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# Genomic mapping of calving ease, gestation length and stillbirth rate in Fleckvieh cattle

Master thesis

of

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# Content

# Introduction

There are many different factors influencing the success in milk production systems that farmers want to get analyzed and take under control. One of the most important topics is the calving process, especially in dairy cattle, as it is a prerequisite for the lactation (Sattlecker 2014).

“Breeding is thinking in generations” (Willam, 2017). This statement means that breeding decisions from today show their results in distant future because of the long generation intervals for cattle. Those decisions should be oriented on future economic conditions. Ideally one would like to produce a high amount of milk with high protein and fat content from healthy cows with good longevity. That means not only milk yield, but also the fitness needs to be considered. Also, the overall cost-effectiveness in dairy farming needs to be considered. Thereby the cost lowering functional traits play an important role. Easy calving and calf vitality are of special importance. Difficult calvings can cause direct costs in form of veterinary services and indirect costs like worse fertility, lower milk yield and a shorter productive life. Furthermore, calving difficulties can lead to the loss of the cow or the calf what means a big economic loss that leads to a reduced replacement possibility (Fürst and Fürst-Waltl 2006).

Calving ease and stillbirth rate are influenced by the dam and the sire of an animal (Luo et al. 1999).

The breeding value estimation for calving ease in Germany and Austria is routinely assessed since 1995 and for stillbirth rate since 1998. Since 2016, the breeding value estimation for the trait calving ease has been calculated for Fleckvieh and Brown Swiss with the helper trait gestation length. Stillbirth rate has been estimated also since 2016 with the trait rearing losses (Fürst 2017).

All considered traits show very low heritabilities, except direct gestation length (Sattlecker 2014).

The importance of fertility is of prime importance, especially if the calving intervals get so high, as the regulated restocking of the female offspring gets problematic. Although fertility traits are lowly heritable, a selection on negatively correlated traits like milk yield, can show massive negative results in fertility (Fürst 2017).

These negative genetic correlations between production traits and functional traits have made it essential for functional traits to be included in national breeding indices to stop undesirable genetic trends on correlated traits (Eaglen et al. 2013).

The aim of this work is to explore regions of the Fleckvieh genome influencing calving ease, gestation length and stillbirth rate. Genome-wide association studies based on dense molecular markers and derived haplotypes will be used to identify potential causative genes.

# Background

## Direct and Maternal effects

Calving in cattle is affected by the calf morphology and by the dam characteristics. It is described by two different traits. The maternal calving ease is the ability to generate dams with good physiological predisposition to calving, and the direct calving ease is the ability to generate calves that are easily born (Bongiorni et al. 2012).

Calving in general is influenced by the two factors, the mother and the calf. On this account it is essential for selection to consider both effects, the direct and the maternal effect.

In general terms, the maternal effects include parameters, i.e. biological properties of the biological mother, which influence the performance of the offspring, for example adequate supply in the womb or uterine size. Furthermore, postnatal effects like mothering abilities, i.e. how well the mother takes care/protects the offspring, and milk yield are also included in maternal effects (Röhe, Krieter, and Preisinger 2000).

Regarding the offspring, the maternal effects must be seen as environmental effects whereas regarding the mother, it is a mix of environmental and genetic effects (Röhe, Krieter, and Preisinger 2000). However, it is known, that the variance of the maternal effect is not only influenced by the environment. The resulting phenotypic expression of calving ease is then the combination of the mother and the offspring, so the direct and maternal effects (Sattlecker 2014).

Maternal effects substantially influence the breeding progress for all the mentioned traits, even if the genetic effect is very low. As a reason behind this tendency is the high genetic correlation between the estimated direct and maternal genetic effects. This is due to the close inheritance of direct and maternal genetic effects via the same genetic pathways in which the maternal genetic effects are expressed one generation later compared to the direct effects (Röhe, Krieter, and Preisinger 2000).

The phenotype of the animal X was shown by (Willham 1963) as follows:

$$\mathbf{P_x = G_{ox} + E_{ox} + G_{mw} + E_{mw}}$$

$P_X$ represents the phenotypic value of individual X. $P_x$ is the sum of two components; one being influenced by the genotypic value of X and the other by the genotypic value of an individual related to X say W. Denote the two components of the character as *o* and *m* symbolizing the offspring component and the maternal effect.

Where $G_{ox}$ and $E_{ox}$ are the genotypic and environmental values of individual X for component *o*, and the terms $G_{mw}$ and $E_{mw}$ are the genotypic and environmental values of individual W for component *m* as expressed in $P_x$. The two terms are defined in terms of the variance of $P_x$ that is causally attributable to them.

In Figure 1 there is illustrated such a model in terms of a path coefficient diagram when W is the dam of X and the (G')'s denote additive genetic values rather than genotypic values.

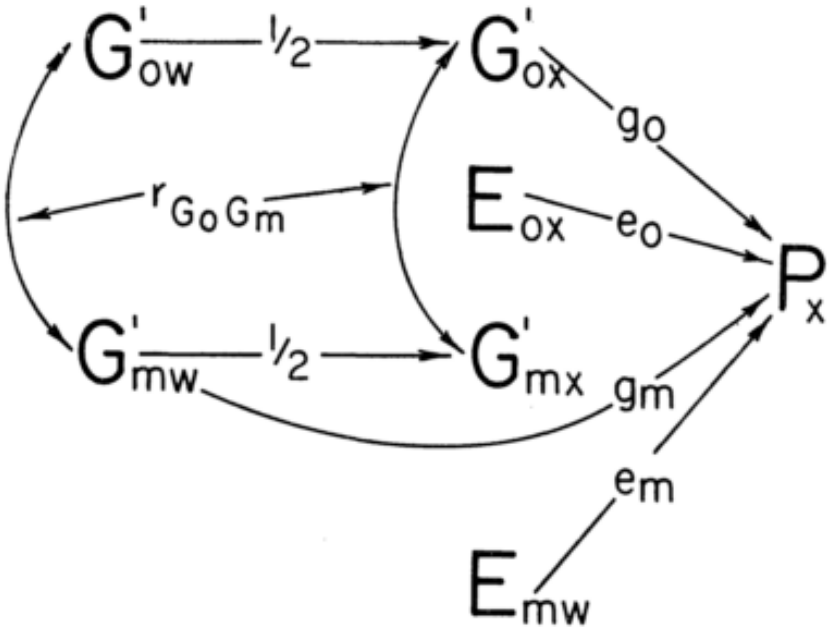


*Figure 1: Path coefficient diagram which describes a phenotypic value influenced by a maternal effect* (Willham 1963)

As the above-mentioned equation already shows, the maternal effect is a characteristic of the mother and is shown in the phenotype of the offspring. The direct effect is shown by birth of the offspring. For female offspring the maternal effect is shown, as soon as it gives birth (Sattlecker 2014).

Furthermore, the cow influences the phenotype of her offspring in two ways. The first way is the genetic performance, so the additive genetic maternal effect and the second way is the transfer of the half of the mothers genes to the offspring what means the half of the additive maternal effect and the additive direct effect when the offspring is female (Sattlecker 2014).

Genome-wide association studies have rapidly spread across the globe over the last few years becoming the de facto approach to identify candidate regions associated with complex diseases. GWAS and genome analysis are powerful tools to provide a handle on genetic variation in a wide range of traits important for human health, agriculture, conservation, and the evolutionary history of life (Gondro, Werf, and Hayes 2013).

## Description of the traits

### Calving ease

The trait calving ease is separated in the following five distinct categories which describe the calving process:

1. **Easy calving**: The cow needs no help to have a successful calving.
2. **Normal calving**: One person is needed for a successful calving.
3. **Difficult calving**: More than one person is needed, or mechanical help is needed.
4. **Caesarean**: A caesarean section is recommended.
5. **Embryotomy**: The calf must be cut into sections, because a normal calving is not possible anymore.

For the breeding value estimation Caesarean and Embryotomy are put together. Depending on the frequency of the individual classes, each of these classes is assigned the average value of a normally distributed random variable (Fürst 2017). This procedure is due to the low frequency of the individual classes.

Table 1 shows the percentage distribution of the above categories for the Austrian cattle populations.

*Table 1: Percentage distribution of calving categories of Austrian cattle populations (Fürst 2017). Fleckvieh (FV), Braunvieh (BV), Holstein Friesian (HF), Pinzgauer (PI), Grauvieh (GV)*

| | FV | BV | HF | PI | GR | all |
|---|---|---|---|---|---|---|
| **1-E*asy calving*** | 53.9 | 57.9 | 56.0 | 39.9 | 53.1 | 54.2 |
| **2-Normal calving** | 43.7 | 39.4 | 41.7 | 57.3 | 43.5 | 43.3 |
| **3-Difficult calving** | 2.4 | 2.6 | 2.2 | 2.5 | 3.4 | 2.4 |
| **4-Caesarean** | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 |
| **5-Embryotomy** | 0.01 | 0.02 | 0.04 | 0.08 | 0.00 | 0.01 |
| **Dead and died within 48 hours** | 3.4 | 4.5 | 6.7 | 4.1 | 2.5 | 3.6 |

According to Fürst (2017), calving ease is influenced by the lactation number, which means calving in later lactations is easier, compared to the first lactation. Also, the sex of the calf has a high impact on calving ease. Male calves lead to more difficult calvings then female calves. For first calving this difference is even higher than for later calvings. The calendar month of calving has only a marginal impact on calving ease.

Calving age has also an impact on calving ease. At the age of around five to six years (third lactation) calving ease is optimal. Very young cows (first or second calving) and very old cows (from the fifth claving on) show calving difficulties more often. These statistics are further detailed in figures 2 and 3.

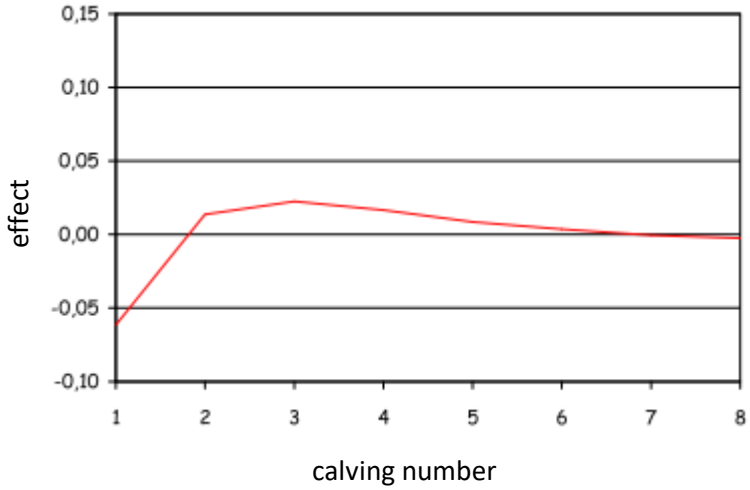


*Figure 2: Effect of the lactation number on calving ease for Fleckvieh (Fürst, 2017).*

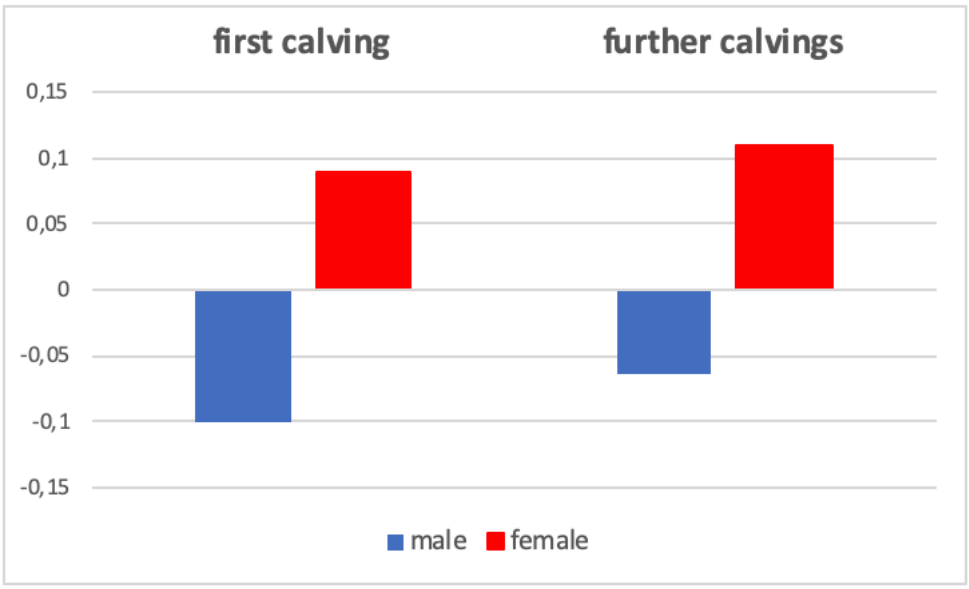


*Figure 3: Effect of the sex of the calve on calving ease for first calving and further calvings (Fürst 2017).*

## Gestation length

The average gestation length in cattle is between 275 and 295 days, however there are significant differences between and within different breeds (Kraßnitzer 2009).

