, Sweden) is a management tool designed to monitor reproduction and health status in dairy herds. It automatically samples and analyzes (in-line) milk P4 at certain time intervals based on a biomodel described by Friggens and Chagunda (2005) (DeLaval International, 2011; Bruinjé et al., 2017; Bruinjé and Ambrose, 2019). The system minimizes the effect of the environment and management decisions by alerting the herd owner to key elements in production, such as resumption of cyclicity after calving, estrus detection, optimal time for insemination, likelihood of conception, and reproductive issues (DeLaval International, 2011; Bruinjé et al., 2017).

Previous studies have mainly focused on how in-line P4 records can be used to assess and improve the resumption of normal cyclicity after calving, and if endocrine fertility traits derived from P4 can be used in addition to, or instead of, classical fertility traits to genetically improve this aspect of dairy cattle fertility (e.g., Tenghe et al., 2015, 2018; Tarekegn et al., 2019). Because sampling continues for approximately 60 d after the last recorded insemination in HN, the system also offers an opportunity to estimate early reproductive losses.

Various definitions of reproductive loss can be found in the literature. Most follow the categories established by the Committee on Bovine Reproductive Nomenclature (1972) to standardize reproductive losses, where embryonic loss is pregnancy loss from 1 d until approximately 45 d after insemination and fetal loss is loss from approximately 45 d after insemination until expected calving. Embryonic losses are further subdivided into early or late, where pregnancy loss during the first 24 d after insemination is considered early, and loss between 25 and 42 to 50 d is considered late embryonic loss (e.g., Santos et al., 2004). Several studies have reported an effect of parity but, on average, 30 to 50% of inseminations end in early embryonic loss, 10 to 15% in late embryonic loss, and around 10% in fetal loss (Bruinjé et al., 2017; Nyman et al., 2018). Previous studies have also reported differences in reproductive outcome depending on the P4 concentration before, during, and after insemination (Båge et al., 2002; Båge, 2003).

To the best of our knowledge, reproductive losses based on P4 have only been analyzed previously using manually sampled P4 data (Nyman et al., 2018) or HN data on small sample sizes (Bruinjé et al., 2017; van Binsbergen et al., 2019). Furthermore, few estimates of genetic parameters for reproductive loss based on P4 data have been reported. If genetic variation exists in these traits, this information could prove useful in routine genetic evaluation to improve fertility and reduce reproductive losses in dairy cattle. Therefore, the aim of this study was to use in-line milk P4 records to assess the extent of reproductive losses and to estimate genetic parameters for pregnancy loss traits in Swedish Red (**SR**) and Swedish Holstein (**SH**) cows.

MATERIALS AND METHODS

In-line milk P4 and milk yield (**MY**) records for 14 Swedish HN herds in the period 2015 to 2019 were obtained. The HN system automatically samples and analyzes milk P4 at frequencies specified by the system's biomodel, which is based on calculations by Friggens and Chagunda (2005). The biomodel is described in detail by Bruinjé et al. (2017, 2019) and validated by Bruinjé and Ambrose (2019). In total, our data set covered 407,794 P4 samples collected from 5,944 lactations in 1,468 SR and 1,876 SH cows. Pedigree, calving, insemination, culling, and disease data for the cows were extracted from the Swedish cow database maintained by Växa Sverige (Stockholm, Sweden).

Filtering Criteria

The P4 data were linearly interpolated to estimate the beginning and end of each estrus cycle, where a P4 concentration above 5 ng/mL was used to define luteal activity. At least 2 consecutive P4 records above the threshold for luteal activity, a luteal phase length of at least 4 d, and an interovulatory interval greater than 4 d were required in each estrus cycle.

The HN system is sometimes used to confirm the reproductive status of cows without a full sampling series during the lactation, and therefore a minimum of 10 P4 samples was required per lactation. Furthermore, the first sample had to be taken by 25 DIM and the last sample after 60 DIM. The cow also had to commence luteal activity by 60 DIM, excluding 19 lactations from the analysis. The mean P4 sampling duration was 136 ± 73 d (mean ± SD), with milk samples taken every 2 ± 4 d during the lactation. On average per cow, 43 ± 26 P4 samples (mean ± SD) were taken during the AI period.

Insemination data were corrected for double inseminations (i.e., if the cow was re-inseminated within 6 d from the first insemination, the later record was used), resulting in exclusion of 511 insemination events. Each insemination was aligned with the P4 records, and only 1 insemination was accepted per cycle. A maximum of 7 inseminations over a period of 147 d was permitted during the first 9 estrus cycles. The final data set is summarized in Table 1.

Table 1. Number of progesterone (P4, ng/mL) samples, inseminations, lactations, and individual Swedish Red (SR) and Swedish Holstein (SH) dairy cows for which data were available in this study

Item	SR and SH	SR	SH
P4 records	330,071	131,004	199,067
Inseminations	10,219	4,399	5,820
1st parity	3,669	1,620	2,049
2nd parity	3,054	1,286	1,768
≥3rd parity	3,496	1,493	2,003
Parity	5,238	2,386	2,852
1	1,903	853	1,050
2	1,542	705	837
≥3	1,793	828	965
Cows	3,304	1,457	1,847

Trait Definitions

Pregnancy loss traits were defined based on the P4 profiles, where an insemination was considered unsuccessful if a cow presented at least 2 consecutive P4 samples below the threshold of luteal activity during gestation. The losses were categorized as early embryonic loss (1–24 d after insemination), late embryonic loss (25–41 d after insemination), fetal loss (42 d after insemination until calving), and total pregnancy loss (d 1 after insemination until calving). Fertilization failures (defined by either absence of onset of luteal phase, or onset of luteal phase followed by P4 concentrations below the threshold value for luteal activity at some time between 1 and 14 d after insemination) were also included as total pregnancy loss, because these events still represent failure of an insemination, although not a pregnancy loss per se. Overall, 413 (~4%) of inseminations were unsuccessful due to fertility failure, in 144 SR and 269 SH cows. The result of each insemination was confirmed using new insemination events, disease, culling, and calving data, and 319 insemination records lacking such information were removed. If a cow was culled due to reproductive failure during gestation, the pregnancy outcome was included in all pregnancy loss traits. However, if a cow was culled due to nonfertility-related causes, the result was included under the respective pregnancy loss trait associated with the given time of culling but not included in the total pregnancy loss. Within the whole data set, 269 cows were culled, 70 due to fertility failures and 199 due to other causes.

The P4 concentrations in 5 time intervals after insemination were also analyzed. The records with the highest P4 value at 10 d (interval 7–13 d after insemination), 20 d (interval 17–23 d after insemination), 30 d (interval 27–33 d after insemination), 40 d (interval 37–43 d after insemination), and 50 d (interval 47–53 d after insemination) were used.

The classical fertility traits included were interval from calving to first service (**CFS**), interval from calving to last service (**CLS**), interval between first and last service (**FLS**), calving interval (**CIN**), and number of inseminations per series (**NINS**). Thresholds were imposed on these traits to handle outliers (mean ± 2SD), where CFS between 20 and 140 d, CLS between 21 and 217, and FLS of maximum 147 d were allowed, whereas CIN greater than 495 d was excluded. Last, MY from the first 305 DIM was analyzed in connection with pregnancy losses, with a minimum threshold of 3,358 kg milk (mean − 2SD). Mean MY in the Swedish HN herds during 2015 to 2019 was 8,352 ± 2,485 kg per lactation in SR and 9,316 ± 2,721 kg per lactation in SH. The national average MY in 2019 was 9,910 kg per lactation and 10,790 kg per lactation in SR and SH, respectively (Växa Sverige, 2020).

Statistical Analysis

Data were analyzed using mixed linear models in SAS (version 9.4, SAS Institute Inc., Cary, NC) to estimate least squares means. Model 1 (Equation 1) was used for pregnancy loss traits and P4 concentrations at certain time points with 1 observation per insemination, and Model 2 (Equation 2) was used for classical fertility traits with 1 observation per lactation. Classical fertility traits were (natural) log-transformed. Heritabilities were estimated based on the variance components estimates from univariate animal models in the DMU software (Madsen and Jensen, 2013) with Model 3 (Equation 3), and the standard errors were computed based on Taylor series of approximation (Madsen and Jensen, 2013; McKinnon Edwards, 2017). Genetic correlations between traits were estimated using bivariate repeatability models. Model 3 was used for pregnancy loss traits, but MY was modeled without the linear regression on 305-d MY, and the classical fertility traits were analyzed without the effect of cycle. Correlations <0.4 were considered weak, 0.4 to 0.7 moderate, and >0.7 strong. The models were as follows:

$$y_{ijklm} = \mu + B_i + P_j + E_k + b_1\text{MY} + hys_l + c_m + e_{ijklm}, \quad [1]$$

$$y_{ijlm} = \mu + B_i + P_j + b_1\text{MY} + hys_l + c_m + e_{ijlm}, \quad [2]$$

$$y_{ijkln} = \mu + B_i + P_j + E_k + b_1\text{MY} + hys_l + a_n + pe_n + e_{ijkln}, \quad [3]$$

where y is the trait analyzed; μ is overall mean; B_i is the fixed effect of the ith breed (SR or SH); P_j is the fixed effect of the jth parity (lactations grouped as 1, 2, and ≥3); E_k is the fixed effect of the kth estrus cycle

Table 2. Number of inseminations (percentage in parentheses) resulting in reproductive losses in 1,457 Swedish Red (SR) and 1,847 Swedish Holstein (SH) cows, based on in-line milk progesterone samples (ng/mL)

Trait[1]	SR and SH	SR	SH
EEL	4,827 (49.2)	2,026 (47.6)	2,801 (50.5)
LEL	606 (12.2)	174 (7.8)	432 (15.7)
FL	448 (10.2)	178 (8.7)	270 (11.7)
TPL	6,095 (60.8)	2,442 (56.5)	3,653 (64.1)

[1]EEL = early embryonic loss, 1–24 d after AI, fertility failures excluded; LEL = late embryonic loss, 25–41 d after AI; FL = fetal loss, 42 d after AI until calving; TPL = total pregnancy loss, 1 d after AI until calving, excluding 199 inseminations from cows culled due to non-fertility-related causes.

number when the insemination took place (k = 1–9); b_1MY is the fixed linear regression on 305-d MY with coefficient b_1; hys_l is the random effect of herd by insemination year and season [with 14 herds, 5 years (2015, 2016, 2017, 2018, and 2019) and 4 seasons (December to February, March to May, June to August, September to November)], and $\sim N\left(\mathbf{0}, \mathbf{I}\sigma^2_{hys}\right)$, where $\mathbf{I}$ is an identity matrix and σ^2_{hys} is the random herd-year-season variance); c_m is the random effect of cow m [$c_m \sim N\left(\mathbf{0}, \mathbf{I}\sigma^2_c\right)$, where σ^2_c is the variance of the cow]; and e is a random error term [$e \sim N\left(\mathbf{0}, \mathbf{I}\sigma^2_e\right)$, where σ^2_e is residual variance]. Model 3 also included the random genetic effect of animal n [$a_n \sim N\left(\mathbf{0}, \mathbf{A}\sigma^2_a\right)$, where $\mathbf{A}$ is the additive genetic relationship matrix and σ^2_a is the additive genetic variance]; and the permanent environmental effect of animal n to account for repeated inseminations within lactation [$pe_n \sim N\left(\mathbf{0}, \mathbf{I}\sigma^2_{pe}\right)$, where σ^2_{pe} is the permanent environmental variance]. Model 3 was used across both breeds and within each breed separately (ignoring the breed effect in the model).

RESULTS

Of the total of 10,219 inseminations used in the analysis, 60.8% led to a reproductive loss (Table 2). The conception rate [calculated by: (no. pregnancies × 100)/no. AI)] was 48.7% at 24 d after AI, 42.8% at 41 d after AI, and 38.4% in total. SH had the highest level of pregnancy losses, regardless of category.

Approximately 45% of all pregnancies led to early embryonic loss, but there was no difference between the breeds ($P = 0.47$; Table 3). SR cows differed from SH in terms of the remaining pregnancy loss traits. The largest difference was observed in late embryonic loss, which was more than twice as high for SH (13.3%) than for SR (6.1%).

