

Slutrapport

Nya mått i aveln för förbättrad fruktsamhet hos nordiska mjölkkor

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Del 1: Utförlig sammanfattning

Idag används information från semineringsdata i aveln för fruktsamhet. Dessa data är i stor utsträckning bestämda av skötsel faktorer och därför inte lika säkra som mer fysiologiska mått på kon själv. Tidigare studier har visat att hormonvärden, så kallade endokrina mått på fruktsamheten, har högre arvbarhet än klassiska fruktsamhetsmått.

Dräktighetsförluster är en viktig anledning till infertilitet hos mjölkkor. Trots att cirka 90 procent av insemineringarna resulterar i befruktning har tidigare studier visat att i genomsnitt bara var tredje insemination resulterar i en livsduglig kalv, vilket pekar på omfattande embryo- och fosterförluster under dräktigheten.

Manuell provtagning och analys har begränsat möjligheterna att använda dessa mått i stor skala. Men registreringar från automatiska system ökar inom mjölkproduktionen. Våra tidigare studier inom området har baserat sig på Holstein eftersom den är den klart viktigaste rasen i de länder vi samarbetar med. I denna svenska studie som är inom ett nordiskt fruktsamhetsprojekt har vi fokuserat på den röda rasen men samtidigt studerat Holstein.

Vi använde automatiskt insamlat och analyserat mjölkprogesteron från 14 Herd NavigatorTM besättningar i Sverige. Vi kombinerade dessa data med basuppgifter från Växa Sveriges kodatabas och samlade uppgifter om kornas härstamning och genotyper. Materialstorleken varierade beroende på studie men maximalt användes 330,071 progesteronobservationer från 5,238 laktationer hos 1,457 SRB-kor och 1847 Holstein.

Nya fruktsamhetsegenskaper baserade på automatiskt registrerade progesteronhalter i mjölk visade sig ha låg till medelhög arvbarhet och generellt högre arvbarhet än klassiska

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fruktsamhetsegenskaper. Genom att exempelvis mäta tiden från kalvning till första förhöjning av mjölkprogesteron (C-LA) kan vi få ett mått på när djuret är redo att semineras oberoende av djurägarens beslut. Vi fann att arvbarheten för detta mått var ca 25% hos både SRB och holstein. Arvbarheten för KFI, som används i den nordiska avelsvärderingen, är ca 5%. Alltså borde vi kunna välja ut avelsdjur på ett säkrare sätt med det nya måttet. Problemet är att detta bara mäts i ett fåtal besättningar, men om dessa djur också har DNA-information finns det möjlighet att skatta sk genomiska avelsvärden, även för djur som inte har hormonmätvärden.

SRB hade högre arvbarheter för de klassiska fruktsamhetsegenskaperna jämfört med holstein. Men för en för rad endokrina mått blev arvbarhetsskattningarna för SRB noll i vår första studie vilket tyder på att vårt SRB-material inte var tillräckligt stort. Det är därför viktigt att också data från automatiska registreringssystem görs tillgängligt för registrerings- och avelsorganisationer och blir inkluderade i kokontrollen. Registreringarna skulle kunna samlas in av husdjursföreningarna på samma sätt som de registreringar som idag används för avelsvärdering.

Vi identifierade en rad genomiska regioner som var associerade med de endokrina och klassiska fruktsamhetsmått som ger nya insikter om den genetiska arkitekturen för de studerade fruktsamhetsegenskaperna hos SRB och Holstein. En utmaning för framtida forskning är att fastställa varför olika regioner är relevanta för olika egenskaper och olika raser, och de praktiska konsekvenserna av detta för genomisk selektion.

SRB hade lägre dräktighetsförluster under sent embryonalt stadium, fosterstadium och totalt sett en bättre fertilitet i jämförelse med holstein. De totala dräktighetsförlusterna i form av embryo- och fosterdöd efter inseminering var cirka 54 procent för SRB och cirka 61 procent för Holstein. Arvbarheterna för dräktighetsförlusterna var låga och jämförbara med de klassiska fruktsamhetsegenskaperna. De skattades till 3 procent för tidig embryonal förlust, 6 procent för sen embryonal förlust och 2 procent för total dräktighetsförlust. Studien ger underlag för hur man bättre ska kunna ta hänsyn till dräktighetsförluster inom avelsarbetet och på så sätt minska deras förekomst.