Sattlecker (2014) determined an average gestation length in Fleckvieh cattle of 286.7 days with a standard deviation of 4.99 days (figure 4).

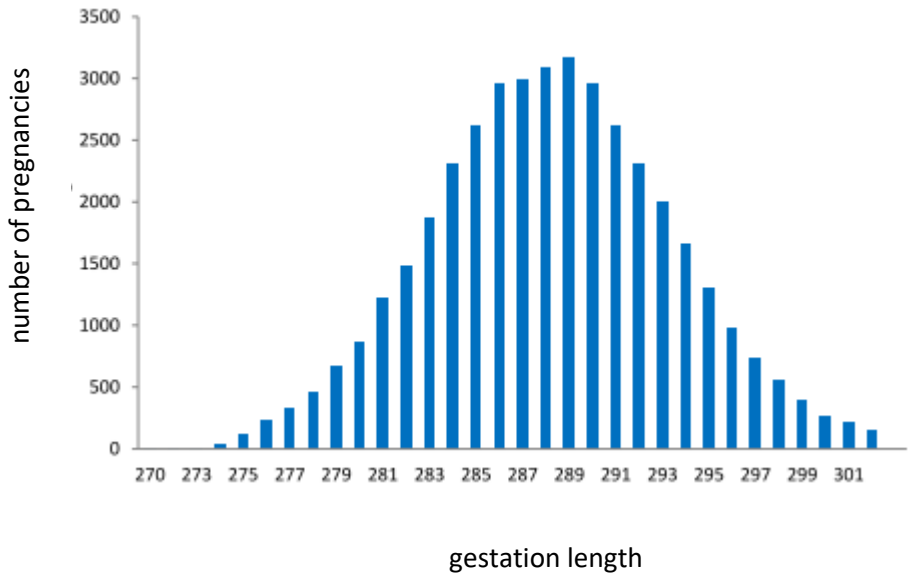


*Figure 4: Distribution of gestation length in Fleckvieh (Sattlecker, 2014)*

Furthermore, also the sex of the calf can have an influence on the length of gestation. Hansen et al. (2004) determined on average, a longer gestation length of 1.1 days for male calves for the Holstein breed in Denmark.

In an Austrian study, a longer gestation length of 1 to 2 days for male calves was determined for Holsteins, for the Fleckvieh breed an average gestation length of plus 0.6 days was found (Kraßnitzer 2009).

Cows which calve for the first time show a shorter gestation than cows with several calvings. Such an association between calving age and gestation length was reported by Kraßnitzer (2009), also shown in the figure 5 (Sattlecker 2014).

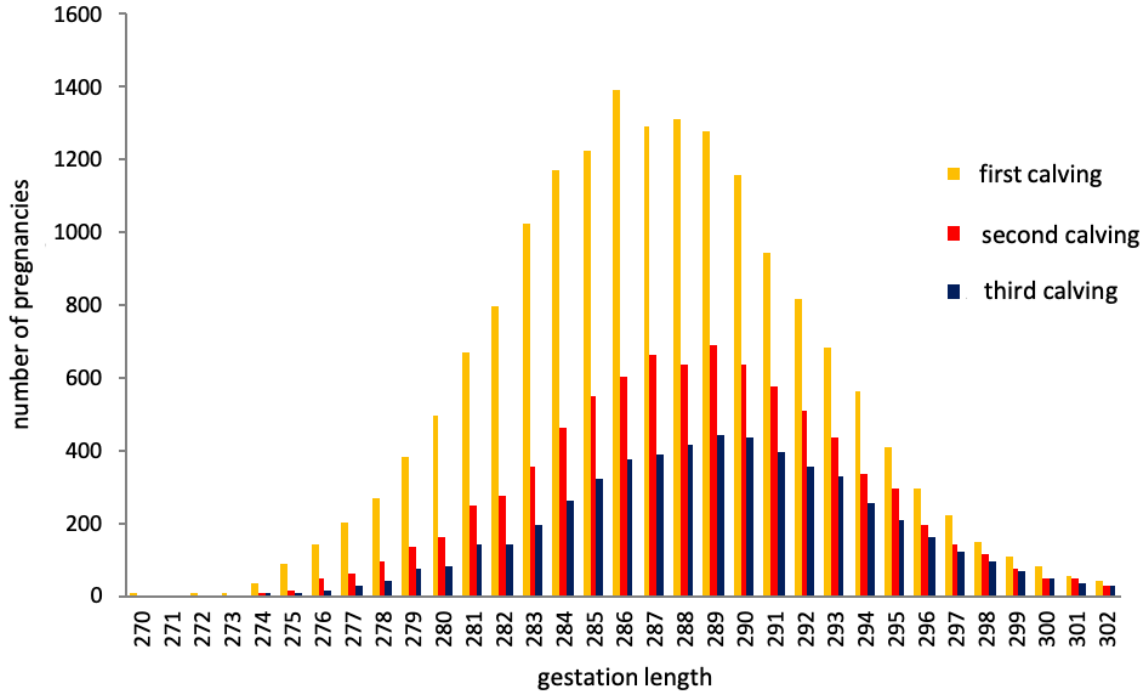


*Figure 5: Distribution of gestation length at first calvings (yellow), second calvings (red) and third calvings (blue) (Sattlecker 2014).*

Furthermore, Kraßnitzer (2009) has found that the calving month has an influence on gestation length at Austrian Braunvieh cattle. This result is also analyzed in the work of Sattlecker (2014) where in the winter season the gestation was longer compared to the summer.

## Stillbirth rate

The stillbirth rate is used as a yes / no characteristic in the breeding value estimation, where else the deaths within 48 hours after birth are also included, as from a veterinary point of view, an infection after birth cannot lead to the death of the calf so quickly. It is known that a longer gestation period leads to larger calves and thus to more birth problems. For every 10 days of prolonged gestation, the heavy-weight-birthrate increases by about 2-3% ( Fürst 2017).

Steinbock et al. (2003) found for Swedish Holstein cattle that around 10% of the calves are born dead or die on the first day at first calving. A considerable difference exists between heifers and the multiparous cows, as the stillbirth rate is around 4% for older cows. Stillbirths and calving difficulties are influenced by both direct and maternal genetic effects, i.e. the genetic constitution of both the sire and the maternal grandsire has an impact on the birth process and its outcome. In the Holstein population, a large variation in stillbirth rates among sires and as maternal grandsires, ranging between 2 and 25%, has been observed in large progeny groups.

The incidences were much higher in first calving for both of the traits. The age of the heifer at calving in connection with sex of the calf has a considerable impact on calving performance. For bull calves there is a considerable difference in stillbirth rate between young and old dams. However, the older heifers with 30 months of age and above, do not have as difficult calvings as the younger ones (Steinbock, Näsholm, et al. 2003).

*Table 2: Incidences in percent of stillbirth, calving difficulty and proportion of dead calves for Swedish Holstein Cattle at normal calvings and numbers of sires and maternal grandsires of calves (Steinbock, Näsholm, et al. 2003).*

| | | Incidence | | | No. of bulls being | | |
|---|---|---|---|---|---|---|---|
| Parity | No. of records | Stillbirths | Calving difficulties | Dead calves at normal calvings | sires | mgs | Total no. of bulls |
| 1 | 411409 | 7.1 | 8.3 | 53 | 2362 | 2468 | 2955 |
| 2 | 281193 | 2.7 | 4.5 | 33 | 806 | 1681 | 1905 |

## Genomic architecture of calving traits

Traits that are controlled by a single gene, such as flower color, have been important in elucidating the mechanisms of heredity, yet most traits that are important in agriculture, medicine and evolution are complex or quantitative traits. These traits include susceptibility to many diseases, such as diabetes in humans or agriculturally important traits (Goddard and Hayes 2009).

Calving traits are complex since they are influenced by the sire-effect through the size of the calf as well as dam effects consisting mainly of the pelvic dimensions (Pausch et al. 2011).

The very low heritabilities of fitness-traits in general make it difficult to achieve breeding progress. Therefore, research on these traits has increased.

Sahana et al. (2011) explored the calving traits for Danish and Swedish Holstein cattle. They identified 22 QTL regions on 19 chromosomes. Nine of the QTL were related to direct calving effects, whereas 5 were related to maternal effects, and 8 affected both direct and maternal calving traits. The QTL for the traits birth index, stillbirth, calving ease, calf survival and calving index were found on 6 chromosomes: BTA4, 6, 12, 18, 20, and 25.

Calving ease has been researched by Pausch et al. (2011). Two associated regions on bovine chromosomes for direct calving ease have been found in this study on BTA 14 and 21. The associated region on BTA 14 is conserved in human chromosome, which has been shown to be associated with adult height. As adult height is positively correlated with fetal size and this is an important determinant of the birthing process, they considered PLAG1, MOS, CHCHD7, RDHE2 (alias SDR16C5), RPS20, LYN, TGS1, PENK as positional and functional candidate genes for the direct calving ease QTL in cattle.

The study of Kipp et al. (2015) reports the identification of a new haplotype associated with calf survival in the Holstein population. Several calves from specific mating showed unspecific symptoms like chronic diarrhea and insufficient development. Affected animals died within the first months of life in spite of symptomatic treatment. A genome-wide case-control-study determined a causal region at BTA 11. Following, homozygosity mapping identified a haplotype affecting calf mortality in the homozygous state.

Goddard and Hayes (2009) summarized the genomic architecture of complex traits as follows:

One of the surprising results in human genome wide association studies has been the small size of the observed effects which implies that many SNPs have effects on complex traits. This conclusion can be supported by the results in domestic animals.

A second surprising result from human and domestic animal genome wide association studies was that the SNPs with validated effects only explain a small proportion of the genetic variance, leading to a question:

Where are the missing genes?

Most QTLs might explain such a small proportion of the variance that even large human genome wide association studies have not the power to find them. This is consistent with the findings that combining data sets from different genome wide association studies leads to the discovery of additional genes.

Some QTLs of larger effect have been discovered, such as the „*DGAT1* polymorphism “, that explains about 40% of the genetic variation in fat content in the milk of Holstein cattle. Therefore, the distribution of effects of QTLs must have many small effects but a big tail with larger, but rare effects. An exponential distribution was suggested. It is likely that a mutation with a large effect on a complex trait will also be under larger selection pressure.