Primiparous cows were significantly different from multiparous cows in fetal loss ($P < 0.001$), with the most pronounced difference being 4.5% of pregnancies lost in primiparous cows compared with 13.3% in cows in parity ≥3. Total pregnancy loss was also less extensive in primiparous cows than in cows in both second parity ($P = 0.002$) and parity ≥3 ($P < 0.001$). However, there were no significant differences ($P > 0.05$) between parities in early or late embryonic loss, and no significant differences between later parities in fetal loss ($P = 0.062$) or total pregnancy loss ($P = 0.307$).

There were significant differences between the breeds in CFS (76 ± 0.8 d in SR, 80 ± 0.7 d in SH; $P < 0.001$), and CLS (137 ± 0.9 d in SR, 140 ± 0.8 d in SH; $P < 0.001$). The CIN was 414 ± 1.2 d in SR and 416 ± 1.2 d in SH. The FLS was 61 d and NINS was approximately 2.9 in both breeds. The CFS, CLS, and CIN were significantly longer in third and later parities than in parity 1 or 2 ($P < 0.02$).

There was a significant association between P4 concentration and pregnancy outcome for most of the time intervals studied during gestation (Table 4). The P4 concentrations from around 10 and 20 d after AI were the only records without an effect on the pregnancy outcome (from 42 d to calving; $P = 0.57$ and $P =$

Table 3. Least squares means (%) ± standard error of pregnancy loss traits estimated from in-line milk progesterone concentrations (ng/mL) in Swedish Red (SR) and Swedish Holstein (SH) cows[1]

Effect	No. of cows	EEL	LEL	FL	TPL
Breed					
SR	1,457	43.4 ± 1.5[a]	6.1 ± 1.2[a]	7.0 ± 1.2[a]	54.4 ± 1.4[a]
SH	1,847	44.8 ± 1.5[a]	13.3 ± 1.1[b]	12.3 ± 1.2[b]	60.6 ± 1.4[b]
Parity					
1	1,903	43.9 ± 1.5[a]	8.5 ± 1.2[a]	4.5 ± 1.2[a]	54.6 ± 1.4[a]
2	1,542	44.0 ± 1.6[a]	10.3 ± 1.3[a]	11.2 ± 1.3[b]	58.3 ± 1.5[b]
≥3	1,793	44.5 ± 1.6[a]	10.3 ± 1.3[a]	13.3 ± 1.3[b]	59.5 ± 1.5[b]

[a,b]Estimates with different superscripts within a column are significantly different ($P \leq 0.05$).

[1]EEL = early embryonic loss, 1–24 d after AI; LEL = late embryonic loss, 25–41 d after AI; FL = fetal loss, 42 d after AI until calving; TPL = total pregnancy loss, 1 d after AI until calving.

Table 4. Least squares means (%) ± SE for progesterone concentrations (ng/mL) in Swedish dairy cattle at 10, 20, 30, 40, and 50 d after insemination, which was used to predict reproductive status on 4 occasions after insemination, in pregnant cows and cows with pregnancy loss (nonpregnant)[1]

Interval	Result	1–24 d	25–41 d	42 d–calving	1 d–calving
d 10	Nonpregnant	18.9 ± 0.19[a]	18.8 ± 0.29[a]	19.7 ± 0.32[a]	18.0 ± 0.20[a]
	Pregnant	19.7 ± 0.18[b]	20.0 ± 0.20[b]	19.9 ± 0.21[a]	19.7 ± 0.20[b]
d 20	Nonpregnant	20.2 ± 0.18[a]	25.8 ± 0.11[a]	26.3 ± 0.11[a]	20.5 ± 0.19[a]
	Pregnant	26.2 ± 0.18[b]	26.4 ± 0.07[b]	26.4 ± 0.07[a]	26.3 ± 0.19[b]
d 30	Nonpregnant		14.8 ± 0.22[a]	26.0 ± 0.10[a]	15.1 ± 0.21[a]
	Pregnant		26.1 ± 0.14[b]	26.3 ± 0.06[b]	26.3 ± 0.21[b]
d 40	Nonpregnant		15.6 ± 0.22[a]	25.0 ± 0.16[a]	22.3 ± 0.18[a]
	Pregnant		25.5 ± 0.14[b]	25.4 ± 0.09[b]	25.3 ± 0.18[b]
d 50	Nonpregnant			19.0 ± 0.24[a]	18.0 ± 0.25[a]
	Pregnant			25.6 ± 0.14[b]	25.8 ± 0.25[b]

[a,b]Estimates with different superscripts within a column are significantly different ($P \leq 0.05$).

[1]1–24 d = indicative of early embryonic loss during the first 24 d after AI; 25–41 d = indicative of late embryonic loss during 25–41 d after AI; 42 d–calving = indicative of fetal loss from 42 d after AI until calving; 1 d–calving = indicative of reproductive loss during the gestation period.

0.33, respectively). Breed-wise comparisons showed several differences in the pregnancy outcome between SR and SH depending on the P4 concentration during gestation, especially during the first few weeks after insemination (Table 5).

Estimated heritability of the pregnancy loss traits ranged from 0.01 to 0.07 (Table 6). The highest heritability estimate for SR was for early embryonic loss (0.04), whereas for SH it was late embryonic loss (0.07). A heritability of 0.00 was estimated for fetal loss in the total data set and in SR cows. The heritability for CFS, CLS, FLS, NINS, and CIN was estimated at 0.04, 0.01, 0.02, 0.03, and 0.02, respectively. Standard error ranged from 0.01 to 0.03 for both pregnancy loss and classical fertility traits.

Early embryonic loss had a strong genetic correlation with FLS, and a moderate correlation with MY, CFS, CLS, and CIN (Table 7). Late embryonic loss was strongly correlated with both CLS and CIN, moderately correlated with FLS and NINS, and weakly correlated with MY and CFS. The standard error ranged from 0.12 to 0.38. Residual correlation between embryonic loss traits, MY, and CFS was around zero. The other traits showed weak positive residual correlations with embryonic loss. These results indicate that the model used in the analysis was able to describe most of the

Table 5. Least squares means (%) ± standard error for progesterone concentrations (ng/mL) in Swedish Red (SR) and Swedish Holstein (SH) cows at 10, 20, 30, 40, and 50 d after insemination, which was used to predict reproductive status on 4 occasions after insemination, in pregnant cows and cows with pregnancy loss (nonpregnant)

Interval	Result	Breed	1–24d[1]	25–41d	42d-calving	1d-calving
10	Nonpregnant	SR	19.5 ± 0.22[a]	19.7 ± 0.45[a]	20.2 ± 0.46[a]	18.8 ± 0.23[a]
		SH	18.2 ± 0.21[b]	18.1 ± 0.33[b]	19.2 ± 0.38[a]	17.2 ± 0.22[b]
	Pregnant	SR	20.2 ± 0.21[a]	20.5 ± 0.23[a]	20.4 ± 0.24[a]	20.2 ± 0.24[a]
		SH	19.1 ± 0.21[b]	19.5 ± 0.23[b]	19.4 ± 0.24[b]	19.2 ± 0.23[b]
20	Nonpregnant	SR	18.6 ± 0.22[a]	25.9 ± 0.17[a]	26.3 ± 0.16[a]	19.1 ± 0.22[a]
		SH	21.5 ± 0.20[b]	25.7 ± 0.12[a]	26.2 ± 0.13[a]	21.6 ± 0.21[b]
	Pregnant	SR	26.6 ± 0.21[a]	26.4 ± 0.08[a]	26.4 ± 0.08[a]	26.6 ± 0.23[a]
		SH	26.0 ± 0.20[b]	26.3 ± 0.08[a]	26.3 ± 0.08[a]	26.1 ± 0.22[b]
30	Nonpregnant	SR		16.0 ± 0.35[a]	26.3 ± 0.15[a]	16.6 ± 0.25[a]
		SH		14.2 ± 0.25[b]	25.8 ± 0.13[b]	13.8 ± 0.23[b]
	Pregnant	SR		26.1 ± 0.16[a]	26.4 ± 0.07[a]	26.3 ± 0.25[a]
		SH		26.1 ± 0.16[a]	26.3 ± 0.07[a]	26.2 ± 0.25[a]
40	Nonpregnant	SR		15.6 ± 0.35[a]	24.6 ± 0.23[a]	22.1 ± 0.22[a]
		SH		15.5 ± 0.25[a]	25.1 ± 0.19[a]	22.4 ± 0.20[a]
	Pregnant	SR		25.6 ± 0.16[a]	25.5 ± 0.10[a]	25.5 ± 0.22[a]
		SH		25.4 ± 0.16[a]	25.3 ± 0.10[b]	25.2 ± 0.21[a]
50	Nonpregnant	SR			18.5 ± 0.35[a]	18.4 ± 0.29[a]
		SH			19.1 ± 0.28[a]	17.6 ± 0.27[a]
	Pregnant	SR			25.7 ± 0.16[a]	25.9 ± 0.30[a]
		SH			25.5 ± 0.15[a]	25.7 ± 0.29[b]

[a,b]Estimates with different superscripts within a column are significantly different ($P \leq 0.05$).

[1]1–24d = indicative of early embryonic loss during the first 24 d after AI; 25–41d = indicative of late embryonic loss during 25–41d after AI; 42d–calving = indicative of fetal loss from 42 d after AI until calving; 1d–calving = indicative of reproductive loss during the gestation period.

Table 6. Estimated heritability (h^2), SE, and additive genetic variance $\left(\sigma_a^2\right)$ of pregnancy loss traits in Swedish Red (SR) and Swedish Holstein (SH) dairy cows

	SR and SH			SR			SH		
Trait[1]	h^2	SE	σ_a^2	h^2	SE	σ_a^2	h^2	SE	σ_a^2
EEL	0.03	0.01	0.007	0.04	0.02	0.01	0.02	0.02	0.006
LEL	0.06	0.02	0.007	0.03	0.03	0.002	0.07	0.03	0.009
FL	0.00	0.02	0.00	0.00	0.02	0.00	0.02	0.02	0.002
TPL	0.02	0.01	0.004	0.03	0.02	0.007	0.01	0.01	0.001

[1]EEL = early embryonic loss, 1–24 d after AI; LEL = late embryonic loss, 25–41 d after AI; FL = fetal loss, 42 d after AI until calving; TPL = total pregnancy loss, 1 d after AI until calving.

genetic and environmental components of the correlations.

DISCUSSION

Impaired fertility is a major concern in the dairy industry. One crucial aspect of fertility is the outcome of pregnancy, and reports of low calving rates indicate high reproductive loss during gestation. It is therefore important to determine genetic variation in reproductive loss and genetically improve the ability to maintain pregnancy to full term, thus increasing favorable pregnancy outcomes. In this study, we evaluated the extent of reproductive loss traits and estimated genetic parameters for these using automatically sampled milk P4 records for Swedish dairy cows in 14 herds. This is the first study to use information on reproductive loss during gestation obtained from a large number of animals of the 2 most common dairy breeds kept in Swedish conditions, which also permitted genetic analysis of the data set.

Genetic Parameters of Pregnancy Loss Traits

The heritability estimates for pregnancy loss traits in this study were of the same order of magnitude as previously reported for classical fertility traits (0.01–0.07, Muuttoranta et al., 2019; NAV, 2020). Estimates of genetic parameters for pregnancy loss traits are scarce in the literature, indicating a need for further research. To the best of our knowledge, only van Binsbergen et al. (2019) have reported estimates for a trait related to reproductive losses derived from P4 data. They estimated the heritability for late embryonic loss to be 0.04 (±0.04), which is comparable to our own results. The low estimates obtained in this study was probably mainly due to large environmental variance in pregnancy loss traits.

Bamber et al. (2009) reported an estimated heritability of 0.16 (±0.11) for late embryonic loss, but their trait was based on pregnancy diagnosis using ultrasound examinations and not on P4 profile recordings. Their estimate is relatively high for a fertility trait, but is associated with a larger standard error, indicating lower precision. Further, they speculated that their results could be due to data recording on few cows in controlled environments by skilled technicians, and that field data would likely yield much lower estimates (Bamber et al., 2009).