Del 2: Improved breeding for fertility in Nordic dairy cows by using new recordings and novel traits

Introduction and background

Because most fertility traits have a low heritability, any improvement in phenotyping that can either lead to a higher heritability or to a higher genetic correlation with the true breeding goal trait, will lead to an improved genetic gain in fertility, all else equal.

Improved phenotyping is focusing on using new measures that enable us to come closer to the underlying physiological background of fertility, it is e.g., better to measure

progesterone in milk rather than the observed interval from calving to first insemination (CFI), since the latter is much influenced by management decisions.

We have previously studied genetic and genomic parameters for progesterone-based measures of fertility for the Holstein breed (Pettersson et al, 2007; Berry et al, 2012; Nyman et al, 2014; Tenghe et al, 2015). We studied Holstein because the countries we collaborated with mainly have this breed (e.g., NL, UK and Ireland). We have mainly focused on traits describing the ability to return to normal cyclicity after calving, being an alternative trait to days from calving to first insemination (CFI), which is used in the fertility index today. We also included other traits describing normal and atypical cycles (Nyman et al. 2014; Tenghe et al. 2015). In conclusion, we found that progesterone records can be used to define several heritable endocrine traits that can be used in genetic improvement of fertility. So far, we have not had large enough material to study the Nordic Red breeds. In the study by Tenghe et al. (2015), we used automated in-line records from Herd Navigator™ (HN) farms in the Netherlands. In the present project we studied the genetic variation in progesterone-based measures of fertility in the two most common breeds in Sweden and especially in the Swedish Red.

Our extensive data set with frequent in-line milk progesterone records also permitted us to study reproductive losses. Pregnancy losses are an important reason to infertility in dairy cattle. Our earlier studies based on manually sampled milk progesterone have shown that although a conception rate of 90% only on average one third of the inseminations result in a living calf (Nyman et al., 2018). Pregnancy loss have not yet been studied using *in-line* progesterone recordings. We assessed the extent of reproductive losses and estimated genetic parameters for these traits.

The overarching hypothesis is that we will be able to define improved fertility traits for use in an updated genetic evaluation for female fertility in the breeding program. All this will provide a significant and sustainable increase in overall herd reproductive performance without excessive management interventions, thereby allowing for increased longevity and better herd profitability.

Materials and Methods

The project start was delayed of several reasons. The most important reason was that our post-doc started first in September 2017 and project start was in the application planned to start in January 2016.

In September 2017 all 22 Swedish herds having the DeLaval Herd Navigator™ system was contacted to ask if they wanted to participate in our study. A collaboration agreement with Lattec which is responsible organisation for the data for HN-data. The first batch of data was obtained in October 2017. Progesterone profiles for all cows and lactations were created and basic data from Växa Sverige were added.

For the genomic studies we genotyped cows in nine of the 14 HN herds who had accepted to participate with their cows. To this we added information from already genotyped cows by Växa Sverige. In Mars 2019 we received all genotypes and also the second batch of data from Lattec and Växa Sverige.

Genetic parameters of endocrine and classical fertility traits

In this study, focusing also on the Swedish Red (SR) breed, genetic parameters were estimated for classical and endocrine fertility traits, the latter based on in-line milk progesterone records obtained for 14 Swedish herds using DeLaval Herd Navigator (DeLaval International, Tumba, Sweden). A total of 210,403 observations from 3,437 lactations of 1,107 SR and 1,538 Holstein cows were used. Mixed linear animal models were used for estimation of genetic parameters.

Genomic regions of endocrine and classical fertility traits

The second study aimed to identify candidate genomic regions associated with classical and endocrine fertility traits in Swedish Red (SR) and Holstein cows. A total of 210,403 observations from 3,437 lactations of 1,107 SR and 1,538 Holstein cows were included in the study. Automatically collected and analyzed in-line milk progesterone (P4) samples from 14 herds having the DeLaval Herd Navigator™ were used. Endocrine traits studied were days from calving to commencement of luteal activity (C-LA), first luteal phase length (LPL), length of inter-luteal interval, length of first inter-ovulatory interval (IOI), luteal activity during the first 60 DIM, and proportion of samples with luteal activity during the first 60 DIM. In addition, classical fertility traits based on insemination data were investigated. A total of 180 SR and 312 Holstein cows were genotyped with a low density SNP chip panel and imputed to 50K SNP chip. We employed a single step genome-wide association approach to explore candidate genomic regions associated with the fertility traits.