It is clear that multi-allelic series exist at major genes, such as for the myostatin gene in cattle for which many double muscling mutations exist, as well as mutations of lesser effect. However, these allelic series may only be able to be discovered when the mutations are positively selected by humans owing to their novelty or practical value. Therefore, if nature selects against most of the QTL mutations, it may be rare for a QTL to have more than two alleles segregating, especially within a breed whose effective population size is small.

# Material and Methods

## Data

Data from the Austrian and German Fleckvieh genotype pool in the form of SNPs (Single nucleotide polymorphism) was provided by ZuchtData EDV Dienstleistungen GmbH. Table 3 lists the number of genotyped animals with each SNP chip type used in this study. The data was available in three different densities like shown below.

*Table 3: Overview of the provided data for density, number of animals and number of SNPs.*

| | **Animals** | **SNPs** |
|---|---|---|
| **Illumina BovineSNP50 v1 BeadChip** | 5340 | 53468 |
| **Illumina BovineSNP50 v2 BeadChip** | 2139 | 54609 |
| **Illumina BovineHD BeadChip** | 192 | 777962 |

### Quality control

After merging the three densities into a joint set and adding the phenotypes in form of estimated breeding values and their reliabilities which were provided by the ZuchtData GmbH, the quality control was done using the PLINK software (Purcell et al. 2007).

With PLINK, very large data sets comprising hundreds of thousands of markers genotyped for thousands of individuals can be rapidly manipulated and analyzed in their entirety.

In a standard quality control procedure only the SNPs residing on autosomes were included. Furthermore, with the command --mind 0.1 animals with more than 10% missing genotypes have been filtered out, as well as all variants with missing call rates exceeding 10% (--geno 0.1). With the command --maf 0.01 there also have been filtered out all variants with minor allele frequency below the threshold of 1%. Also, all variants with Hardy-Weinberg equilibrium exact test p-value below 0.00001 were filtered out.

After the quality control 7416 animals with 42041 SNPs remained for the analysis.

## Genome wide association study using SNPs

The available genotype and phenotype data were analyzed via genome wide association study (GWAS). Two versions of this popular technique were used, the first of which was a SNP wise GWAS using the GEMMA software (Zhou 2016).

Gemma can fit a univariate linear mixed model in the following form (Zhou 2016):

$$\mathbf{y} = \mathbf{W}\boldsymbol{\alpha} + \mathbf{x}\beta + \mathbf{u} + \boldsymbol{\epsilon}; \quad \mathbf{u} \sim \mathrm{MVN}_n(0, \lambda\tau^{-1}\mathbf{K}), \quad \boldsymbol{\epsilon} \sim \mathrm{MVN}_n(0, \tau^{-1}\mathbf{I}_n)$$

where y is an n-vector of quantitative traits (or binary disease labels) for n individuals; W = ($w_1$ , · · · , $w_c$ ) is an n × c matrix of covariates (fixed effects) including a column of 1s; α is a c-vector of the corresponding coefficients including the intercept; x is an n-vector of marker genotypes; β is the effect size of the marker; u is an n-vector of random effects; ε is an n-vector of errors; τ−1 is the variance of the residual errors; λ is the ratio between the two variance components; K is a known n × n relatedness matrix and In is an n × n identity matrix. MVNn denotes the n-dimensional multivariate normal distribution.

In this study, there were no fixed effects in the model.

GEMMA tests the alternative hypothesis H1 : β ≠ 0 against the null hypothesis H0 : β = 0 for each SNP in turn, using one of the three commonly used test statistics (Wald, likelihood ratio or score). GEMMA obtains either the maximum likelihood estimate (MLE) or the restricted maximum likelihood estimate (REML) of λ and β, and outputs the corresponding p value.

In addition, GEMMA estimates the PVE by typed genotypes or “chip heritability”.

GEMMA is the software that is implementing the Genome-wide Efficient Mixed Model Association algorithm for a standard linear mixed model and some of its close relatives for genome-wide association studies (Zhou 2016).

The Bonferroni threshold was used as a significance limit for base p value of 0.05, computed as:

$$\alpha' = \alpha / k$$

Where α' is the updated set of p-values, α is the initial threshold of the p-value (0.05) and k is the number of performed tests, equal to the number of SNPs remaining after quality control. For adequate visualization the qqman R package was used to visualize the results with Manhattan and QQ plots (Turner 2017).

## Genome wide association study using haplotypes

The halotype based analysis was used as the second approach for our GWAS study. For this the software SHAPEIT (Delaneau et al. 2013) and the software GHap (Utsunomiya et al. 2016) have been used. SHAPEIT has been used for phasing the data. The precise sequence or phase of alleles in each homologous copy of a chromosome is not directly observed by genotyping and must be inferred by statistical methods. Once this is estimated, the haplotypes can be used to infer for example ancestry, detect selection or detect causal variants (Delaneau et al. 2013). The phased haplotypes from SHAPEIT were further analysed by GHap used for the handling of haplotypes and the GWAS analysis. The quality cotrol has been done in the same way and with the same parameters as for the SNP-GWAS.

The chromosomes were divided using a sliding window approach, with non-overlapping segments of 5 SNPs. These segments were then later analysed in a genome wide association study with GHap.

The linear mixed model procedure in **GHap** is implemented under a Bayesian framework.

The model fitted by the ghap.blmm() function is:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{Zp} + \mathbf{e}$$

where **X** is a n x p matrix relating the n records in **y** to the p fixed effects in **b**, **Z** is a n x m incidence matrix relating the n records in **y** to the m random effects in **u** (with variance-covariance **K**σu2) and **p** (with variance-covariance **I**σp2), and **e** is the vector of residuals (with variance-covariance **W**σe2). Here we assume residuals are independent, such that **W** = diag(wi). Also, if we let **K** be the HapAllele relationship matrix, then **u** becomes the HapAllele-based polygenic effects/breeding values, and σu2 becomes the variance due to HapAlleles. Importantly, any arbitrary **K** matrix is admitted, such that one may also use the ghap.blmm to fit models combining pedigree and haplotype relationships (e.g., single-step GWAS analysis). No permanent environmental effects were included in the analysis.

# Results and Discussion SNP GWAS

## Calving ease

### Direct calving ease

The most significant finding in the SNP-GWAS study was on BTA 14 at 24.1 Mb.

The significant interval of about 20.3 Mb to 25.4 Mb on bovine BTA 14 is conserved in human chromosome 8q21, which has been shown to be associated with adult height, while adult height is positively correlated with fetal size and this is an important factor during the birth. The region contains growth and body size related genes such as PLAG1, TGS1, RPS20, LYN and SOX17 as shown in Pausch et al. (2011) and Mészáros et al.(2013). This Interval is also partly shown in this study.

The second obvious peak was on BTA 21 from 2.1 to 2.4 Mb. This regions correspond to the Prader-Willi-Syndrome and the Angelman-Syndrome Pausch et al. (2011), Mészáros et al. (2013). This syndrome can manifest as genital hypoplasia, incomplete pubertal development, and, in most, infertility (Cassidy et al. 2008).

With SNP ARS-BFGL-NGS-104268 and BTB-01417924 on the positions 25.4Mb and 24.2 Mb on BTA 14, there are 2 further highly significant SNPs very close to each other. In this region there are several genes located, such as FAM110B, UBXN2B, or TOX associated with puberty related traits (Seabury et al. 2017).

These signals also correspond to Pausch et al. (2011) and Mészáros et al. (2013).

Three interesting genes are located in this significant region: LAP3, at 38.0Mb on BTA 6 which is encoding for a leucine aminopeptidase, responsible for the oxytocin hydrolysis; NCAPG, encoding for a non-SMC condensing I complex, in cattle associated with fetal growth and carcass size; LCORL, encoding for a ligand dependent nuclear receptor co-repressor- like, affecting height in humans and also controlling stature in cattle, and is highly correlated to NCAPG (Bongiorni et al. 2012).

Bongiorni et al. (2012) also considered LAP3, NCAPG and LCORL as potential positional and functional candidate genes on BTA 6 for direct calving ease.

A further significant region was detected on BTA 18, with the ARS-BFGL-NGS-110287 SNP at the position 13.0 Mb. In this region the Gene FBXO31 is located, with no apparent association to reproductive traits. Also Müller et al. (2017) detected a QTL on BTA 18 for calving ease but on another region compared to our study.

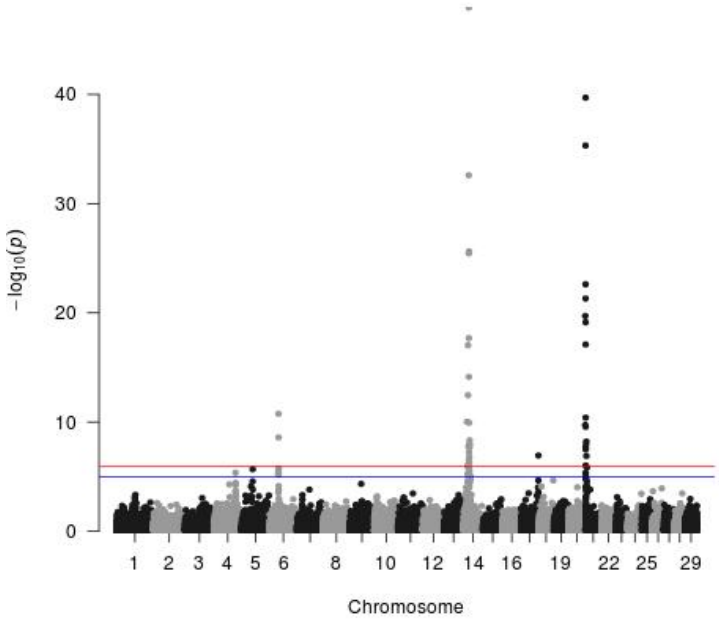


*Figure 7: Manhattan plot of direct calving ease.*

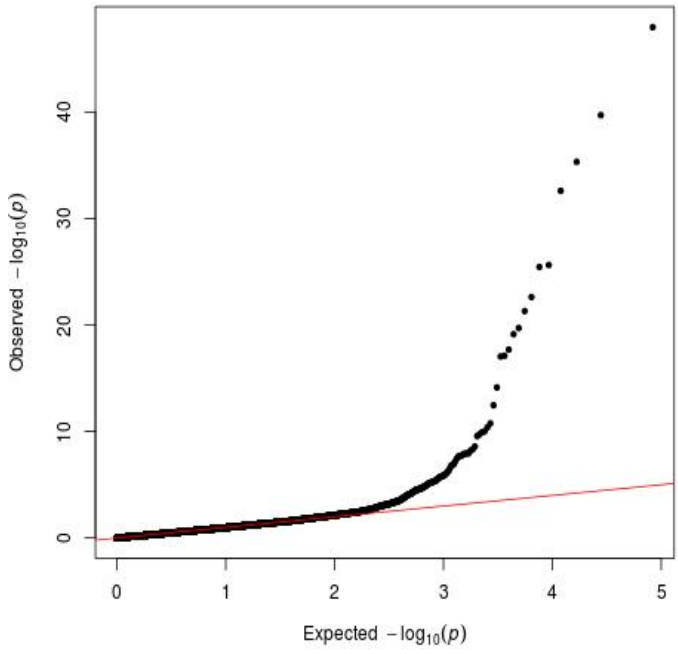


*Figure 6: QQPlot of direct calving ease.*

In the figures 6 and 7 the Manhattan plot (figure 6) and the QQ-plot (figure 7) of direct calving ease are shown.

The QQ-plot also indicates that most of the SNPs are exactly on the observed vs expected line, with only the significant ones deviating from it. This visualization confirms that the population structure correction was done correctly.