Other studies have also used ultrasound diagnostics; for example, Carthy et al. (2015, 2016) estimated heritability to be 0.02 for reproductive loss from 21 d after AI until end of gestation. This is comparable with our heritability estimates for fetal loss in SH cows

Table 7. Estimated genetic (r_g) and residual (r_e) correlation, with standard error (subscript), between pregnancy loss traits, milk yield, and classical fertility traits in Swedish Red and Swedish Holstein cows[1]

Trait[2]	MY	CFS	CLS	FLS	CIN	NINS[3]
r_g						
EEL	$0.52_{0.20}$	$-0.46_{0.28}$	$0.45_{0.29}$	$0.85_{0.16}$	$0.43_{0.38}$	NC
LEL	$0.39_{0.19}$	$0.35_{0.28}$	$0.92_{0.15}$	$0.51_{0.32}$	$0.91_{0.12}$	$0.52_{0.30}$
r_e						
EEL	$-0.02_{0.01}$	$0.00_{0.01}$	$0.38_{0.01}$	$0.40_{0.01}$	$0.38_{0.01}$	NC
LEL	$-0.01_{0.02}$	$0.03_{0.02}$	$0.38_{0.01}$	$0.32_{0.02}$	$0.48_{0.02}$	$0.36_{0.02}$

[1]MY = milk yield from 305-d lactation, kg; CFS = interval from calving to first service, d; CLS = interval from calving to last service, d; FLS = interval from first to last service, d; CIN = calving interval, d; NINS = number of inseminations per AI-period.

[2]EEL = early embryonic loss, 1–24 d after AI; LEL = late embryonic loss, 25–41 d after AI.

[3]NC = not converged.

and reproductive losses in total. However, we estimated zero heritability for fetal loss in the full data set and in SR cows. These results were most likely due to small sample size, as we only had access to 448 cases of fetal loss in total. Thus, further studies on larger data sets are necessary to obtain reliable estimates for this trait.

Embryonic losses were moderately genetically correlated with MY (0.39–0.52) in this study, which is in agreement with reports of higher number of AI required per successful pregnancy in high-yielding dairy cattle (Nyman et al., 2018). However, van Binsbergen et al. (2019) reported a much lower genetic correlation between late embryonic loss and MY (-0.02 ± 0.05), and attributed this to the nature and precision of P4 recordings. In comparison, classical fertility traits are calculated from calving and insemination data, and therefore risk being biased due to recording errors and management practices that can affect the perceived fertility of the cow (van Binsbergen et al., 2019). Carthy et al. (2016) found reproductive loss to be genetically correlated with fat content (-0.17 ± 0.099), fat-protein ratio (-0.22 ± 0.103), and SCS (0.32 ± 0.119).

A study by van Binsbergen et al. (2019) found a lower genetic correlation between late embryonic loss and CIN (0.34 ± 0.08) than observed in this study (0.91 ± 0.12). We also found stronger associations between late embryonic loss and FLS (0.51 ± 0.32) and NINS (0.52 ± 0.30) than van Binsbergen et al. (2019) (0.31 ± 0.13 and 0.37 ± 0.11, respectively), although our estimates had high standard error. Differences in recording practices, management, and data filtering could have contributed to the differences in results. Genetic correlations between late embryonic loss and CFS were of similar magnitude in the 2 studies. These results indicate that cows with impaired fertility have difficulties conceiving and in supporting early embryo development and survival.

The low number of cases on fetal loss in our data set influenced the analysis, as genetic correlations were not estimable for any of the bivariate analyses associated with fetal loss. However, estimates from Carthy et al. (2015) suggest that reproductive losses from 21 d after AI are strongly genetically correlated with CIN (0.8 ± 0.097), moderately correlated with CFS (0.55 ± 0.165) and days open (0.44 ± 0.141), and weakly correlated with NINS (0.33 ± 0.183).

Descriptive Evaluation of Pregnancy Loss

Our estimates for early embryonic loss (~45%) are in line with those in Bruinjé et al. (2017) who reported 59% pregnancy losses within 30 d of insemination in Canadian cows. Both are much higher than the 29% estimated by Nyman et al. (2018) for Swedish SH and SR. However, these differences are probably explained by different sampling methods and filtering criteria imposed on the data. Nyman et al. (2018) estimated pregnancy losses in Swedish dairy cows based on manually sampled P4 records collected on the day of insemination and at 10 and 21 d after each insemination, until the cows were confirmed pregnant. Continuous sampling through the lactation was used by Bruinjé et al. (2017) and in the present study. Furthermore, the sampling frequency is determined by the HN biomodel based on the P4 profile, and on cow- and lactation-specific characteristics (Friggens and Chagunda, 2005; Bruinjé and Ambrose, 2019). Although using more data points to assess the result of insemination provides a more precise description of the reproductive status of the cow through the lactation, the fertility outcome is still affected by farm management decisions (e.g., voluntary waiting periods, preferential treatment of high-yielding cows) and does not depend solely on the biomodel.

Bruinjé et al. (2017) estimated that a further 12.9% of pregnancies were lost between 31 and 55 d after insemination, which is similar to the late embryonic losses reported by Nyman et al. (2018), van Binsbergen et al. (2019), and this study. In total, Bruinjé et al. (2017) found that 28% of inseminations resulted in pregnant cows at 55 d after insemination, which is comparable to our estimates. Although the majority of reproductive losses in dairy cattle happen during the early embryonic stage of the pregnancy (observed as cows returning to heat), losses in later gestation have higher negative economic effect on production due to the cost of keeping unproductive animals in the herd (Santos et al., 2004; Diskin et al., 2012).

Nyman et al. (2018) reported significantly less total pregnancy loss in SR than SH (62.4 and 67.9%, respectively). This is in line with our own findings, but we observed significant breed differences for all pregnancy loss traits except early embryonic loss. Similarly to Nyman et al. (2018), we observed a significant increase in fetal loss and total pregnancy loss with increasing parity.

We observed a stable level of early embryonic loss, regardless of age of the cow, but Bruinjé et al. (2017) observed an effect of parity, with an increase of 12.6% in non-pregnant rate from primiparous to second-parity cows. They also reported an 11% increase in late embryonic loss from first to second parity. Although these results are interesting, their study was based on a smaller data set and they do not specify whether their results are statistically significant, possibly because their main focus was on changes in P4 concentrations in relation to insemination success.

We detected an increase in number of inseminations used in HN herds (2.2) compared with the average (1.8)

reported by Växa Sverige (2020) for non-HN herds in Sweden. Increased number of data points during estrus (Bruinjé et al., 2019; Bruinjé and Ambrose, 2019), estimated likelihood of insemination success for a given estrus (Blavy et al., 2018; Bruinjé and Ambrose, 2019), and a stronger emphasis on following the recommendations for timing of insemination identified by the HN system could possibly decrease the number of inseminations outside the optimal time for conception in HN herds.

P4 Concentration Indicative of Reproductive Loss

An abnormal endocrine pattern, monitored as fluctuations in P4, and negative energy balance have a negative effect on oocyte quality, follicular development, and uterine environment, and thus compromise early embryo survival (Diskin et al., 2012; Bruinjé et al., 2017; Blavy et al., 2018). For instance, the P4 concentration in certain intervals after insemination has been found to significantly affect the outcome of insemination. Swedish dairy cows suffering pregnancy losses from late embryonic stage onwards had significantly higher P4 concentrations on the day of insemination than cows that calved successfully (Båge, 2003; Nyman et al., 2018). It would be interesting to study this using continuously sampled data from Sweden, but the sampling frequency in HN herds is based on the biomodel, which meant that fewer than 15% of inseminations in our data set could be connected to a P4 sample on the day of service.

We were able to investigate the effect of P4 concentration on pregnancy outcome during 5 intervals from 10 to 50 d after insemination. Similarly to Bruinjé et al. (2017) and Nyman et al. (2018), we found significantly lower P4 concentration during the intervals in nonpregnant compared with pregnant cows from late embryonic stage onwards. This indicates the importance of continuously high P4 concentrations during gestation to support development of the embryo and fetus. We also observed several breed differences in P4 concentration, depending on the outcome of the insemination. These indicate reproductive physiological differences between SR and SH, which should be considered when setting up HN and refining the system.

Application in Future Breeding Strategies

Endocrine-derived traits are promising for describing reproductive loss in dairy cattle (Bruinjé et al., 2017, 2019; van Binsbergen et al., 2019). One of the main issues associated with traits derived from P4 data is that these data are not available in the national cow database. Another concern is the limited number of HN units in use in the Nordic countries today, currently around 40 herds, which is primarily due to the high running costs of the system. The low number of animal records currently available is most likely not enough to benefit selection or genetic evaluation based on these traits (Tenghe et al., 2016, 2018; van Binsbergen et al., 2019). However, the collective HN data could serve as a reference population for genomic evaluation of reproductive loss (Tenghe et al., 2016, 2018; Tarekegn et al., 2019).

The strong associations between traits for embryonic loss and FLS (0.82), CLS (0.92), and CIN (0.91) suggest that these traits express much of the same variation. This is encouraging, because these classical fertility traits are already included in Nordic breeding programs (NAV, 2020). The current fertility index in the Nordic programs is mainly focused on the genetic ability of dairy cows to resume cyclicity after calving, show estrus, and become pregnant after insemination (NAV, 2020). Although there are numerous studies relating to the first 2 aspects of the fertility index, more research is required on the ability of high-yielding cows to maintain their pregnancy to full term.

Use of biosensor technology is likely to increase on farms with the move toward more automated production systems. Future studies should explore how biosensor data can be used efficiently to improve genetic and genomic evaluations.

CONCLUSIONS

Analysis of in-line milk progesterone records revealed that approximately 45% of all inseminations resulted in early pregnancy loss, 12% in late embryonic loss, and 10% in fetal loss. SR cows had significantly lower pregnancy loss during late embryonic stage, fetal stage, and in total, and had better fertility than SH cows. Diagnosing reproductive loss early in gestation could reduce losses in production, decrease the risk of premature culling, and increase herd profitability. The heritability estimates obtained for pregnancy loss traits were low and of the same order of magnitude as those for classical fertility traits. Embryonic loss showed moderate to strong genetic correlations with milk production and several classical fertility traits. These results could be valuable for determining genetic variation in reproductive loss and its potential usefulness as an alternative fertility trait in genetic or genomic evaluations. Further studies are required for better predictive estimates of these novel traits, to modernize breeding strategies and exploit modern biosensor technologies for genetic improvements of dairy cattle fertility.

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Genetic parameters of pregnancy loss in dairy cows estimated from pregnancy-associated glycoproteins in milk

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ABSTRACT

This study examined the feasibility of using pregnancy-associated glycoproteins (PAG) in milk within breeding for pregnancy maintenance and assessed the genetic variation in pregnancy loss traits. A total of 374,206 PAG samples from 41,889 Swedish Red (SR) and 82,187 Swedish Holstein (SH) cows were collected at monthly test-day milkings in 1,119 Swedish herds. Pregnancy status was defined based on PAG levels and confirmed by data on artificial insemination (AI), calving, and culling from d 1 postinsemination to calving. Pregnancy loss traits were defined as embryonic loss (diagnosed 28 d to 41 d after AI), fetal loss (42 d after AI until calving), and total pregnancy loss. Least squares means (± standard error, %) and genetic parameters were estimated using mixed linear models. Heritability was estimated to be 0.02, 0.02, and 0.03 for embryonic loss, fetal loss, and total pregnancy loss, respectively. Cows with pregnancy loss had lower PAG concentrations than cows which successfully maintained pregnancy and calved. PAG recording was limited to monthly test-day milking, resulting in low estimated embryonic loss (17.5 ± 0.4 and 18.7 ± 0.4 in SR and SH, respectively) and higher fetal loss (32.8 ± 0.5 and 35.1 ± 0.5 in SR and SH, respectively). Pregnancy loss might have occurred earlier but remained undetected until the next test-day milking, when it was recorded as fetal loss rather than embryonic loss. Estimated genetic correlation between embryonic and fetal pregnancy loss traits and classical fertility traits were in general high. Identification of novel genetic traits from PAG data can be highly specific, as PAG are only secreted by the placenta. Thus, PAG could be useful indicators in selection to genetically improve pregnancy maintenance and reduce reproductive losses in milk production. Further studies are needed to clarify how these results could be applied in breeding programs concurrent with selection for classical fertility traits.