Genetic parameters for reproductive losses

The aim of this study was to assess pregnancy losses and estimate associated genetic parameters for these traits in dairy cattle using in-line milk progesterone records collected by DeLaval's Herd Navigator™ in 14 Swedish herds. A total of 325,782 progesterone samples were connected to 9,517 inseminations (AI) from 4,935 lactations of 1,391 Swedish Red (SR) and 1,818 Holstein (H) cows. Pregnancy loss traits were defined as early embryonic loss (1-24 days after AI), late embryonic loss (25-60 days after AI), fetal loss (61 days after AI to calving) and total pregnancy loss (from day 1 after AI to 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, and calving interval. The traits were analyzed with a mixed linear model in SAS. The genetic analysis was run using the software program DMU.

Results and discussion

Genetic parameters of endocrine and classical fertility traits

Least squares means analysis showed that Holstein cows had a 2.5-d-shorter interval from calving to commencement of luteal activity (C-LA) and longer length of first inter-ovulatory interval (IOI) than SR cows. The highest mean interval for C-LA, IOI, and first luteal phase length (LPL) was observed in the fourth parity. The incidence of short (24 d) IOI was 29.3, 40.7, and 30%, respectively. Genetic analysis indicated moderate heritability (h^2) for C-LA ($h^2 = 0.24$), luteal activity during the first 60 d in milk (LA60, $h^2 = 0.15$), proportion of samples with luteal activity (PLA, $h^2 = 0.13$), and calving to first heat (CFH, $h^2 = 0.18$), and low heritability estimates for LPL ($h^2 = 0.08$) and IOI ($h^2 = 0.03$) in the combined data set for both breeds. Similar heritability estimates were

obtained for each breed separately except for IOI and LPL in SR cows, for which heritability was estimated to be zero. Swedish Red cows had 0.01 to 0.06 higher heritability estimates for C-LA, LA60, and PLA than did Holstein cows. Calving interval had moderate heritability among the classical traits for Holstein and the combined data set, but h^2 was zero for SR. Commencement of luteal activity had a strong genetic correlation with LA60 (mean \pm SE; -0.88 ± 0.06), PLA (-0.72 ± 0.11), and CFH (0.90 ± 0.04). Similarly, CFH had a strong genetic correlation with IOI (0.98 ± 0.20). Number of inseminations per series showed a weak genetic correlation with all endocrine traits except IOI. Overall, endocrine traits had higher heritability estimates than classical traits in both breeds, and may have a better potential to explain the actual reproductive status of dairy cows than classical traits. This might favor inclusion of some endocrine fertility traits—especially those related to commencement of luteal activity—as selection criteria and breeding goal traits if recording becomes more common in herds. Further studies on genetic and genomic evaluations for endocrine fertility traits may help to provide firm conclusions. A prerequisite is that the data from automatic devices be made available to recording and breeding organizations in the future and included in a central database.

Genomic regions of endocrine and classical fertility traits

The second study aimed to identify candidate genomic regions associated with classical and endocrine fertility. The results revealed 990 and 415 SNPs above the threshold ($-\log(p\text{-value}) \geq 4$) for SR and Holstein cows, respectively. The breeds shared only eight SNPs significantly associated with fertility traits. Annotation analysis revealed 281 SNPs located in 241 genes. Functional enrichment analysis using DAVID tools reduced the number to 80 genes, which were mediated in various biological processes and KEGG pathways in multiple functions, including folliculogenesis, embryogenesis, uterine growth and development, immune response, and ovarian cysts. Of the 80 genes, 67 were associated with fertility traits in SR cows and 13 in Holstein. Most genes were associated with LPL and IOI in SR cows, but in Holstein the only association with an endocrine trait was with C-LA. Twenty QTL regions that embedded 40 genes were associated with fertility traits in both breeds. All the QTLs detected, except at BTA2 and BTA19 are novel QTL regions that were not reported previously. These novel QTL regions embedded the candidate genes that include ARHGAP20, PHLDB1, CACNA1D, ATG7, CCNE1, GPI, CDH13, ECT2, PLD1, FBN2, KIF3A, FGF12, KCNMB2, GJA1, MAN1A1, KCNN2, SMAD6, MAPK8IP1, PHF21A, LPXN, MMRN1, KCNIP4, NID2, PCDHGA8, GRIA1, PCDHGB4, PHLDB2, STXBP5L, PPP3CA, PTPRR, SRGAP1, SNX27, SPTA1, S100A10, TBC1D20 and ITCH. The candidate regions may help to improve genetic progress in female fertility if used in selection decisions. A challenge for future research is to determine why different regions seem relevant for different traits and breeds, and the practical implications for genomic selection.