*Table 4: List of significant SNPs for direct calving ease.*

| BTA | SNP name | position in Mb | number of missing values | p value |
|---|---|---|---|---|
| 14 | BTA-91250-no-rs | 24.15 | 13 | 1.10E-42 |
| 21 | ARS-BFGL-NGS-108925 | 2.46 | 0 | 1.99E-34 |
| 21 | ARS-BFGL-NGS-53975 | 2.15 | 0 | 4.82E-30 |
| 14 | ARS-BFGL-NGS-104268 | 24.06 | 19 | 2.57E-27 |
| 14 | Hapmap46735-BTA-86653 | 25.40 | 19 | 2.35E-20 |
| 14 | BTB-01417924 | 24.18 | 2 | 3.61E-20 |
| 21 | ARS-BFGL-NGS-114372 | 2.38 | 49 | 2.41E-17 |
| 21 | Hapmap52072-rs29018920 | 2.33 | 10 | 5.02E-16 |
| 21 | BTB-01171141 | 0.83 | 8 | 1.95E-14 |
| 21 | ARS-BFGL-NGS-10260 | 2.69 | 3 | 7.36E-14 |
| 14 | BTB-01532239 | 24.44 | 4 | 2.07E-12 |
| 21 | ARS-BFGL-NGS-31507 | 2.59 | 1 | 7.90E-12 |
| 14 | ARS-BFGL-NGS-28867 | 20.32 | 1 | 9.22E-12 |
| 14 | Hapmap59686-rs29020689 | 24.37 | 5 | 7.38E-09 |
| 14 | ARS-BFGL-NGS-112623 | 20.64 | 3 | 3.48E-07 |
| 6 | Hapmap26308-BTC-057761 | 38.58 | 0 | 1.76E-05 |
| 21 | ARS-BFGL-NGS-92774 | 2.94 | 1 | 3.99E-05 |
| 14 | UA-IFASA-7112 | 16.11 | 2 | 9.70E-05 |

## Maternal calving ease

The GWAS analysis has been also executed for maternal traits.

For maternal calving ease the region with the most significant SNPs is between about 2.15 to 2.71 Mb, on BTA 21, similarly to the direct effect.

In this region the gene UBE3A is located which is called Ubiquitin-Protein-Ligase E3A. Ubiquitination is a specific process that is responsible for adding ubiquitin molecules to cellular substrates. The loss of this function has been shown to have an important role in the development of severe physiological conditions and neurological disorders as the Angelmann Syndrome (Tomaić and Banks 2015).

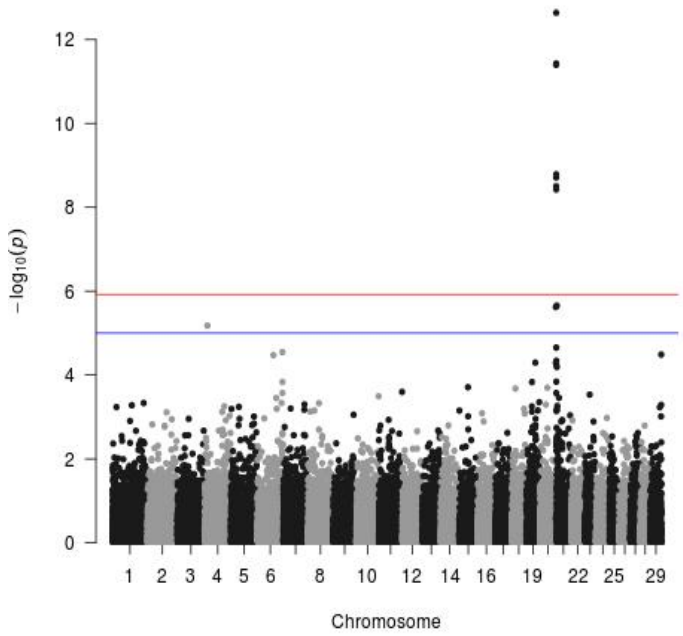


*Figure 8: Manhattan Plot for maternal calving ease.*

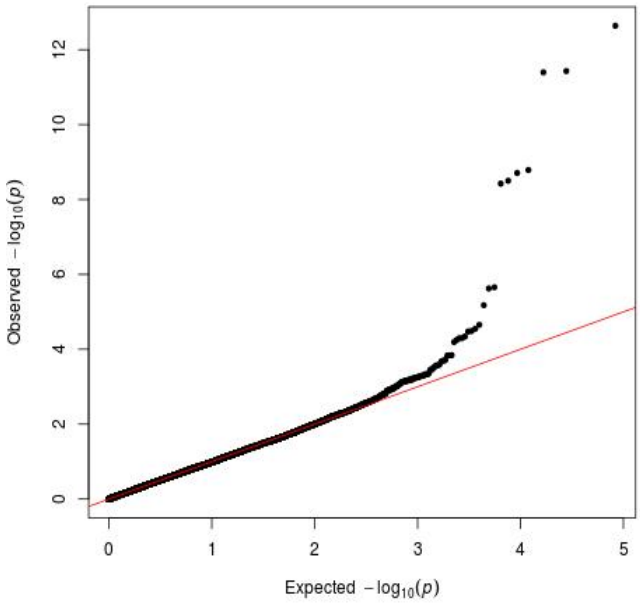


*Figure 9: QQPlot for maternal calving ease.*

Figure 9 shows the QQPlot for maternal calving ease after correction for population structure.

Compared the direct calving ease, it is obvious that maternal calving ease does not show such strong signals. The peaks on BTA 21 are similar, with exactly the same two most significant SNPs on the positions 2.2 Mb and 2.5 Mb.

The peaks on BTA 6 and 14 from direct calving ease are not visible for maternal calving ease. A single SNP was over the indicative significance threshold BTA 4 AT 13.1 Mb for maternal calving ease, not visible on the direct trait, but there could have not been found an associated gene with our traits.

Table 5 shows the significant SNPs for maternal calving ease, a similar picture like for direct calving ease, especially the region on chromosome 21.

*Table 5: Significant SNPs for maternal calving ease.*

| BTA | SNP name | position in Mb | number of missing values | p value |
|---|---|---|---|---|
| 21 | ARS-BFGL-NGS-108925 | 2.46 | 0 | 2.29E-07 |
| 21 | ARS-BFGL-NGS-10260 | 2.69 | 3 | 3.73E-06 |
| 21 | ARS-BFGL-NGS-53975 | 2.15 | 0 | 4.03E-06 |
| 21 | BTA-54983-no-rs | 2.71 | 12 | 1.63E-03 |
| 21 | ARS-BFGL-NGS-114372 | 2.38 | 49 | 1.96E-03 |
| 21 | Hapmap52072-rs29018920 | 2.33 | 10 | 3.15E-03 |
| 21 | ARS-BFGL-NGS-31507 | 2.59 | 1 | 3.77E-03 |

## Gestation length

### Direct gestation length

The Manhattan plot for direct gestation length (Figure 10) shows significant regions on the BTA 4, 7 and 21, and a slight peak also on chromosome 19.

It is known, that a longer gestation length leads to bigger calves and further to more problems while giving birth. A 10 days longer gestation length leads to a higher rate of dystocia of about 2 – 3 % (Fürst 2017).

On BTA 21 there is again a highly significant region found with exact the same SNP as the most significant one, ARS-BFGL-NGS-108925, on Mb position 2.461, This region contains the UB3A gene, which is associated with the Angelman-syndrome, what is already mentioned above, similarly to calving ease (Pausch et al. 2011).

The significance of the same region in both traits further underlines their connectedness, as shown in the literature. Direct gestation length shows a very high heritability of 0.57. Also the genetic correlation of direct gestation length and direct calving ease for first calvings is relatively high with 0.41, and 0.45 for the following calvings (Fürst 2017).

Holmberg and Andersson-Eklund (2006) found 13 suggestive QTL that were mapped to BTAs 6, 7, 9, 11, 13, 15, 20, and 29 for calving traits and fertility, but not exactly gestation length, in Swedish Holstein cattle. These results partly confirm the findings of our study for the signals on BTA 7 and 19.

In general, the gestation length is a rarely researched trait in animal breeding, when it comes to genomic analyses.

A further significant region could have been observed on BTA 4 at about 94.7 Mb to 94.2 Mb. In this region are the genes CPA1, CPA5 and CEP41 located. There could have not been found any association with fertility traits in cattle.

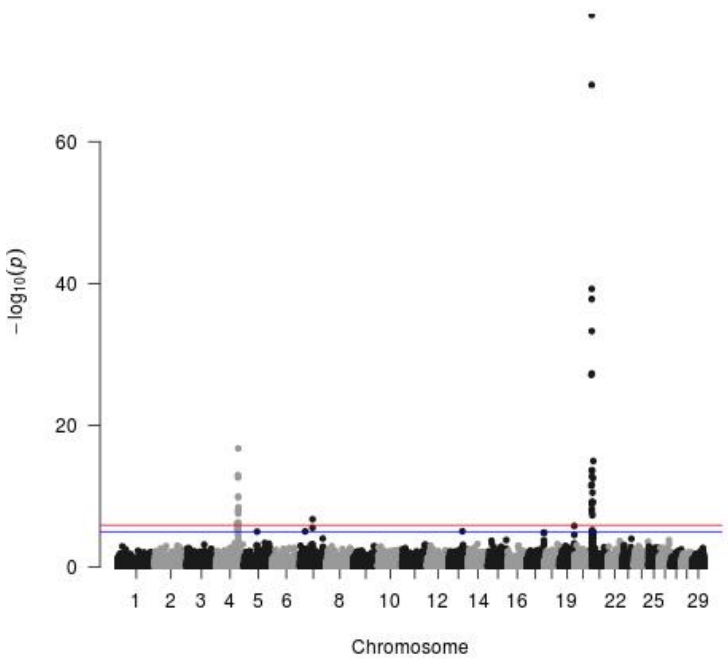


*Figure 10: Manhattan Plot for direct gesation length.*

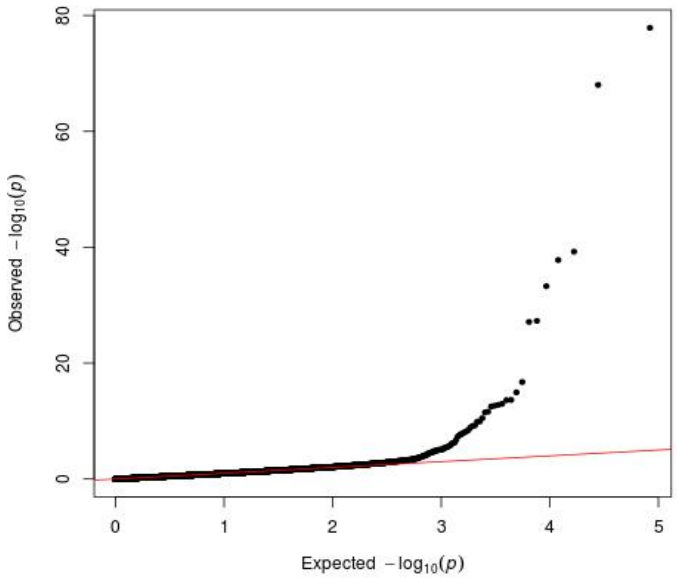


*Figure 11: QQPlot for direct gestation length.*

*Table 6: List of significant SNPs for direct gestation length.*

| BTA | SNP name | position in Mb | number of missing values | p value |
|---|---|---|---|---|
| 21 | ARS-BFGL-NGS-108925 | 2.46 | 0 | 1.37E-72 |
| 21 | ARS-BFGL-NGS-53975 | 2.15 | 0 | 9.84E-63 |
| 21 | Hapmap52072-rs29018920 | 2.33 | 10 | 5.85E-34 |
| 21 | ARS-BFGL-NGS-114372 | 2.38 | 49 | 1.64E-32 |
| 21 | ARS-BFGL-NGS-10260 | 2.69 | 3 | 5.37E-28 |
| 21 | ARS-BFGL-NGS-31507 | 2.59 | 1 | 5.07E-22 |
| 21 | BTB-01171141 | 0.83 | 8 | 7.84E-22 |
| 4 | UA-IFASA-7564 | 95.07 | 3 | 1.87E-11 |
| 21 | ARS-BFGL-NGS-86740 | 9.10 | 43 | 1.10E-09 |
| 21 | Hapmap40822-BTA-86024 | 3.86 | 0 | 2.25E-08 |
| 21 | BTA-54983-no-rs | 2.71 | 12 | 2.48E-08 |
| 4 | Hapmap40578-BTA-71629 | 94.15 | 0 | 1.09E-07 |
| 21 | ARS-BFGL-NGS-92774 | 2.94 | 1 | 1.64E-07 |
| 4 | Hapmap33892-BES6_Contig314_677 | 94.18 | 2 | 2.32E-07 |
| 21 | ARS-BFGL-NGS-16681 | 7.90 | 40 | 3.03E-07 |
| 21 | ARS-BFGL-NGS-60326 | 2.83 | 45 | 2.47E-06 |
| 21 | BTB-01171128 | 0.80 | 0 | 3.22E-06 |
| 21 | ARS-BFGL-NGS-1498 | 5.74 | 13 | 3.10E-05 |

## Maternal gestation length

The maternal gestation length is not as highly heritable as direct gestation length. Direct gestation length shows a heritability of about 0.56 and the maternal gestation length shows a heritability of 0.10 in the study of Sattlecker (2014).