Key words: pregnancy-associated glycoprotein, pregnancy loss, heritability, genetic correlation

INTRODUCTION

Previous work on improving fertility in dairy cattle has focused on the genetic ability to resume cyclicity after calving, show signs of estrus, and become pregnant when inseminated (Muuttoranta et al., 2019; NAV, 2021). The Nordic countries have been selecting for fertility for decades, but extensive pregnancy losses (54–73%) are still being reported based on progesterone profiles (Nyman et al., 2018; Ask-Gullstrand et al., 2021), highlighting the importance of pregnancy maintenance. Impaired fertility is the most commonly reported reason for culling in Sweden, accounting for 17.8% of culled cows (Växa, 2021).

Accurate and early pregnancy diagnosis is a vital part of reproductive management in dairy herds. Pregnancy is generally confirmed by rectal palpation or transrectal ultrasonography, but alternative methods such as chemical pregnancy detection have been developed to improve efficiency in herd management (Lawson et al., 2014; Pohler et al., 2016). In addition to optimizing fertility and productivity in herds by refining reproductive management practices, increasing the genetic progress of these traits is an important step toward improving overall fertility. Classical fertility traits generally have low heritability, which hampers genetic progress (Berry et al., 2014; Muuttoranta et al., 2019). Endocrine fertility traits have been proposed as an alternative indicator for fertility (Friggens and Chagunda, 2005; Petersson et al., 2008; Tenghe et al., 2015). These traits have higher heritability, partly because they reflect the cow's reproductive physiology more directly and are less biased by management decisions than classical fertility traits, which are defined from conventional reproductive parameters such as calving and insemination (Tenghe et al., 2015).

Pregnancy diagnosis by analysis of pregnancy-associated glycoproteins (**PAG**) is routine in many

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dairy herds. PAG are secreted in the placenta, by cells deriving from fetal membranes. PAG can be detected in plasma and milk from approximately 3 wk of gestation and concentrations remain elevated throughout gestation, and may serve as an early pregnancy-specific marker in ruminant species (Zoli et al., 1992; Lawson et al., 2014; Ricci et al., 2015). The physiological function of PAG is still uncertain, but their spatio-temporal gene expression and secretion patterns (Green et al., 2000; Garbayo et al., 2008) suggest that they are involved in key components of gestation, such as placental formation, embryonic growth and development (Patel et al., 2004; Mercadante et al., 2016), pregnancy maintenance (Santos et al., 2018), and preparing the uterine environment for parturition (Patel et al., 2004). In the event of embryonic or fetal loss, placental function (and thus secretion of PAG) is disturbed and PAG concentrations decline over time, returning to nonpregnant levels within 7–14 d (Ricci et al., 2015).

Enzyme-linked immunosorbent assays have been developed to detect PAG in milk and are easily incorporated into routine milk recording schemes, as a convenient early indicator and monitor of pregnancy status in dairy herds (Lawson et al., 2014; Ricci et al., 2015; Santos et al., 2018). The industry has been accumulating large quantities of PAG data from recording schemes since the commercial pregnancy tests became available. Most research has, however, focused on the accuracy and efficiency of measuring plasma and milk PAG, and the usefulness of routine milk recording samples for large-scale pregnancy diagnosis compared with conventional measurements (e.g., Lawson et al., 2014; Ricci et al., 2015; Mercadante et al., 2016). If PAG data are useful indicators of pregnancy maintenance, they could be a valuable tool in determining genetic variation in pregnancy loss traits, as a relevant complement in genetic or genomic evaluations seeking to improve dairy cattle fertility and reduce losses in production. Furthermore, no scientific publications on PAG in Swedish dairy cattle have been published to date, even though PAG-based pregnancy diagnosis has been in routine use in Swedish commercial dairy herds since 2014. The aims of this study were: i) to assess the potential for using PAG information from Swedish routine milk recording in breeding to improve pregnancy maintenance and ii) to examine factors associated with PAG levels in milk during gestation in Swedish Red (**SR**) and Swedish Holstein (**SH**) cows.

MATERIALS AND METHODS

This study used already-collected data from a cow database, and no handling of the already-recorded cows was required.

The PAG records for 1,119 Swedish dairy herds were extracted from the database maintained by Växa (Stockholm, Sweden). These PAG data were derived from milk samples collected in routine (monthly) milk recording in herds during 2014 to 2020. The PAG were analyzed using an enzyme-linked immunosorbent assay (IDEXX Laboratories Inc.). A total of 439,565 PAG observations from 241,780 lactations in 45,709 SR and 90,957 SH cows were used. Additional information, such as pedigree, calving, insemination, culling, and test-day milk records, was also extracted from the database. Mean milk yield per lactation at national level in Swedish milk-recorded herds in 2020 was 10,152 kg in SR and 11,064 kg in SH cows (Växa, 2021).

Trait Definitions

Pregnancy status at the monthly test-day milking was determined based on threshold values set by the commercial kit manufacturer, with the PAG value derived from optical density of the sample and corrected for reference wavelength of the sample and a negative control (Ricci et al., 2015). Pregnancy loss was defined based on PAG profiles, where an insemination was considered unsuccessful if the PAG value was less than 0.1, requiring re-check if the PAG value was in the range 0.1 to 0.25, and successful (pregnancy) if the PAG value was higher than 0.25 (Lawson et al., 2014; Ricci et al., 2015). Pregnancy loss was not dependent on a preceding high PAG value. Three definitions of pregnancy loss (embryonic, fetal, total) were used in the present study based on the nomenclature established by the Committee on Bovine Reproductive Nomenclature (1972), and were based on the pregnancy status derived from PAG sampling in the monthly milk recording scheme. The start of PAG recording in Swedish herds is set to 28 d after insemination at the earliest, embryonic loss was therefore defined as failure to maintain pregnancy during the first 28 to 41 d of pregnancy, whereas fetal loss was defined as pregnancy loss detected based on the PAG sampling from 42 d until calving. These definitions of pregnancy loss reflect the restrictions of PAG sampling on a monthly basis. According to previous studies, the majority of pregnancy losses occur during the embryonic period (Walsh et al., 2011; Nyman et al., 2018; Ask-Gullstrand et al., 2021), and an unknown proportion of embryonic loss will be detected as fetal loss when using monthly-recorded PAG data. Total pregnancy loss was defined as losses during the whole sampling period from 28 d after insemination to calving.

The 3 traits were compared with other direct or indirect information, such as manual pregnancy diagnosis, repeated inseminations, culling, and calving records to

confirm gestation or pregnancy loss. The outcome of the pregnancy diagnosis was also dependent on whether the cows were culled due to poor fertility or for other reasons. If a cow was culled due to reproductive failure during gestation, the pregnancy outcome was included in all pregnancy loss traits. However, culling for reasons not relating to fertility was not included in total pregnancy loss, so as not to penalize the fertility of the individual cow. A total of 86,856 cows were culled during the study period, of which 22,182 (25.5%) were culled for fertility-related causes.

Table 1. Number of pregnancy-associated glycoprotein (PAG) analysis records, inseminations, lactations, and Swedish Red (SR) and Swedish Holstein (SH) dairy cows for which data were available in this study

Item	SR and SH	SR	SH
Cows	124,076	41,889	82,187
Lactations	214,134	73,340	140,794
Inseminations	264,009	88,748	175,261
First parity	101,512	32,150	69,362
Second parity	76,810	25,063	51,747
≥Third parity	85,687	31,535	54,152
PAG records	374,206	125,824	248,382
First parity	144,458	45,674	98,784
Second parity	108,795	35,591	73,204
≥Third parity	120,953	44,559	76,394

Editing Criteria

Gestation stage at PAG sampling was restricted to 28 to 302 d, i.e., the interval from insemination to first sample was limited to 28 d and the maximum time allowed for a given gestation period was 302 d. The mean interval from insemination to first PAG sample was 50 ± 24.3 d, from insemination to last PAG sample 78 ± 42.6 d, and from first to last PAG sample 28 ± 39.0 d. A maximum of 3 PAG samples (mean number of PAG samples per insemination ± 2SD for all cows) were included per insemination, a criterion that excluded 3,625 PAG samples from 656 inseminations. Furthermore, at least 5 PAG samples were required per contemporary group for inclusion in the final data set, affecting 5,622 PAG samples. On average, 1.5 ± 0.55 PAG samples were taken per gestation. Approximately 36% of all inseminations were never monitored by PAG sampling, and these inseminations were excluded from the analysis. On average, the interval between repeated inseminations was 51 ± 54.3 d in cows that returned to heat. Manual pregnancy diagnosis, with or without transrectal ultrasound, was performed in 14.3% of inseminations, with an overall interval from insemination to examination of 57 ± 47.4 d. Gestation period ranged from 260 to 302 d, with an average gestation length of 279 ± 7.9 d.

Data on manual pregnancy diagnosis, repeated inseminations (25%), calving (63% of gestations), and culling (68% of cows) were used to evaluate the pregnancy status of individual cows in each gestation. A total of 33,633 PAG samples (8.76%) were excluded because their records were open at the time of data extraction in the herds and therefore lacked the necessary information. A further 5,625 PAG samples were excluded because they lacked test-day milk yield records (these samples were taken solely for pregnancy diagnosis and no milk parameters were recorded). Lastly, 255 PAG samples were removed due to double insemination within the same cycle (within ≤ 6 d). The final data set comprised 374,206 PAG samples, which were linked to 264,009 inseminations from 214,134 lactations in 41,889 SR and 82,187 SH cows (Table 1).

Five classical fertility traits were also analyzed: interval from calving to first service (**CFS**), interval from calving to last service (**CLS**), interval between first and last service (**FLS**), calving interval (**CVI**), and number of inseminations per series (**NINS**). Thresholds were set to handle outliers (mean ± 2SD) in these traits, with CFS between 42 and 169 d, CLS between 42 and 278 d, and FLS of maximum 173 d permitted, and CVI greater than 536 d excluded.

Statistical Analysis

The accuracy of PAG analysis in measuring pregnancy status was estimated using 5 parameters: (1) sensitivity, i.e., percentage of samples from confirmed pregnant cows identified by the analysis as pregnant; (2) specificity, i.e., percentage of samples from confirmed open cows identified by the analysis as nonpregnant; (3) positive predictive value, i.e., percentage of samples identified by the analysis as pregnant that were from confirmed pregnant cows; (4) negative predictive value, i.e., percentage of samples identified by the analysis as not pregnant that were from confirmed open cows; and (5) accuracy, i.e., percentage of samples from confirmed open/pregnant cows accurately identified as open or pregnant by the analysis. The agreement between insemination records and individual cow pregnancy status according to PAG analysis was determined by calculating the kappa (κ) statistic, where $\kappa > 0.80$ indicates a high level of agreement.

Pregnancy loss traits were analyzed using mixed linear models in SAS (version 9.4, SAS Institute Inc., 2017) to estimate least squares means. Model 1 [1] was used for pregnancy status (pregnant or open), and model 2 [2] to analyze PAG value during gestation. The PAG values for cows with successful pregnancies were also analyzed to test the effect on PAG levels in

milk of calving ease, calf survival, calf sex, and number of calves born. Analysis was performed across both breeds and within each breed separately (ignoring the breed effect in the model). Classical fertility traits were analyzed without the effect of insemination number, and were (natural) log-transformed. The heritability estimates were based on variance components estimated from univariate animal models using model 3 [3] in DMU (Madsen and Jensen, 2013), and standard error of heritability was computed based on Taylor series of approximation (Madsen and Jensen, 2013; McKinnon Edwards, 2017). Heritability was estimated as $\sigma_a^2/(\sigma_a^2+\sigma_{pe}^2+\sigma_e^2)$. The genetic correlations between traits were estimated using bivariate repeatability models, where correlations <0.4 were considered weak, 0.4–0.7 moderate, and >0.7 strong. The models were as follows:

$$y_{ijklm} = \mu + B_i + P_j + I_k + hys_l + c_m + e_{ijklm}, \quad [1]$$

$$y_{ijklmno} = \mu + B_i + P_j + I_k + MY_n + ISP_o + hys_l + c_m + e_{ijlkmno}, \quad [2]$$

$$y_{ijklm} = \mu + B_i + P_j + I_k + hys_l + a_m + pe_m + e_{ijklm}, \quad [3]$$

where y is the trait analyzed; μ is overall mean; B_i is the fixed effect of the ith breed (SR or SH); P_j is the fixed effect of the jth parity (lactation 1 to 7, grouped as 1, 2 and ≥3); I_k is the fixed effect of the kth insemination number (k = 1–4); MY is daily milk at monthly test-day milking, categorized in 10 levels based on deciles; ISP is the interval from service to when PAG sample was taken, categorized in 10 levels based on deciles; hys_l is the random effect of herd by insemination year and season (with 1,052 herds, 7 years (2014–2020), and 4 seasons (Dec–Feb, Mar–May, Jun–Aug, Sep–Nov) and $\sim N(0, \mathbf{I}\sigma_{hys}^2)$, where $\mathbf{I}$ is an identity matrix and σ_{hys}^2 is the random herd-year-season variance); c_m is the random effect of cow m ($c_m \sim N(0, \mathbf{I}\sigma_c^2)$, where σ_c^2 is the variance of the cow); and e is a random error term ($e \sim N(0, \mathbf{I}\sigma_e^2)$, where σ_e^2 is residual variance). Model 3 also included the random genetic effect of animal m ($a_m \sim N(0, \mathbf{A}\sigma_a^2)$, where $\mathbf{A}$ is the additive genetic relationship matrix and σ_a^2 is the additive genetic variance); and the permanent environmental effect of animal m to account for repeated inseminations within lactation ($pe_m \sim N(0, \mathbf{I}\sigma_{pe}^2)$, where σ_{pe}^2 is the permanent environmental variance).