Genetic parameters for reproductive losses

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 $6.1 \pm 1.2\%$ compared with $13.3 \pm 1.1\%$, fetal loss of $7.0 \pm 1.2\%$ compared with $12.3 \pm 1.2\%$, and total pregnancy loss of $54.4 \pm 1.4\%$ compared with $60.6 \pm 1.4\%$. 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. 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). 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.

Conclusions

Most of the endocrine traits studied, including C-LA, LA60, PLA, and CFH, were found to be moderately heritable and strongly correlated with each other. Classical traits had lower heritability estimates and weak genetic correlations with endocrine traits. Our results confirm previous findings that endocrine traits might have the potential to better describe the actual reproductive status of dairy cows than classical traits, and suggests that some endocrine fertility traits, especially those related to commencement of luteal activity, may increase the rate of genetic improvement if used as additional selection criteria and in the breeding goal. Swedish Red cows appeared superior to Holstein in some fertility traits, but showed zero heritability for LPL, IOI, CLAFS, and CFH, which warrants further studies on a larger sample of Red cattle. It is important that endocrine progesterone data from automatic devices be made available to recording and breeding organizations in the future and included in a central database.

Our study of genomic regions was yet another study to confirm that fertility is a complex trait influenced by many small polygenic effects and by environmental factors. In the present study, we identified a wide range of genomic regions associated with endocrine and classical fertility traits. The LPL, IOI, CI, and CLI traits were found to be influenced by many genes. Moreover, based on the significantly differentiated SNPs, we identified genes with pleiotropic effects that control and regulate two or more endocrine and classical fertility traits. The results obtained suggest that the endocrine fertility traits LPL and IOI may have the potential to improve fertility in dairy cows, particularly SR cows. A total of 20 QTLs, of which 18 of them are novel, were detected. Potential candidate genes to be further studied in SR and Holstein cows are EFNA5, GNAQ, NID2, SLC25A17, CREB5, ATF2, CACNAD1, CREB5, and KIF3A. Our findings offer new insights into the genetic basis of endocrine and classical fertility in SR and Holstein dairy breeds. A challenge for future research is to determine why different regions seem relevant for different traits and breeds, and the practical implications of this for genomic selection.

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.

Relevance for the industry and recommendations

Overall, endocrine traits had higher heritability estimates than classical traits in both breeds, and may have a better potential to explain the actual reproductive status of dairy cows than classical traits. This might favor inclusion of some endocrine fertility traits - especially those related to commencement of luteal activity - as selection criteria and breeding goal traits if recording becomes more common in herds.

We identified a wide range of genomic regions associated with endocrine and classical fertility traits. Our study offers new insights into the genetic basis of endocrine and classical fertility in SR and Holstein dairy breeds. A challenge for future research is to determine why different regions seem relevant for different traits and breeds, and the practical implications of this for genomic selection.

The reproductive losses are extensive. A more direct measure in the breeding index to maintain the pregnancy to full term would enable a more effective breeding reducing the pregnancy losses. A prerequisite is that the data from automatic devices be made available to recording and breeding organizations in the future and included in a central database.

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Del 3: Resultatförmedling

Vetenskapliga publiceringar	<p>Ask-Gullstrand P, E. Strandberg, R. Båge, JM. Christensen, B. Berglund. 2021. Genetic parameters for pregnancy losses estimated from in-line milk progesterone profiles in Swedish dairy breeds. <i>Journal of Dairy Science</i> 104:3231-3239, https://doi.org/10.3168/jds.2020-9385</p>
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