The results of the trait maternal gestation length (Figure 12) show affinity to the results of direct calving ease. On BTA 4 and 21 are the highest peaks, same as for direct calving ease.

A new slight signal on BTA 13 shows us the SNP ARS-BFGL-NGS-110904 at the position 59.1 Mb. In this region the BMP7 gene is located, which is one of the genes of the bone morphogenetic proteins. Regan et al. (2018) found an association of them with the regulation of the ovarian functions.

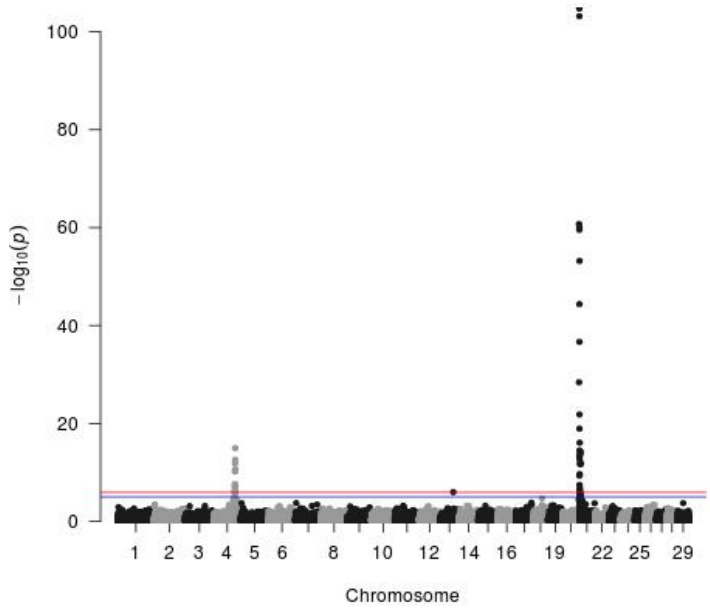


*Figure 12: Manhattan plot for maternal gestation length.*

In figure 13 shows the QQ-plot of the trait maternal gestation length. Similarly, as in previous cases, the population structure seems to be corrected well, with strong signals shown by the big deviations of the observed values from the expected ones.

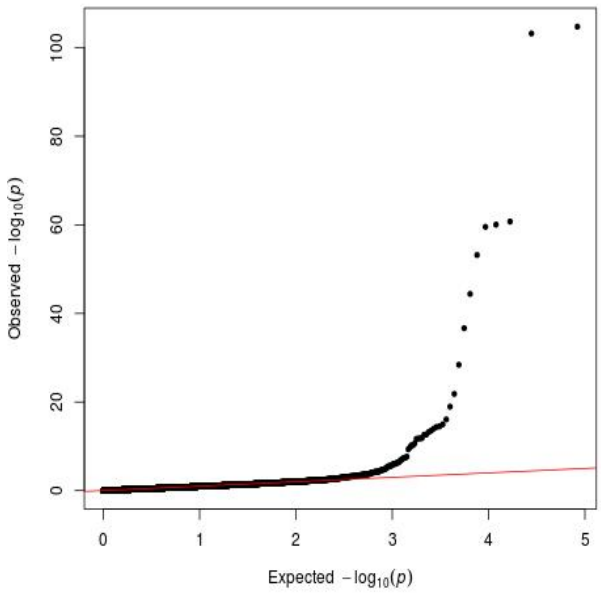


*Figure 13: QQPlot for maternal gestation length.*

Regions on BTA 13 already showed significance in other scientific papers such as Höglund et al. (2012). The regions there are from 16.9 Mb to 17.4 Mb and from 55.6 to 60.5 Mb. In this study QTL have been found for stillbirth; calving ease; calf survival and calving index in Swedish and Danish Holstein cattle.

Furthermore, the exact p values of the most significant SNPs from our study are presented in table 7.

*Table 7: Significant SNPs for maternal gestation length.*

| BTA | SNP name | position in Mb | number of missing values | p value |
|---|---|---|---|---|
| 21 | ARS-BFGL-NGS-53975 | 2.15 | 0 | 1.79E-99 |
| 21 | ARS-BFGL-NGS-108925 | 2.46 | 0 | 6.51E-98 |
| 21 | BTB-01171141 | 0.83 | 8 | 1.84E-55 |
| 21 | Hapmap52072-rs29018920 | 2.33 | 10 | 8.99E-55 |
| 21 | ARS-BFGL-NGS-114372 | 2.38 | 49 | 2.85E-54 |
| 21 | ARS-BFGL-NGS-10260 | 2.69 | 3 | 6.37E-48 |
| 21 | ARS-BFGL-NGS-31507 | 2.59 | 1 | 4.06E-39 |
| 21 | BTA-54983-no-rs | 2.71 | 12 | 2.22E-31 |
| 21 | BTB-01171128 | 0.80 | 0 | 3.96E-23 |
| 21 | ARS-BFGL-NGS-92774 | 2.94 | 1 | 1.52E-16 |
| 21 | ARS-BFGL-NGS-60326 | 2.83 | 45 | 1.14E-13 |
| 21 | Hapmap40822-BTA-86024 | 3.86 | 0 | 8.50E-11 |
| 4 | UA-IFASA-7564 | 95.70 | 3 | 1.11E-09 |
| 21 | ARS-BFGL-NGS-104603 | 2.92 | 2 | 3.25E-09 |
| 21 | BTA-52244-no-rs | 5.48 | 6 | 4.40E-09 |
| 21 | ARS-BFGL-NGS-16681 | 7.90 | 40 | 1.04E-08 |
| 21 | ARS-BFGL-NGS-37987 | 2.99 | 3 | 3.01E-08 |
| 21 | ARS-BFGL-NGS-102425 | 3.09 | 6 | 6.15E-08 |
| 21 | ARS-USMARC-Parent-DQ995976-no-rs | 3.09 | 1 | 2.19E-07 |

## Stillbirth rate

### Direct stillbirth rate

For the Austrian breeding value estimation stillbirth rate is a part of the trait “rearing losses” and is defined as calves that are born dead or die within 48 hours (Fürst 2017).

For the trait direct stillbirth rate (figure 14) two peaks have been found: a clear signal on BTA 14 around 24.1 Mb and another signal on BTA 21, around 2.5 Mb. Both of the signals has already showed significance in previous calving traits.

Also some SNPs on BTA 18 are just near to the significance level. Kühn et al. (2003) explored QTLs for functional traits in the German Holstein cattle population and found significant regions for length of functional life on BTA 2 and 18.

Length of functional life is directly related to calving traits, while problems at calving can lead to reduced productive life in cows.

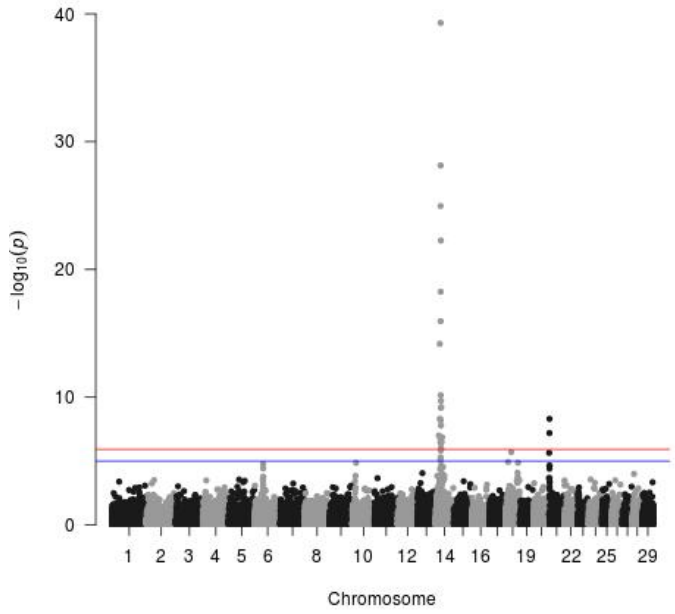


*Figure 14: Manhattan plot for direct gestation length.*

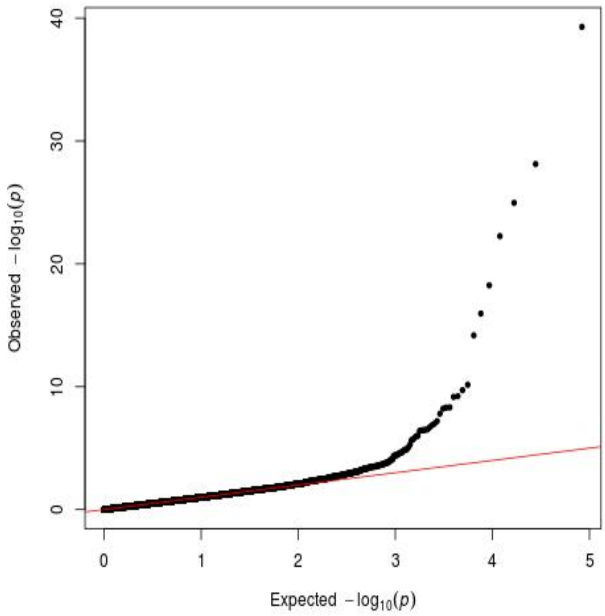


*Figure 15: QQPlot for direct stillbirth rate.*

Table 8: List of significant SNPs for direct stillbirth rate.

| BTA | SNP name | position in Mb | number of missing values | p value |
|---|---|---|---|---|
| 14 | BTA-91250-no-rs | 24.15 | 13 | 5,12E-34 |
| 14 | ARS-BFGL-NGS-104268 | 24.06 | 19 | 7,44E-23 |
| 14 | BTB-01417924 | 24.18 | 2 | 1,09E-19 |
| 14 | Hapmap46735-BTA-86653 | 25.40 | 19 | 5,57E-17 |
| 14 | BTB-01532239 | 24.44 | 4 | 5,65E-13 |
| 14 | Hapmap59686-rs29020689 | 24.37 | 5 | 1,12E-10 |
| 14 | ARS-BFGL-NGS-28867 | 20.32 | 1 | 6,68E-09 |
| 14 | BTB-00557532 | 24.64 | 0 | 7,00E-05 |
| 14 | Hapmap39876-BTA-97370 | 25.86 | 17 | 1,93E-04 |
| 14 | Hapmap23172-BTC-011263 | 26.69 | 2 | 5,93E-04 |
| 14 | Hapmap46986-BTA-34282 | 25.31 | 1 | 6,82E-04 |
| 14 | ARS-BFGL-NGS-112623 | 20.64 | 3 | 4,88E-03 |
| 21 | ARS-BFGL-NGS-108925 | 2.46 | 0 | 5,02E-03 |
| 14 | ARS-BFGL-NGS-102351 | 24.41 | 49 | 6,18E-03 |
| 14 | ARS-BFGL-NGS-8308 | 25.99 | 7 | 1,60E-02 |
| 21 | ARS-BFGL-NGS-53975 | 2.15 | 0 | 6,44E-02 |
| 14 | UA-IFASA-7112 | 16.11 | 2 | 1,02E-01 |
| 14 | ARS-BFGL-BAC-8535 | 31.06 | 39 | 1,37E-01 |
| 14 | ARS-BFGL-BAC-8052 | 23.89 | 4 | 1,99E-01 |

## Maternal stillbirth rate

The GWAS on the trait maternal stillbirth rate showed no significant regions, as shown in the Manhattan plot (figure 16) and the QQ plot (figure 17).