RESULTS

For the 264,009 inseminations represented by the data, pregnancy loss was reported for 100,858 inseminations during gestation (Table 2). The overall sensitivity, specificity, positive predictive value, and negative predictive value of the PAG analysis was found to be 99%, 77%, 91%, and 97%, respectively, in both SR and SH cows. The accuracy of the assay was 93% in SR cows and 92% in SH cows. The overall κ value was 0.81 ± 0.001.

Around 70% of all pregnancy losses were detected within the first 70 d post-AI. Regardless of time period, pregnancy loss was significantly more frequent ($P < 0.0001$) in SH than in SR cows (Table 3). Pregnancy loss also increased significantly with parity ($P < 0.0001$) for all 3 traits (embryonic, fetal, total pregnancy loss). Overall, PAG levels were significantly lower in cows that suffered pregnancy loss than in cows that successfully maintained pregnancy and calved (Table 4). The PAG level increased with gestational stage, i.e., the longer the cow had been pregnant the higher the PAG level in the milk sample, resulting in average PAG level in pregnant cows varying from 0.77 to 1.41. In gestations with subsequent calving, PAG levels were significantly higher in younger animals than in multiparous cows, and in pregnant SR compared with pregnant SH (both $P < 0.0001$). The PAG value also varied depending on

Table 2. Number of inseminations (percentage in brackets) resulting in pregnancy losses in 41,889 Swedish Red (SR) and 82,187 Swedish Holstein (SH) cows, based on pregnancy-associated glycoprotein analysis records

Trait[1]	SR and SH	SR	SH
EL	33,168 (15.7)	10,909 (15.3)	22,259 (16.0)
FL	67,690 (29.4)	22,146 (28.6)	45,544 (29.9)
TPL	71,015 (30.5)	22,897 (29.3)	48,118 (31.1)

[1]EL = embryonic loss, 28–41 d after AI; FL = fetal loss, 42 d after AI until calving; TPL = total pregnancy loss, diagnosed 28 d after AI until calving, excluding inseminations from cows culled due to non-fertility–related causes.

Table 3. Number of cows (N) and LSM (%) ± SE of pregnancy loss traits estimated from monthly pregnancy-association glycoprotein analysis in Swedish Red (SR) and Swedish Holstein (SH) cows

		Trait[1]		
Effect	N	EL	FL	TPL
Breed				
SR	41,889	17.5 ± 0.4[a]	32.8 ± 0.5[a]	31.2 ± 0.5[a]
SH	82,187	18.7 ± 0.4[b]	35.1 ± 0.5[b]	34.4 ± 0.5[b]
Parity				
1	82,769	15.7 ± 0.4[a]	29.2 ± 0.5[a]	30.1 ± 0.5[a]
2	61,882	18.3 ± 0.4[b]	34.1 ± 0.5[b]	33.5 ± 0.5[b]
≥3	69,483	20.2 ± 0.4[c]	38.5 ± 0.5[c]	34.9 ± 0.5[c]

[a–c]Estimates with different superscripts are significantly different ($P \leq 0.05$).

[1]EL = embryonic loss, 28–41 d after AI; FL = fetal loss, 42 d after AI until calving; TPL = total pregnancy loss, diagnosed 28 d after AI until calving.

Table 4. Least squares means ± SE of pregnancy-associated glycoprotein levels in milk in Swedish Red (SR) and Swedish Holstein (SH) cows at first sample used to predict pregnancy status after insemination, in pregnant cows and cows with pregnancy loss (nonpregnant)

Effect	28–41 d[1]	42 d–calving	28 d–calving
Breed			
Pregnant[2]			
SR	1.10 ± 0.007[a]	1.14 ± 0.008[a]	1.14 ± 0.007[a]
SH	1.05 ± 0.007[b]	1.09 ± 0.007[a]	1.09 ± 0.007[b]
Nonpregnant			
SR	−0.16 ± 0.013[a]	0.39 ± 0.009[a]	0.14 ± 0.009[a]
SH	−0.11 ± 0.012[b]	0.41 ± 0.009[b]	0.18 ± 0.008[b]
Parity			
Pregnant			
1	1.14 ± 0.007[a]	1.18 ± 0.008[a]	1.18 ± 0.007[a]
2	1.08 ± 0.007[b]	1.12 ± 0.008[b]	1.11 ± 0.007[b]
3	1.01 ± 0.007[c]	1.05 ± 0.008[c]	1.04 ± 0.007[c]
Nonpregnant			
1	−0.18 ± 0.013[a]	0.36 ± 0.009[a]	0.14 ± 0.009[a]
2	−0.12 ± 0.013[b]	0.41 ± 0.009[b]	0.17 ± 0.009[b]
3	−0.11 ± 0.013[c]	0.43 ± 0.009[c]	0.18 ± 0.009[c]

[a–c]Estimates with different superscripts are significantly different ($P \leq 0.05$).
[1]28–41 d = indicative of early embryonic loss during the first 41 d after AI; 42 d–calving = indicative of fetal loss from 42 d after AI until calving; 28 d–calving = indicative of pregnancy loss during the gestation period.
[2]Optical density readings (adjusted for background) were reported as an indication of the PAG in milk samples.

calf variables in successful pregnancies. Calf survival and calf sex had significant effects on milk PAG in SR cows ($P = 0.0232$ and $P = 0.010$, respectively), while number of calves influenced milk PAG level in both SR and SH cows, with higher PAG in twin births ($P < 0.0001$). Milk yield at monthly test-day recording also influenced milk PAG level, with higher milk yield associated with lower PAG level in samples. The PAG level in the lowest milk yield decile was 0.95 and decreased to 0.68 in the highest milk yield decile.

The heritability estimates of pregnancy loss traits were low, ranging between 0.02 and 0.05 (Table 5). The HYS variances ranged from 0.01 to 0.17. Embryonic loss and fetal loss both had a strong genetic correlation (0.80–0.99) with CLS, FLS, CVI and NINS, but a weak association (0.10–0.27) with CFS (Table 6).

There were significant differences (LSM ± SE) between the breeds in CFS (81 ± 0.2 d in SR, 84 ± 0.1 d in SH; $P < 0.001$), CLS (121 ± 0.3 d in SR, 129 ± 0.3 d in SH; $P < 0.001$), and FLS (35 ± 0.2 d in SR, 38 ± 0.2 d in SH; $P < 0.001$). CVI was significantly longer in SH than in SR, 396 ± 0.3 d compared with 390 ± 0.3 d. NINS was approximately 1.9 ± 0.005 in both SR and SH. CFS, CLS, FLS, and CVI were significantly longer ($P < 0.001$) in multiparous cows than in primiparous cows.

DISCUSSION

Poor reproductive performance is a major concern in the dairy industry. Reports of high fertilization rate and low calving rates indicate high reproductive loss during gestation (Nyman et al., 2018), which increases the risk of premature culling and compromises herd profitability. Studies based on progesterone profiles have confirmed high pregnancy losses in Nordic dairy cattle (Nyman et al., 2018; Ask-Gullstrand et al., 2021). In this study, we evaluated the extent of pregnancy loss and estimated genetic parameters for pregnancy loss traits based on data for PAG pregnancy analysis from the monthly milk recording scheme on Swedish dairy herds. The data set provided valuable information for

Table 5. Estimated heritability (h^2), SE, and additive genetic variance (σ^2_a) of pregnancy loss traits in 41,889 Swedish Red (SR) and 82,189 Swedish Holstein (SH) dairy cows in 17,334 contemporary groups

	SR and SH			SR			SH		
Trait[1]	h^2	SE	σ^2_a	h^2	SE	σ^2_a	h^2	SE	σ^2_a
EL	0.02	0.002	0.002	0.02	0.004	0.003	0.02	0.003	0.002
FL	0.02	0.002	0.004	0.02	0.004	0.004	0.02	0.003	0.005
TPL	0.03	0.002	0.006	0.05	0.006	0.009	0.03	0.003	0.006

[1]EL = embryonic loss, 28–41 d after AI; FL = fetal loss, 42 d after AI until calving; TPL = total pregnancy loss, diagnosed 28 d after AI until calving.

Table 6. Estimated genetic (r_g) correlation, with SE (subscript), between pregnancy loss traits and classical fertility traits in Swedish Red and Swedish Holstein cows[1]

	Trait				
Trait	CFS	CLS	FLS	CVI	NINS
EL	$0.10_{0.056}$	$0.92_{0.019}$	$0.99_{0.018}$	$0.80_{0.043}$	$0.89_{0.033}$
FL	$0.27_{0.062}$	$0.98_{0.005}$	$0.97_{0.014}$	$0.89_{0.019}$	$0.89_{0.022}$

[1]EL = early embryonic loss, diagnosed 28–41 d after AI; FL = fetal loss, 42 d after AI to calving; CFS = interval from calving to first service, d; CLS = interval from calving to last service, d; FLS = interval from first to last service, d; CVI = calving interval, d; NINS = number of inseminations per AI period.

determining genetic variation in pregnancy loss and for assessing the utility of pregnancy loss traits based on PAG recordings. Pregnancy loss is an interesting alternative trait to be considered in genetic or genomic evaluations, to genetically improve cow fertility and reduce pregnancy loss in production.

Circulating Concentrations of PAG

The PAG are produced by the placenta during pregnancy and concentrations continue to increase in circulation as gestation proceeds (Green et al., 2000; Garbayo et al., 2008; Santos et al., 2018). Cows in this study that suffered embryonic or fetal loss had reduced PAG levels in milk during gestation, which suggests that PAG is a useful biomarker for placental function and prediction of pregnancy maintenance (e.g., Lawson et al., 2014; Mercadante et al., 2016; Pohler et al., 2016). Furthermore, Ricci et al. (2015) observed similar PAG levels in pregnant cows that successfully maintained pregnancy and calved and cows with subsequent pregnancy loss, and concluded that cows will test positive for PAG as long as there is a viable pregnancy. We also identified higher PAG levels in milk from primiparous cows than in milk from multiparous cows in successful pregnancies with subsequent calving ($P \leq 0.0001$), which is in line with previous findings (Ricci et al., 2015; Mercadante et al., 2016). We found that SR cows had higher PAG levels in their milk samples than SH cows.

The PAG have a long half-life and can still be detected in circulation for a couple of weeks after the latest AI (Green et al., 2000; Ricci et al., 2015), but with a decrease in circulating PAG between approximately 46 and 72 d post-AI (Lawson et al., 2014; Ricci et al., 2015). Using a PAG-based pregnancy test during this part of the gestation period might therefore increase the number of cows being classified as open or re-check, requiring additional sampling later in gestation for reliable determination of pregnancy status (Lawson et al., 2014, Ricci et al., 2015). In this study we identified 5.95% of the PAG records as re-check, which is similar to the proportion found in previous studies (LeBlanc, 2013; Lawson et al., 2014). It is also important that cows identified as pregnant or re-check early in the gestation period are re-tested later, due to the similarities between truly pregnant cows diagnosed as re-check or open during the 46–72 d period, and cows undergoing pregnancy loss but with the PAG level not fully receded. Failing to do so increases the risk of missing open cows, which also extends FLS, delays CVI, and adds to the production costs (Ricci et al., 2015).

Application in Future Breeding Strategies

The heritability estimates for pregnancy loss traits based on PAG records in this study were low, and similar to those of classical fertility traits (Muuttoranta et al., 2019; NAV, 2021). The low estimates are possibly due to infrequent recording of milk PAG levels (on which the pregnancy loss traits are based) and the traits did not fully capture the genetic variation underlying fertility traits (Berry et al., 2014; Muuttoranta et al., 2019). Santos et al. (2018) found moderate genetic control of milk PAG levels (h^2 = 0.11–0.23), but other estimates of genetic parameters of pregnancy loss traits based on PAG data are scarce in the literature. Similarly, studies based on progesterone data have obtained low heritability estimates for pregnancy loss traits (0.02–0.06; van Binsbergen et al., 2019; Ask-Gullstrand et al., 2021).

Increases in CLS, FLS, CVI, and NINS were genetically associated with increased pregnancy loss. The correlation between pregnancy loss traits and classical fertility traits was generally much higher than that found in previous studies based on in-line progesterone measurements, with both van Binsbergen et al. (2019) and Ask-Gullstrand et al. (2021) reporting moderate genetic correlations (0.31–0.52) between embryonic loss and classical fertility traits. Ask-Gullstrand et al. (2021) also found a high genetic correlation (0.91 ± 0.12) between late embryonic loss and CVI. To our knowledge, no previous study has reported a genetic correlation between fetal loss and classical fertility traits, possibly due to data limitations. The estimates for fetal loss in this study were of the same order of magnitude as those for embryonic loss. The strong genetic correlation observed between pregnancy loss traits and several classical fertility traits suggests that cows with impaired fertility have difficulties supporting embryo and fetal development and survival. Endocrine traits may provide a better definition of fertility because they reflect the cow's reproductive physiology more closely and are less biased by management. However, using endocrine traits in genetic or genomic evaluations has

long been inhibited by laborious techniques and high costs associated with data collection. Automatic in-line sampling methods, such as progesterone recording using the Herd Navigator (DeLaval International, Tumba, Sweden), offer high resolution of pregnancy status in early gestation and are less laborious, but are still associated with high running costs to gather sufficient data per lactation for correct recording of pregnancy maintenance (Tenghe et al., 2015). Furthermore, progesterone is an indirect indicator of pregnancy and is more accurate at finding open cows (i.e., cows with low progesterone concentration) and returning them to service, rather than confirming pregnancy, because cows can have high progesterone concentrations without being pregnant (Lawson et al., 2014; Tenghe et al., 2015). PAG analysis data from monthly milk recording schemes offer an alternative for cost-effective large-scale recording. Whereas PAG recording was primarily developed as a management tool to simplify pregnancy diagnosis in the herds, pregnancy loss traits based on PAG analysis could contribute to better trait definition because they are a direct marker of placental function (Zoli et al., 1992; Patel et al., 2004; Mercadante et al., 2016), and thus an indicator of a cow's ability to maintain pregnancy. However, the current sampling strategy for PAG (at monthly test-day milking) limits measurements to a couple of events per gestation, resulting in imprecise classification of the occurrence of pregnancy loss and risking delay in pregnancy diagnosis. It could be interesting to increase PAG sampling frequency to around the level used for progesterone sampling with in-line milking systems. The potential to develop such a system exists, but the cost and accuracy of higher-frequency of PAG sampling need to be determined.

The fertility index used in the Nordic breeding program focuses on the genetic ability of the dam to resume cyclicity after calving, show sign of estrus, and conceive after insemination (NAV, 2021). PAG records are currently being used as an indicator in calculating conception rate in breeding evaluation of fertility (Muuttoranta et al., 2019; NAV 2021), but this low-heritability trait focuses on the cow's ability to conceive, rather than actual pregnancy maintenance. Endocrine fertility traits could be useful in selecting for improved fertility, with PAG as a potential indicator to define novel fertility traits. An updated genomic evaluation for female fertility could then consider the ability of high-yielding cows to maintain pregnancy to full term. Of the 2,147 herds affiliated with the Swedish milk recording scheme (Växa, 2021), 1,119 herds use PAG analysis for pregnancy diagnosis. The amount of data collected from these herds is sufficient for traditional genetic evaluation of pregnancy loss traits, and it would also constitute a large population for genomic evaluation, where cows from subscribing herds could form the reference population. It would be possible to increase the size of the training population, thus improving genomic predictions, through cooperation within the Nordic countries (Lund et al., 2011; Tenghe et al., 2018; Muuttoranta et al., 2019), with Sweden, Denmark, and Finland contributing to the joint Nordic fertility evaluation (Lund et al., 2011; Muuttoranta et al., 2019). However, the added information in addition to that already available from, e.g., CLS, seems to be low, given the high genetic correlation. Collecting PAG data solely for the use in genomic evaluation would also yield more expensive trait recording compared with classical fertility traits, which are estimated based on calving and insemination data. However, PAG analysis carry the added benefit of replacing manual pregnancy diagnosis with or without ultrasound, which is laborious and therefore costlier than PAG sampling.

The PAG Pregnancy Assay

In this study, estimated embryonic loss (diagnosed from 28 to 41 d after AI) ranged between 15.7 and 20.2% and estimated fetal loss (42 d post-AI onwards) between 29.2 and 38.5%. In a previous study based on PAG data, Pohler et al. (2016) found 12–20% pregnancy loss during 31–59 d post-AI, and 19% from 59 d to parturition. However, Mercadante et al. (2016) reported much lower incidence of embryonic loss of 4.3% at 32–46 d post-AI, 5.8% loss from 46 d to 74 d, and 6.4% loss from 74 d onward. Those results are similar to those of van Binsbergen et al. (2019), who found pregnancy loss ranging from 8 to 23% based on in-line progesterone recording. The differences between studies could be explained by sampling method and frequency, trait definition, and data editing.

In contrast to Mercadante et al. (2016), we found a significant effect of parity on all pregnancy loss traits ($P < 0.0001$), with higher incidence of pregnancy loss with increasing parity, suggesting that age of dam increases the likelihood of pregnancy loss.

The PAG pregnancy assay performed well and test parameters were comparable with previous findings (LeBlanc, 2013; Lawson et al., 2014; Ricci et al., 2015). The high negative predictive value reported for the assay (range 81–100% in various studies) indicates that it is efficient in finding open cows that should be returned to service. Reported positive predictive values are somewhat lower (range 79–91% in various studies), indicating that a few cows are still at risk of losing their pregnancy later in term. The κ value of pregnancy outcomes based on insemination records and the PAG analysis was 0.81, which is similar to the 0.77 reported by Ricci et al. (2015), but somewhat lower than the

0.98 reported by Lawson et al. (2014). Apart from being used as an indicator of pregnancy, PAG pregnancy analysis offers a reliable alternative for dairy herds that have limited access to skilled technicians or veterinarians who can perform manual pregnancy diagnosis (Lawson et al., 2014; Pohler et al., 2016). Moreover, the milk pregnancy test minimizes the amount of handling of animals compared with nonchemical pregnancy diagnostic methods, as sampling is done concurrently with the monthly test-day milk recording (LeBlanc, 2013).

Herds subscribing to Växa's milk PAG pregnancy analysis in Sweden are offered the following sampling strategies, with the aim of detecting open cows as early as possible and re-inseminating them: 1) one sample after 28 d post-AI, 2) one sample after 28 d post-AI and a second sample after 60 d post-AI to confirm an initial positive sample (recommended routine), 3) one sample in preparation for drying off, and 4) an additional individual sample at some point during gestation. The infrequent recording, at monthly test-day milking, might explain why so few embryonic losses were observed in the present study. If a cow is, for example, scheduled for a PAG sample at 28 d post AI but misses it due to a herd test-day at e.g., 24 d post AI, the sample will be rescheduled for the next month's test-day. This would result in a longer interval and no recording for embryonic loss unless individual samples are performed outside of the test-day. In the event of a pregnancy loss, the loss might in fact have occurred earlier, but the long sampling interval meant that the pregnancy loss could not be detected until the next monthly test-day milking, thus contributing to the high estimated fetal loss in this study. One way to overcome this issue would be to perform additional PAG sampling (outside the monthly test-day milk recording) in early pregnancy or to use other diagnostic tools, such as heat detection and manual pregnancy diagnosis. This also highlights the need for optimized reproductive management strategies in individual herds.

CONCLUSIONS

Using PAG data to define novel pregnancy loss traits in dairy cattle is an interesting prospect because these proteins are only produced and secreted by the placenta during gestation. Consequently, they are a more direct reflection of the cow's reproductive physiology in terms of pregnancy maintenance than the classical fertility traits currently used in the Nordic breeding program. Assessing the quality of PAG analysis and the genetic variation in novel pregnancy loss traits was a first step in determining whether these data can help increase genetic progress in cow fertility. Due to low heritabilities and strong genetic correlation to classical fertility traits, the potential usefulness of these pregnancy loss traits in selection is probably limited given the current sampling strategies. The cost of PAG sampling must also be taken into consideration as PAG derived traits will be more expensive than classical fertility traits in the current breeding program. Further research is needed to identify candidate genomic regions associated with pregnancy loss, and determining the accuracy of PAG derived pregnancy traits to ascertain their usability in genomic prediction of fertility. Selection for reduced losses would increase herd reproductive performance without excessive management interventions, allowing for increased longevity and better herd profitability.

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THE EFFECT OF GENETIC DEFECTS ON PREGNANCY LOSS IN SWEDISH DAIRY CATTLE

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ABSTRACT

The effect of carrier status of 10 lethal recessive genetic defects on pregnancy maintenance in Swedish dairy cattle was examined. The genetic defects were: Ayrshire Haplotype 1, Ayrshire Haplotype 2, Bos taurus autosome 12 (BTA12), Bos taurus autosome 23, and Brown Swiss Haplotype 2 in Red Dairy Cattle (RDC), and Holstein Haplotype 1, 3, 4, 6, and 7 (HH1-HH7) in Holstein. Effects of carrier status of BTA12 and HH3 on conception rate (CR), interval from first to last service (FLS), and milk production were also examined. Data were obtained for 1,429 herds in the Swedish milk recording system, while information on carrier status of genetic defects was obtained from the Nordic Cattle Genetic Evaluation. In total, data on 158,795 inseminations in 28,432 RDC and 22,018 Holstein females were available. Data permitted separate analyses of BTA12 and HH3, but carrier frequencies of other defects were too low to enable further analysis. Pregnancy loss was defined as failure to maintain pregnancy, where pregnancy status was confirmed with manual and chemical pregnancy diagnosis, insemination, calving, sales and culling data. Odds ratios (OR) and probabilities of pregnancy loss and CR were estimated using generalized linear mixed models, while pregnancy loss, CR, FLS, milk, protein, and fat yields were analyzed using linear mixed models. Pregnancy losses were reported on average within the first month post-AI. At-risk matings were more prone to suffer pregnancy loss in BTA12 (OR = 1.79) and HH3 carriers (OR = 1.77) than not-at-risk matings. At-risk matings also had lower CR (OR = 0.62 and 0.63 for BTA12 and HH3, respectively) than not-at-risk matings. Carrier females of BTA12 had longer FLS and higher milk production than non-carriers. Conception rate and pregnancy maintenance could be improved by avoiding at-risk matings. This finding could help reduce pregnancy loss due to genetic defects in the breeding program for improved fertility.
Keywords: pregnancy loss, genetic defects, Bos taurus autosome 12, Holstein Haplotype 3

INTRODUCTION

In the past, lethal recessive genetic defects were determined based on clinical cases and inheritance traced through breeding trials. However, this approach fails to identify most genetic defects that cause embryonic or fetal losses, because the phenotype is not clearly observed. Due to the rapid increase in genotyping of dairy cattle, lethal genetic defects can now be identified via tracking haplotypes that show lower than expected homozygotes in living animals in the population, and associated impaired fertility (VanRaden et al., 2011; Fritz et al., 2013). The carrier status of these defects could be used in selection to avoid at-risk matings (i.e., carrier male mated with carrier female) and reduce carrier frequencies (VanRaden et al., 2011; Cole et al., 2016; Bengtsson et al., 2022). The information could also be useful when making culling decisions in the herd (Cole et al., 2016).