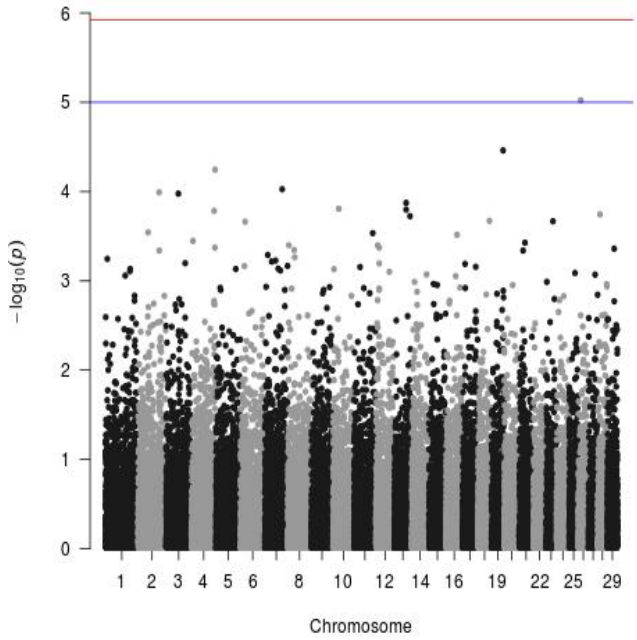


*Figure 16: Manhattan Plot for maternal stillbirth rate.*

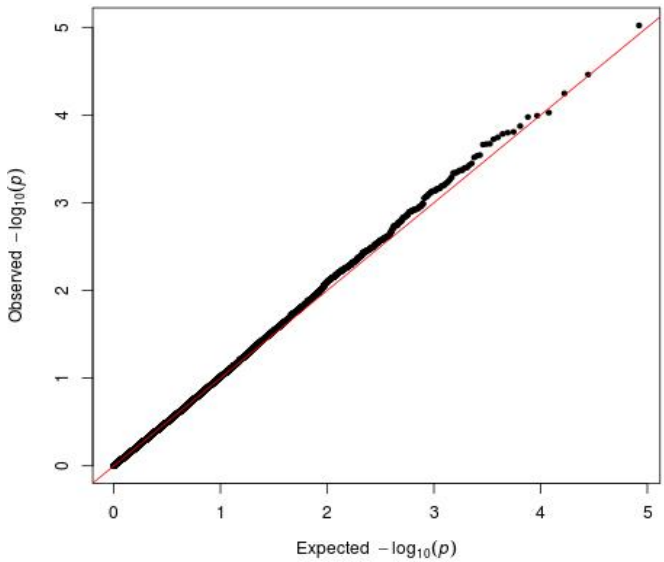


*Figure 17: QQPlot for maternal stillbirth rate.*

# Results and Discussion HapGwas

## Calving ease

### Direct calving ease

With the haplotype based GWAS we have found significant SNPs at the BTA 4, 14 and 21. The signals on the BTA 14 and 21 are repeated from the SNP based GWAS, the signal on BTA 4 is new.

The region on BTA 4 at 97 Mb contains the EXOC4 gene which plays a role in the GWAS study of Buzanskas et al. (2017) for male and female reproductive traits in Canchim beef cattle. The gene EXOC4 plays a role in insulin processing, metabolism of proteins, and peptide hormone metabolism pathways. An association with scrotal circumference has been noted.

Gonda et al. (2004) conducted a GWAS study, searching for ovulation rate QTLs. His research was based on the potential to improve production efficiency in beef cattle by increased twinning rate. The subsequent interval mapping strengthened support for the presence of an ovulation rate QTL on BTA 14. He mentioned a QTL at the position 27.7 Mb, which is near our significant region at the GHap for direct calving ease.

In figure 18, here are summed up the results of the two GWAS approaches. At the GWAS with SNPs, every dot represents a SNP, however at the GWAS with haplotypes, every dot represents a haplotype. As we decided a haplotype length of 5 SNPs, every dot represents 5 SNPs.

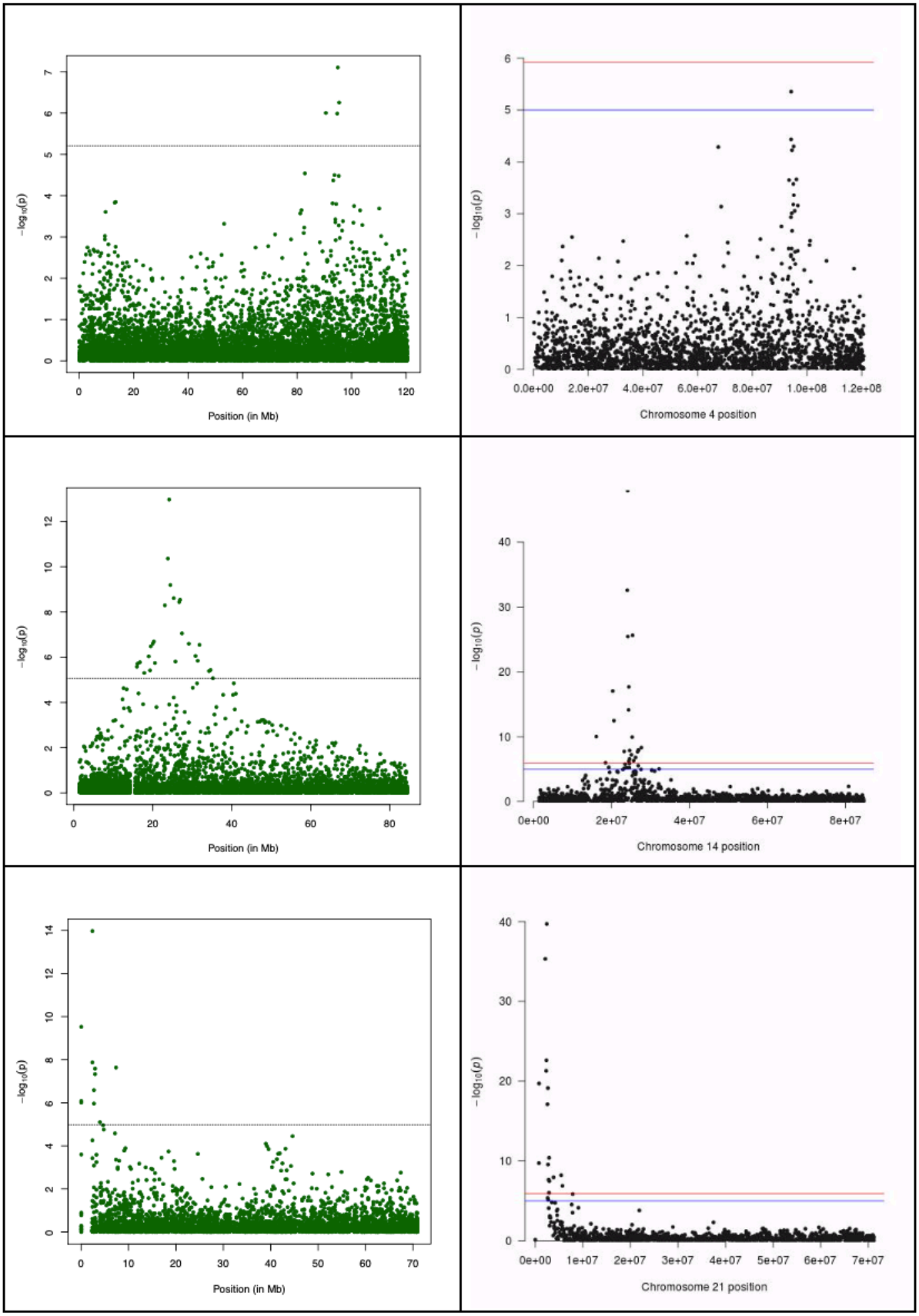


*Figure 18: Comparison of the GWAS results with haplotypes on the left and SNPs on the right side for the BTA 4, 14 and 21.*

## Maternal calving ease

For the Maternal Calving ease the results deviate totally from the SNP based GWAS. On BTA 11, 12 and 20 there have been found significant haplotypes, what show a very slight signal, compared to the SNP based GWAS analysis with a very clear signal on BTA 21.

The literature underlines the signal on chromosome 21 like ,among others, Pausch et al. (2011).

In our significant GHap region was from 90 Mb to 95 Mb on BTA 11, containing among others the DENND1A gene. This gene is associated with the Polycystic ovary syndrome that affects approximately 5–7% of reproductive age women placing it among the most common female endocrine disorders (Mittal et al. 2015).

The region at about 10 Mb on BTA 12 has not been showing genes with apparent connection to calving traits in cattle.

Furthermore, some significant haplotypes were also found on BTA 20 at the region of about 32 Mb. In this region the GHR gene has been found. According to Di Stasio et al. (2005) the growth hormone (GH) exerts its effects on growth and metabolism by interacting with a specific receptor (GHR) on the surface of the target cells. Therefore, GHR has been suggested as candidate gene for traits related to growth and meat production in cattle.

The clear signal from SNP based GWAS on BTA 21 was not found with the haplotype analysis.