Genomic tests are currently available for 10 recessive genetic defects associated with pregnancy loss, which are included in the SNP chip used for genotyping by the Nordic Cattle Genetic Evaluation (**NAV**, http://www.nordicebv.info) of Nordic Red Dairy Cattle (**RDC**, including Swedish Red, Danish Red, and Finnish Ayrshire) and Swedish Holstein (**SH**). The defects evaluated are Ayrshire Haplotype 1 (**AH1**), Ayrshire Haplotype 2 (**AH2**), Bos taurus autosome 12 (**BTA12**), Bos taurus autosome 23 (**BTA23**), and Brown Swiss Haplotype 2 (**BH2**) in RDC, and Holstein Haplotype 1, 3, 4, 6, and 7 (**HH1-HH7**) in SH. The most common defect in RDC is BTA12, which according to Kadri et al. (2014) primarily affects pregnancy maintenance during the first 5 mo of gestation. They identified a 660-kb deletion encompassing 4 genes as the causative variant, out of which the ribonuclease H2 subunit B (*RNASEH2B*) gene was suggested as the candidate

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gene since it is known to cause embryonic lethality when knocked-out in mice. However, it is possible that the other coding genes in the deletion (GUCY1B2 and FAM124A) or DLEU7 and the 2 noncoding RNA genes (DLEU7-AS1; LINC00371) are partly responsible for the embryonic lethality as well.

The most frequent defect in the US Holstein population is HH3, with pregnancy loss occurring during the first 60 d post-artificial insemination (**AI**) (McClure et al., 2014). The deleterious effect is due to a missense variant of the structural maintenance of chromosomes 2 (*SMC2*) gene, where phenylalanine has been replaced by serine (p.Phe1135Ser) on chromosome 8 (McClure et al., 2014; Häfliger et al., 2022).

Pregnancy loss is currently not directly considered in genetic evaluation in the Nordic countries (NAV, 2023), despite reports of high incidence (Nyman et al., 2018; Ask-Gullstrand et al., 2021). If pregnancy losses due to genetic defects are substantial, carrier status could be used in the breeding program to optimize mating plans and avoid at-risk matings (Bengtsson et al., 2022). Considering pregnancy loss in routine genetic and genomic evaluations of fertility in dairy cattle could genetically improve cow fertility and reduce pregnancy loss in production, while also preventing economic losses arising from extended service period and calving interval, and premature culling due to infertility.

The aims of this study were to estimate the frequency of different genetic defects in Swedish dairy cattle (RDC and SH); to analyze the effect of these genetic defects on conception rate (**CR**) and pregnancy maintenance in RDC and SH; and to assess the impact of carrier status of BTA12 and HH3 on interval from first to last insemination (**FLS**). The starting hypothesis was that at-risk matings result in a decrease in CR and in favorable pregnancy outcomes, and longer FLS in carrier females.

MATERIALS AND METHODS

Pedigree, calving, insemination, sales, and culling data were extracted for RDC and SH females in 1,429 Swedish dairy herds in the cow database maintained by Växa (Stockholm, Sweden). Data on a total of 1,974,494 insemination events from 2014 to 2021 were available, with up to 7 insemination records per lactation for virgin heifers and cows up to third parity. The insemination data were corrected for double inseminations within the same cycle (within $\leq$ 6 d), excluding 417 inseminations. A minimum of 5 insemination records was required per contemporary group for inclusion in the final data set.

Data on carrier status of genetic defects were obtained from the Nordic Cattle Genetic Evaluation (NAV, 2020), which uses the Illumina 50k chip (Illumina Inc.) to analyze for genetic defects and FImpute software to impute genotypes of animals with lower-density chips to 50k. Information about carrier status was first made available in October in 2018 in Sweden, and the genomic tests are currently available for everyone who genotypes their animals. Information on genetic defects was available for 57,536 animals (1,259 males and 56,277 females). Only animals with known carrier status (carrier or non-carrier) for 10 genetic defects (AH1, AH2, BTA12, BTA23, BH2, HH1-HH7) were included in the final data set, which comprised 158,795 inseminations in 28,432 RDC and 22,018 SH females (Table 1). A total of 402 and 154 at-risk matings for BTA12 and HH3, respectively, were available for analysis. The remaining defects had too low carrier frequencies to enable further analysis (Table 2).

Trait definitions

Conception rate was defined in line with the NAV trait definition rules used in genetic evaluation of fertility (Muuttoranta et al., 2019; NAV, 2023). Accordingly, each insemination was assigned a phenotypic value of failure to conceive (0) or successful conception (1), which was evaluated based on subsequent inseminations, pregnancy diagnoses (manual and chemical), calving records, data on sales of animals during the service period, and culling data, to assess the pregnancy outcome. Pregnancy status was determined based on manual and chemical pregnancy diagnosis performed in the herds during gestation until expected calving date. Pregnancy outcome was compared with subsequent inseminations, calving, and sales and culling records, to confirm gestation or pregnancy loss. A maximum service period of 163 d (mean + 2SD) was allowed for FLS.

Statistical analysis

Generalized linear mixed models [eq. 1] were fitted using SAS 9.4 Proc GLIMMIX (SAS Institute Inc., Cary, NC) to study pregnancy outcome (success or fail-

Table 1. Number of inseminations in Nordic Red Dairy Cattle (RDC) and Swedish Holstein (SH) for which data were available in this study

	RDC and SH	RDC	SH
Females	50,450	28,432	22,018
Inseminations	158,795	97,551	61,244
Heifers	69,776	42,301	27,475
1st parity	48,944	30,444	18,500
2nd parity	27,514	16,905	10,609
3rd parity	12,561	7,901	4,660

Table 2. Number of inseminations with different carrier status in Red Dairy Cattle (AH1-BH2) and Swedish Holstein (HH1-HH7) in the data set

Carrier status[1]	AH1[2]	AH2	BTA12	BTA23	BH2	HH1	HH3	HH4	HH6	HH7
NCM x NCF	95,586	96,769	81,344	96,357	97,341	57,779	55,605	59,525	60,942	61,150
NCM x CF	1,581	392	3,541	563	186	1,722	2,872	1,541	302	94
CM x NCF	381	389	12,264	6,331	24	1,704	2,613	171	0	0
CM x CF	3	1	402	0	0	39	154	7	0	0

[1]NCM = non-carrier male; NCF = non-carrier female; CM = carrier male; CF = carrier female.
[2]AH1 = Ayrshire Haplotype 1; AH2 = Ayrshire Haplotype 2; BTA12 = Bos taurus autosome 12; BTA23 = Bos taurus autosome 23; BH2 = Brown Swiss Haplotype 2; HH1 = Holstein Haplotype 1; HH3 = Holstein Haplotype 3; HH4 = Holstein Haplotype 4; HH6 = Holstein Haplotype 6; HH7 = Holstein Haplotype 7.

ure) and CR, with a binary distribution and logit link function. The model took the following form:

$$\ln [p/(1-p)] = \mathbf{X\beta} + \mathbf{Zu} \quad [1]$$

where p is probability of pregnancy loss; $\mathbf{X}$ is the design matrix for the fixed effects; $\boldsymbol{\beta}$ is a vector of the effects associated with the columns of $\mathbf{X}$; $\mathbf{Z}$ is the design matrix for random effects; and $\mathbf{u}$ are vectors of the random effects ($\mathbf{u} \sim N(0, \sigma^2_u)$). The model for pregnancy loss and CR in RDC included the fixed effects of type of mating of BTA12 (not-at-risk or at-risk mating), parity, insemination number, and the random effect of service bull. The model for pregnancy loss and CR in SH included the fixed effects of type of mating of HH3, parity, insemination number, and the random effect of female. The models for the 2 breeds differed because of insufficient memory of SAS to handle the effect of female in RDC, due to a larger data set compared with SH.

Linear mixed models were used to estimate least squares means (LS-Means), where model 2 [eq. 2] was used for pregnancy loss and conception rate, and model 3 [eq. 3] was used for FLS, and milk, protein, and fat yield.

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Z}_h\mathbf{h} + \mathbf{Z}_a\mathbf{a} + \mathbf{Z}_s\mathbf{s} + \mathbf{Z}_p\mathbf{p} + \mathbf{e} \quad [2]$$

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Z}_h\mathbf{h} + \mathbf{Z}_a\mathbf{a} + \mathbf{Z}_p\mathbf{p} + \mathbf{e} \quad [3]$$

where $\mathbf{y}$ is a vector of phenotypic observations; $\boldsymbol{\beta}$ is a vector of fixed effects with corresponding incidence matrix $\mathbf{X}$; $\mathbf{h}$ is the random effect of herd by insemination year and season (with 1,429 herds, 8 years (2014–2021), and 4 seasons (Dec-Feb, Mar-May, Jun-Aug, Sep-Nov) where $\mathbf{h} \sim N(0, \mathbf{I}\sigma_h^2)$, with variance σ_h^2); $\mathbf{s}$ is the random effect of service bull with $\mathbf{s} \sim N(0, \mathbf{I}\sigma_s^2)$ where σ_s^2 is variance of the service bull; $\mathbf{a}$ is the random effect of the female with $\mathbf{a} \sim N(0, \mathbf{I}\sigma_a^2)$ where σ_a^2 is the additive genetic variance; $\mathbf{p}$ is the random effect of permanent environment with $\mathbf{p} \sim N(0, \mathbf{I}\sigma_p^2)$ where σ_p^2 is the permanent environment variance; $\mathbf{Z}_h$, $\mathbf{Z}_a$, $\mathbf{Z}_s$, and $\mathbf{Z}_p$ are incidence matrices of herd-year-season, service bull, female, and permanent environment, respectively; and $\mathbf{e}$ is a residual vector, where $\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2)$ and σ_e^2 is residual variance. Model 2 included type of mating (not-at-risk or at-risk mating), parity (0–3), and number of insemination (1–7) as fixed effects, while model 3 included fixed effect of carrier status (non-carrier or carrier), effect of parity, and linear regression of calving interval.

RESULTS

Carrier frequency of BTA12 and HH3 was 12.8% and 3.1%, respectively, in males, and 15.4% and 4.2%, respectively, in females during 2020 (Table 3). We observed a decrease in carrier frequencies from 32.2% in BTA12 in 2014 to 12.8% in 2020 in sires, but an increase from 0% in 2014 to 15.4% in females in 2020. The increase in carrier frequency in HH3 was less pronounced. Pregnancy losses were reported on average within the first 36 ± 27.1 d (mean ± SD) and 32 ± 14.7 d post-AI in BTA12 and HH3 at-risk matings, respectively, with 95% of pregnancy losses occurring before 79 d and 61 d post-AI in BTA12 and HH3 at-risk matings, respectively (Figure 1).

At-risk matings were more prone to result in pregnancy loss in both BTA12 carriers (odds ratio (**OR**) = 1.79) and HH3 carriers (OR = 1.77) than not-at-risk matings (Table 4). Estimated probabilities of pregnancy loss obtained using generalized linear mixed models were very close to estimated LS-Means from linear mixed models, especially for HH3. The difference in probability of pregnancy loss between at-risk matings and not-at-risk matings for BTA12 was 13.4% and the difference between LS-Means from the linear model was 14.1%. For HH3, the corresponding differences were 14.0% and 14.9%, respectively.

Conception rates were considerably lower in BTA12 and HH3 at-risk matings (0.31 and 0.37, respectively) than in not-at-risk matings (0.42 and 0.49, respectively) (Table 5).