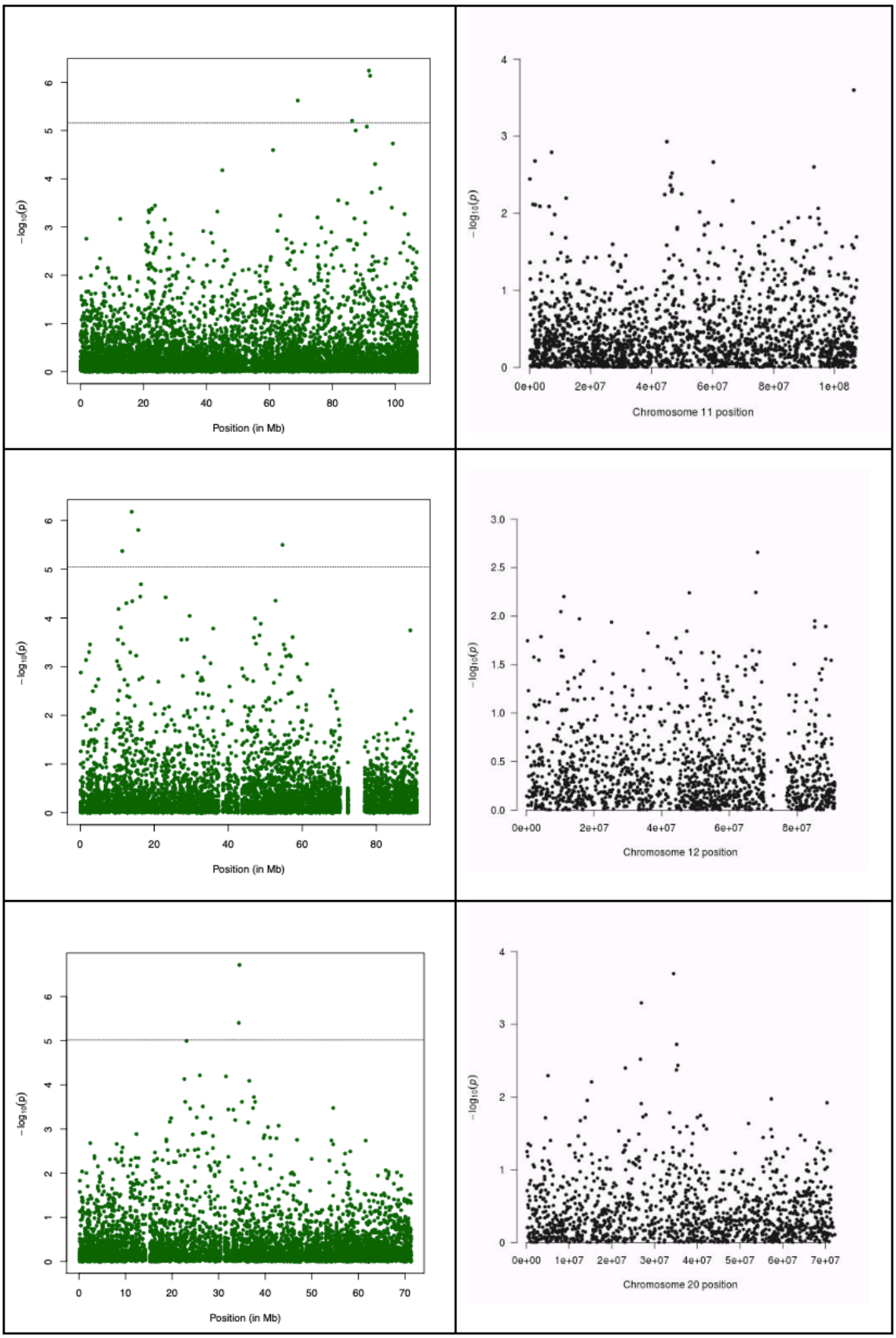


*Figure 19: Comparison of the haplotype based GWAS on the left and the SNP based GWAS on the right side with the BTA 11, 12 and 20.*

## Gestation length

### Direct gestation length

For the trait direct gestation length there have been found two strong signals common with SNP, one on BTA 4 and one on chromosome 21.

These signals could have also been detected by the haplotype approach, as shown in figure 20.

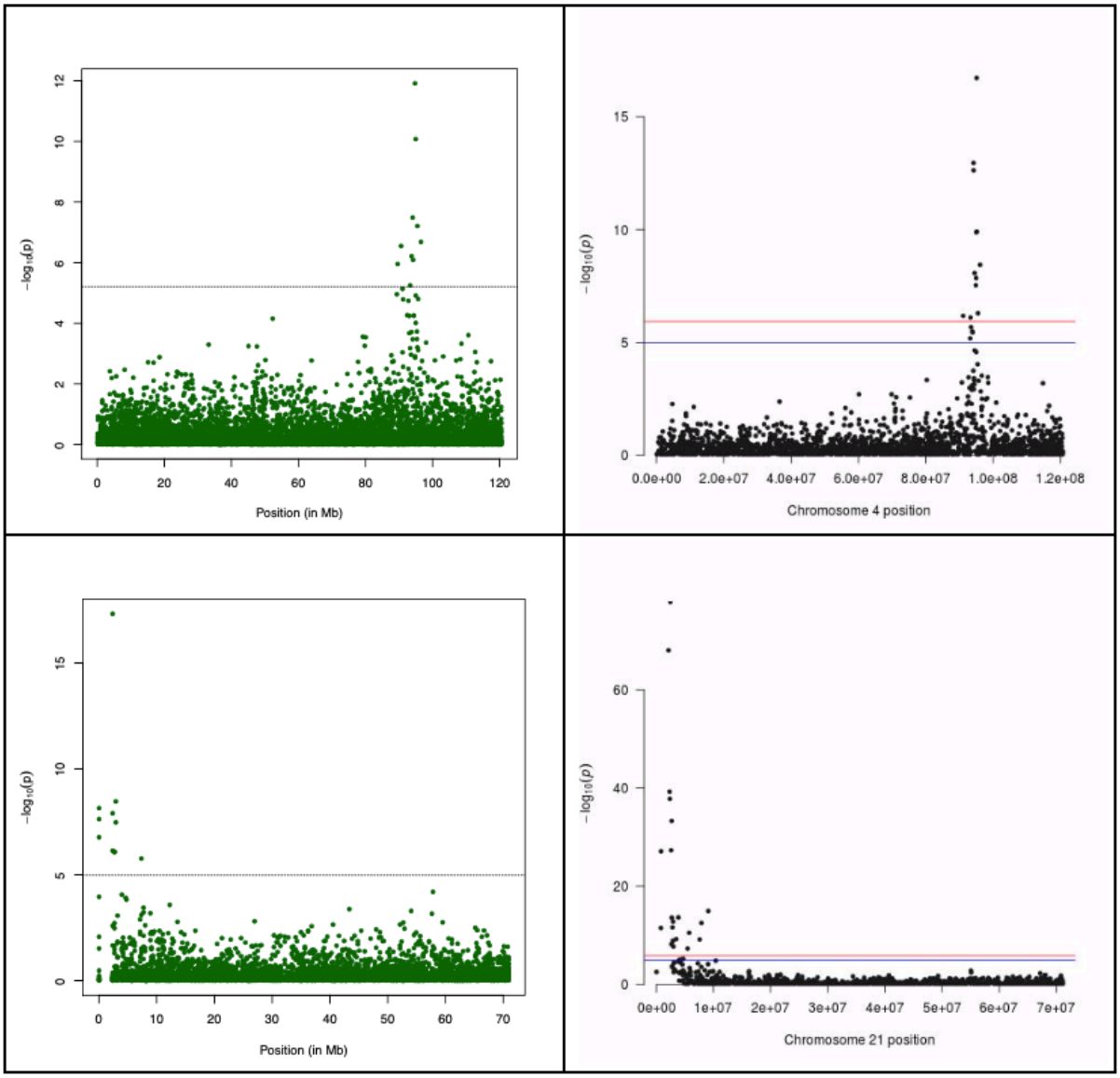


*Figure 20: Results of the two GWAS approaches for chromosome 4 and 21. The haplotype-based results are shown on the left side, while the SNP based results are shown on the right side.*

## Maternal gestation length

The significant regions on the BTA 4 and 21 can also been found at the GWAS with SNPs. The signal on BTA 6 is new.

Searching this significant region on BTA 6 from 8 Mb to 11 Mb there were no genes found with apparent association with calving traits.

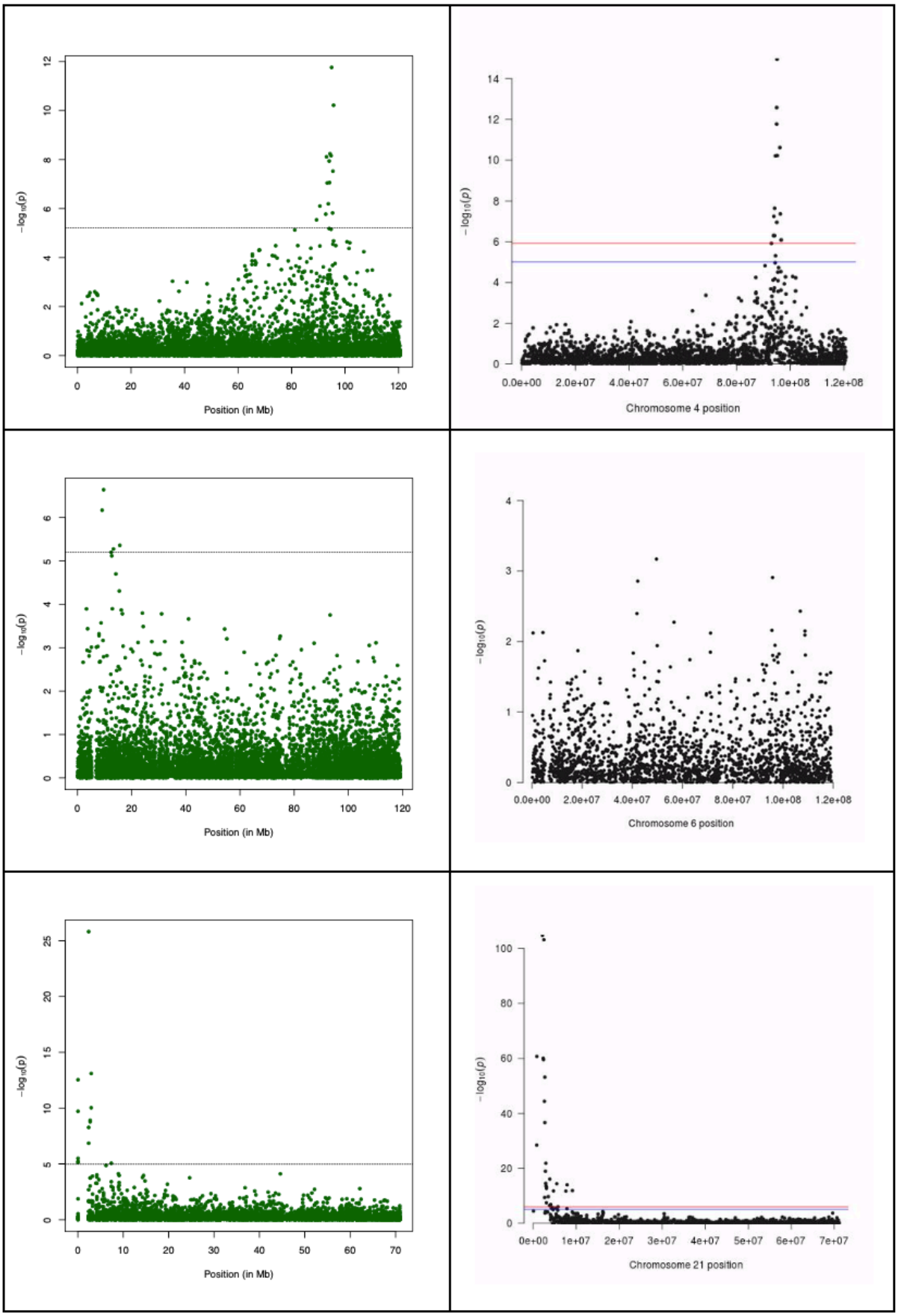


*Figure 21: Results of the two GWAS approaches. GHap results on the left and Gemma results on the right for the chromosomes 4, 6 and 21.*

## Stillbirth rate

### Direct stillbirth rate

The results for the traits direct stillbirth rate from the two approaches differ completely. While there have been found two clear signals on BTA 14 and 21 at the SNP GWAS, only a very low signal could have been found on BTA 5 at the haplotype GWAS.

The results are compared directly in figure 22.