Table 3. Carrier frequencies in percent of ten genetic defects in Red Dairy Cattle (AH1-BH2) and Swedish Holstein (HH1-HH7) during the study period 2014 to 2021

	2014	2015	2016	2017	2018	2019	2020	2021
AH1								
males	2.27	1.22	0.68	0.27	0.34	0.06	0.08	0.13
females	0.6	0.37	0.51	2.5	3.03	2.27	1.35	1.55
AH2								
males	0	1.15	2.73	0.25	0.05	0.02	0	0
females	0.05	0	0	0.02	0.18	0.95	1.49	2.19
BTA12								
males	32.16	17.23	11.38	13.73	17.16	12.73	12.75	4.3
females	0	0.44	0.64	0.77	0.69	8.3	15.44	13.76
BTA23								
males	0.04	1.59	0.39	3.02	0.71	0	0	0.04
females	0.05	0.15	0.1	0.1	0.18	1.56	1.81	1.42
BH2								
males	0.86	0	0	0	0	0	0	0
females	0.05	0.15	0	0.1	0.28	0.4	0.32	0.43
HH1								
males	0	3.1	1.37	2.61	7.27	2.74	1.74	0.8
females	0	0.39	3.09	3.03	2.07	2.2	3.92	3.52
HH3								
males	0	0.97	0.75	5.03	2.94	3.93	3.13	5.95
females	2.21	2.71	4.46	5.7	4.36	4.87	4.23	3.73
HH4								
males	0	0	3.71	0.69	0.44	0.04	0	0
females	0	1.16	5.15	2.83	2.86	1.88	1.52	1.34
HH6								
males	0	0	0	0	0	0	0	0
females	0	0	0.07	0.17	0.9	0.68	0.45	0
HH7								
males	0	0	0	0	0	0	0	0
females	0	0	0	0.14	0.03	0	0.28	0.29

[1]AH1 = Ayrshire Haplotype 1; AH2 = Ayrshire Haplotype 2; BTA12 = Bos taurus autosome 12; BTA23 = Bos taurus autosome 23; BH2 = Brown Swiss Haplotype 2; HH1 = Holstein Haplotype 1; HH3 = Holstein Haplotype 3; HH4 = Holstein Haplotype 4; HH6 = Holstein Haplotype 6; HH7 = Holstein Haplotype 7.

Carrier females of BTA12 had significantly (P = 0.0004) longer FLS than non-carriers, 37 ± 1.5 d and 31 ± 0.3 d, respectively. However, there was no difference (P = 0.0779) in FLS depending on carrier status in SH (39 ± 1.5 d in carriers compared with 37 ± 0.4 d in non-carriers).

There were significant differences in milk production traits depending on carrier status of BTA12, where carriers had on average higher yield (Table 6). However, carrier status of HH3 did not result in any difference in milk yield.

DISCUSSION

Increasing conception rates and reducing pregnancy losses in dairy cattle is important to increase reproductive performance and production efficiency. This study examined the pattern of pregnancy loss related to BTA12 and HH3 at-risk matings, estimated the effect of type of mating (at-risk or not-at-risk mating) on pregnancy outcome and CR, and evaluated the effect of carrier status of females on FLS and 3 milk production traits in RDC and SH.

For carriers of genetic defect HH3, 95% of pregnancy losses occurred before 61 d post-AI, which is comparable to values reported in previous studies (McClure et al., 2014). However, the majority of pregnancy losses in BTA12 at-risk matings occurred much earlier in gestation in the present study (95% by d 79) than in a study by Kadri et al. (2014), where only 20–25% of embryonic losses occurred by 35 d post-AI and 79–88% of pregnancies failed by 150 d post-AI. This difference was seen despite a delay in recording when pregnancy loss actually occurred in the present study, as pregnancy status was primarily evaluated based on pregnancy diagnosis and subsequent insemination data, rather than on estrus detection in the herd. While embryonic losses are more frequently observed (Nyman et al., 2018; Ask-Gullstrand et al., 2021), the economic impact of pregnancy loss increases when it occurs later in the gestation period, owing to an extension of the service period and causing a delay to next lactation (Cole et al., 2016). Prolonged calving interval and unproductive drying-off period also increase the risk of premature culling, further affecting herd profitability.

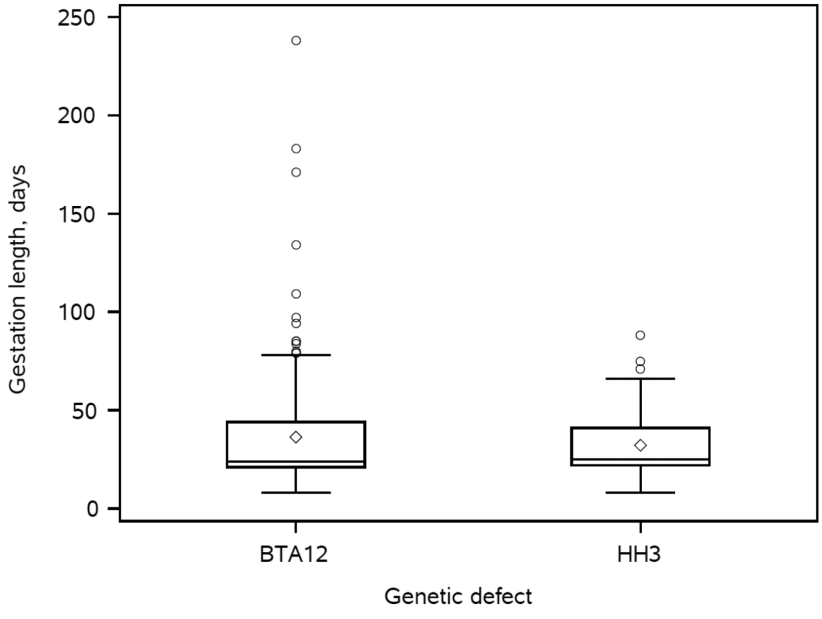

Figure 1. Distribution of pregnancy losses occurring within the gestation period in at-risk matings in Red Dairy Cattle females carrying Bos taurus autosome 12 (BTA12) and Swedish Holstein females carrying Holstein Haplotype 3 (HH3).

Table 4. Odds ratios (OR) with 95% confidence interval (CI), probability, and least squares means (LS-Means) of pregnancy loss in at-risk and not-at-risk matings in Red Dairy Cattle females carrying the BTA12 lethal haplotype and Swedish Holstein females carrying Holstein Haplotype 3 (HH3)

	OR (95% CI)	Probability ± S.E., %	LS-Means ± S.E., %
BTA12			
At-risk matings	1.79 (1.44–2.22)	69.9 ± 2.43	66.0 ± 2.76[a]
Not-at-risk matings		56.5 ± 0.78	51.9 ± 0.76[b]
HH3			
At-risk matings	1.77 (1.25–2.50)	62.7 ± 4.23	62.1 ± 4.28[a]
Not-at-risk matings		48.7 ± 0.97	47.2 ± 0.99[b]

[a-b]Values with different superscripts are significantly different ($P \leq 0.05$).

Table 5. Odds ratios (OR) with 95% confidence interval (CI), probability, and least squares means (LS-Means) of conception rate in at-risk and not-at-risk matings in Red Dairy Cattle females carrying the BTA12 lethal haplotype and Swedish Holstein females carrying Holstein Haplotype 3 (HH3)

	OR (95% CI)	Probability ± SE.	LS-Means ± SE.
BTA12			
At-risk matings	0.62 (0.50 – 0.78)	0.31 ± 0.025	0.36 ± 0.027[a]
Not-at-risk matings		0.42 ± 0.008	0.47 ± 0.008[b]
HH3			
At-risk matings	0.63 (0.45 – 0.89)	0.38 ± 0.042	0.36 ± 0.042[a]
Not-at-risk matings		0.49 ± 0.009	0.50 ± 0.010[b]

[a-b]Values with different superscripts are significantly different ($P \leq 0.05$).

Table 6. Interval between first and last service (FLS), and milk, protein, and fat yield[1] in 305-d lactation in Red Dairy Cattle females carrying/not carrying the BTA12 lethal haplotype and Swedish Holstein females carrying/not carrying Holstein Haplotype 3 (HH3)

	FLS	MY[1]	PY	FY
BTA12				
Carriers	37 ± 1.5[a]	9,781 ± 59.8[a]	359 ± 2.1[a]	428 ± 2.6[a]
Non-carriers	31 ± 0.3[b]	9,634 ± 15.8[b]	353 ± 0.6[b]	421 ± 0.7[b]
HH3				
Carriers	39 ± 1.5	11,081 ± 67.6	392 ± 2.2	454 ± 2.6
Non-carriers	37 ± 0.4	11,091 ± 22.8	391 ± 0.8	452 ± 0.9

[1]MY = milk yield, kg; PY = protein yield, kg; FY = fat yield, kg.
[a-b]Values with different superscripts are significantly different ($P \leq 0.05$).

Carrier frequency of BTA12 was 12.8% in males and 15.4% in females in 2020 in the present study, and 12.9% in a study by Wu et al. (2020). However, Kadri et al. (2014), who first reported the BTA12 haplotype, found higher carrier frequencies for RDC (13%, 23%, and 32% in Danish, Swedish, and Finnish Red Cattle, respectively). In 2014, the carrier frequency of BTA12 in males in Sweden was 32.2%, however, it was 0% in females possibly due to lack of genotyping of older cows during this time. Kadri et al. (2014) suggested that while BTA12 is deleterious in homozygotes, it is still maintained at high frequency in the population because it is associated with a positive effect on milk yield and composition in carriers. A positive effect on milk yield was observed in the present study. However, in the long term, the negative effect on fertility could outweigh the increase in production efficiency in the individual herd.

The carrier frequency of BTA12 in males has more than halved in 7 years. This is probably due to increased genotyping and re-genotyping of older bulls with the newer SNP chip, and information on carrier status becoming available, enabling avoidance of carrier bulls and undesirable at-risk matings. The availability of data on carrier status has probably also facilitated continued use of carrier bulls that have high genetic gain in other desirable traits (Cole et al., 2016; Bengtsson et al., 2022) or have valuable pedigrees, rather than excluding these completely from selection (Bengtsson et al., 2022). Further, while the overall carrier frequency of BTA12 has been declining, Bengtsson et al. (2022) reported large variation in Swedish herds, where some herds completely lacked carriers while others had up to 36% carrier frequency among their females.

Beneficial effects on milk production traits in BTA12 carriers were observed in the present study, however, we observed no significant change in milk production in HH3 carriers. This is in contrast to Cole et al. (2016) who observed lower milk and protein yield in HH3 carrier cows. For HH3, lower haplotype frequencies have also been reported, ranging between 2.9 and 3.1% (Fritz et al., 2013; Cole et al., 2016).

This study defined pregnancy loss due to genetic defects as a potential trait to be included in genetic evaluations, this trait definition is scarce in literature. In line with our results, previous studies have observed a loss of fertility in at-risk matings of both BTA12 and HH3 carrier heifers and cows. Cole et al. (2016) found a decrease in daughter pregnancy rate, heifer CR, and cow CR in HH3 carrier cows compared with non-carriers. Likewise, Fritz et al. (2013) observed a negative effect of HH3 on calving rates in both heifers and cows in matings between carrier bulls and daughters of carrier bulls. Further, significantly lower non-return rates have been reported for HH3 and BTA12 at-risk matings in the Nordic dairy cattle population (Wu et al., 2019; Wu et al., 2020), and for HH3 at-risk matings in German Holstein (Segelke et al., 2016). This reduction in fertility in carriers in at-risk matings, despite higher milk yield, results in an economic loss because production efficiency is influenced by a delay of the next lactation owing to the increased number of inseminations necessary for a successful pregnancy, thus increasing the calving interval and the risk of premature culling due to infertility.

CONCLUSIONS

This study found that pregnancy loss was more likely in at-risk matings between carriers of genetic defects than in not-at-risk matings in both RDC and SH. The majority of pregnancy losses in BTA12 and HH3 at-risk matings were reported to the Swedish cow database within the first 3 mo post-AI. At-risk matings were also associated with a large negative effect on CR. Carrier females of BTA12 (RDC) had longer FLS, but higher milk yield, than non-carriers. Carrier status can therefore be used to avoid at-risk matings and prevent economic losses arising from extended service period and calving interval. These initial results indicate a way to reduce pregnancy loss due to genetic defects in the breeding program for improved fertility in Swedish dairy cattle.

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The aim of this thesis was to study new phenotypes of pregnancy loss, identify candidate genes for pregnancy loss traits, and estimate the effect of genetic defects on pregnancy maintenance in dairy cattle. Pregnancy loss was extensive and had low heritability. Several genes associated with embryonic and fetal development were found, and mating carriers of defects adversely affected fertility. Considering pregnancy loss in routine genetic and genomic evaluations of fertility could improve the ability to maintain pregnancy to full term.

Patricia Ask-Gullstrand received her postgraduate education at the Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences. She received her undergraduate degree from the Swedish University of Agricultural Sciences.

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