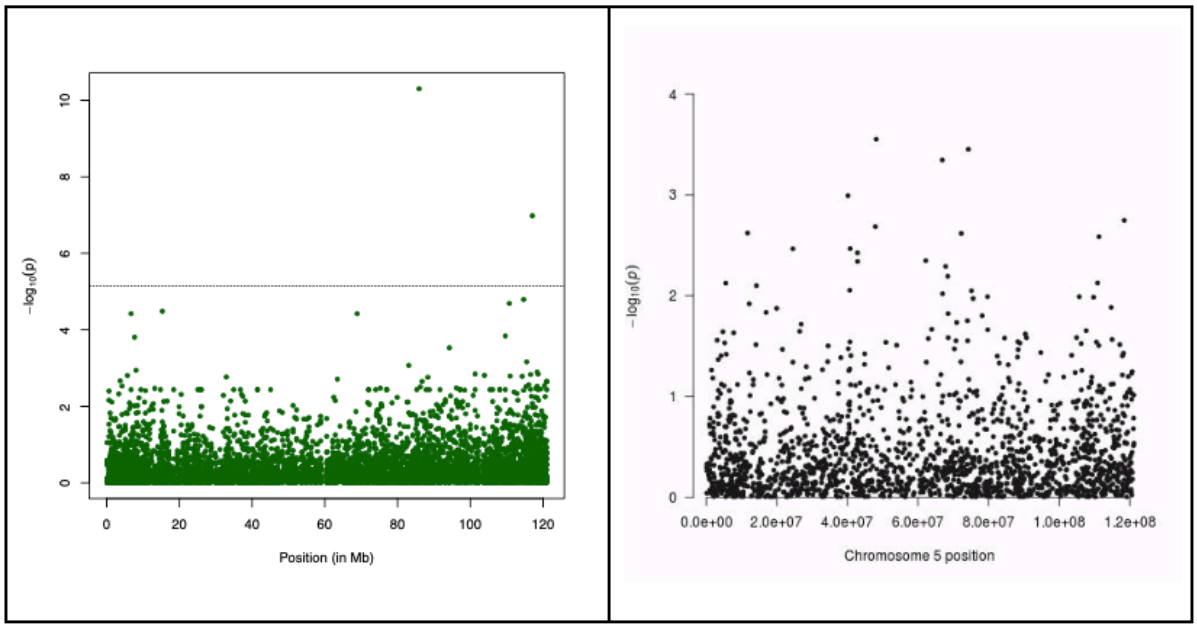


*Figure 22: GHap (left) and Gemma (right) results of the trait direct stillbirth rate for chromosome 5.*

## Maternal stillbirth rate

We did not find any significant regions with neither at the SNP based GWAS nor at the haplotype GWAS for maternal stillbirth rate.

The only slight signal was one haplotype at BTA 5 that was over the significance level.

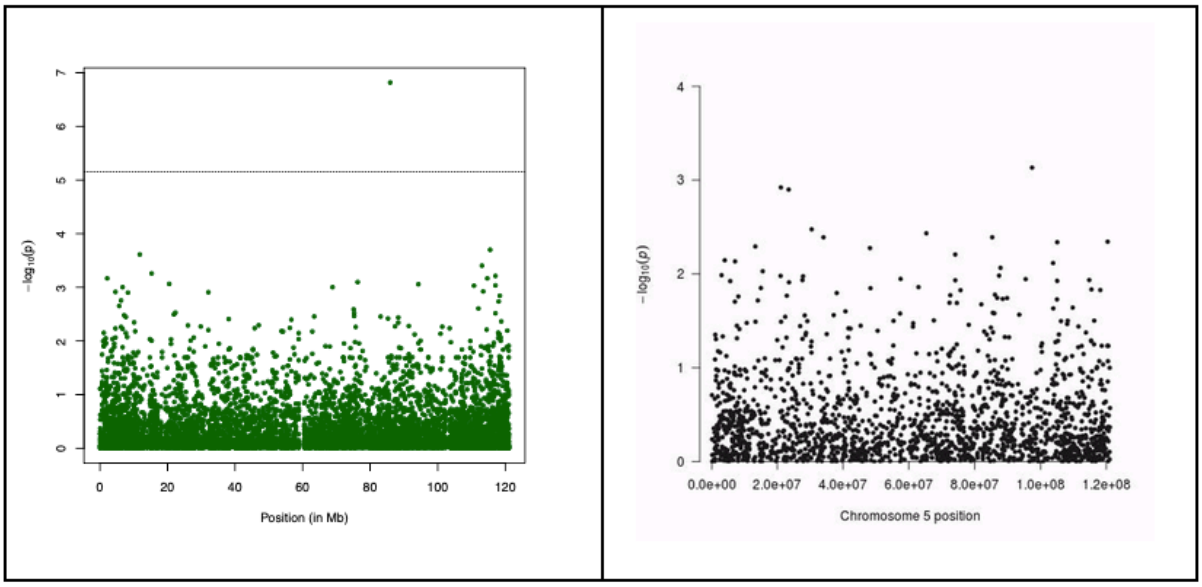


*Figure 23: Results of the two GWAS approaches for maternal stillbirth rate for chromosome 5. GHap results are presented on the left and GEMMA results are presented on the right.*

# Conclusions

The regular reproduction of the herd is essential for economic success in cattle farming for any production sector. Due to this high economic value of calving traits, research efforts have substantially increased for these traits.

The aim of this study was the identification of significant regions on the cattle genome for the traits calving ease, gestation length and stillbirth rate in Fleckvieh cattle, based on two different approaches, single SNP and phased haplotype data.

At the trait calving ease two highly significant regions could have been detected on BTA 14 at 24.1 Mb and BTA 21 at 2.1 Mb. The region on BTA 14 includes genes like PLAG1, TGS1, RPS20, LYN and SOX17 that are associated with growth and body size.

The region on BTA 21 is associated with the Prader-Willi-Syndrome and the Angelman-Syndrome, which can lead to genital hypoplasia, incomplete pubertal development and infertility.

At the haplotype GWAS an additional peak on BTA 2 could have been found, what is not associated to calving traits, but to mastitis and clinical mastitis.

The results for the trait gestation length show significant regions on the BTA 4, 7 and 21 and a slight peak on BTA 19. The signal on BTA 21 is exactly the same region compared to calving ease. The significance of the same region further underlines the relationship of the two traits.

The signals on BTA 14 and 21 are repeated again in direct stillbirth rate.

The maternal traits also showed clear significant signals, especially for calving ease and gestation length. While some of the signals were overlapping with those of the direct traits, there were numerous differences between the two sets of traits.

The haplotype based GWAS confirmed the most important regions for the calving traits, but the signals from the two approaches were not corresponding to full extent.

# Summary

An essential factor for economic success in dairy farming and meat production is the regular reproduction in the herd.

Calving traits show very low heritabilites and high environmental influence, what makes breeding success extremely difficult. Due to this fact, the research on calving traits is gaining in popularity.

Calving in general is influenced by the two factors, the mother and the calf. For this it is essential for selection to consider both effects, the direct and the maternal traits. The direct trait describes the property of the calf and the maternal trait show the abilities of the cow once giving birth.

The aim of this work was to identify significant regions for three traits calving ease, gestation length and stillbirth rate in Fleckvieh, using single SNP and haplotype based GWAS approaches.

As the major result, it can be said, that the region about 2.4 Mb on BTA 21 has a high influence on calving traits for Fleckvieh cattle.

In principle, the core results of the two different GWAS approaches based on SNPs and haplotypes were similar. Especially the results of direct gestation length fit very well. Indeed, some of the peaks which shown in the SNP approach were missing at the haplotype approach and vice versa.

The trait calving ease shows in both computations' strong signals on BTA 14 and 21. These two chromosomes show also significant regions on other traits. These results were also supported by the literature. At the GHap analyses a small signal on BTA 4 could was found, but not supported by previous results in the literature.

For gestation length ttwo strong signals were found: on BTA 4 and 21. The genes on BTA 4 were not associated with calving traits according to the literature. A small signal with no relevant associations could was found on BTA 6 for maternal gestation length.

For the trait direct stillbirth rate, results of the two different approaches differed a lot. The strong signals from the SNP-GWAS on the BTA 14 and 21 were not find in haplotype analysis.

Maternal stillbirth rate shows no signals for SNP or haplotype based GWAS, excepta very slight signal is only visible on BTA 5 at the HapGWAS.

In summary it can be said, that the regions around 24.1 Mb on BTA 14 and 2.5 Mb on BTA 21 have a large effect in calving traits in general.

Furthermore, it has been shown, that the two different GWAS approaches based on SNP data and haplotype data show similar results, with some differences in individual traits.

# Zusammenfassung:

Einen essentiellen Faktor für wirtschaftlichen Erfolg in der Milchwirtschaft, aber sowohl in der Rindfleischproduktion, stellt die regelmäßige Reproduktion der Herde dar.

Kalbemerkmale zeigen geringe Vererblichkeiten und hohe Umwelteinflüsse, was die Verwirklichung von Zuchtfortschritt enorm schwierig darstellt. Aufgrund dessen, bekommt die Forschung an diesen Merkmalen immer mehr an Bedeutung.

Die Abkalbung ist im generellen von zwei Faktoren beeinflusst, der Mutter und dem Kalb. Deshalb ist es essentiell, bei der Selektion beide Effekte in Betracht zu ziehen, den direkten und den maternalen Effekt. Der direkte Effekt beschreibt die Eigenschaften des Kalbes im Mutterleib, während der maternale Effekt die Voraussetzungen des Kalbes bei den eigenen Geburten zeigt.

Das Ziel dieser Arbeit ist die Identifikation von signifikanten Regionen für die drei Merkmale Kalbeverlauf, Trächtigkeitsdauer und Totgeburtenrate beim Fleckvieh. Dafür werden zwei verschiedene Methoden verwendet, die einerseits auf SNP Daten und andererseits auf Haplotypen basieren.

Grundsätzlich kann gesagt werden, dass die Region um 2.4 Mb auf dem Chromosom 21 einen hohen Einfluss auf die Kalbemerkmale beim Fleckvieh haben.

Prinzipiell kann gesagt werden, dass die Hauptresultate der beiden verschiedenen Methoden basierend auf SNP und Haplotypen die gleichen sind. Vor allem die Resultate des Merkmals direkte Trächtigkeitsdauer passen sehr gut zusammen. Dennoch, manche Signale, die bei Methode basieren auf SNPs zu erkennen sind, fehlen bei der Methode mit Haplotypen und umgekehrt.

Das Merkmal Kalbeverlauf zeigt in beiden Berechnungen starke Signale auf den Chromosomen 14 und 21. Diese beiden Chromosomen zeigen auf signifikante Regionen bei anderen Merkmalen. Diese Resultate konnten auch in der Literatur bestätigt werden. Bei der Methode mit Haplotypen konnte außerdem ein kleines Signal auf Chromosom 4 gefunden werden, welches allerdings in der Literatur nicht wiedergefunden werden konnte.

Beim Merkmal Trächtigkeitsdauer konnte zwei starke Signale gefunden werden, auf den Chromosomen 4 und 21. Die gefundenen Gene in der Region auf Chromosom 4 konnten nicht durch andere Literatur unterstützt werden. Ein kleines Signal ohne relevante Verbindungen konnte auf Chromosom 6 für maternale Trächtigkeitsdauer gefunden werden.

Für das Merkmal Totgeburtenrate unterscheiden sich die Resultate der beiden Methoden sehr. Während ein starkes Signal bei der Methode basieren auf SNP Daten auf den Chromosomen 14 und 21 gefunden werden konnte, waren keine signifikanten Regionen aufgezeigt worden bei der Methode basierend auf Haplotypen, außer einem sehr geringen Signal auf Chromosom 5.

Maternale Totgeburtenrate zeigt keine Signale bei beiden Methoden, SNP basierende Assoziationsstudie und Haplotypen basierende Assoziationsstudie. Ein ganz leichtes Signal konnte bei der Haplotypen-Analyse auf Chromosom 5 gefunden werden.

Zusammenfassend kann gesagt werden, dass die Region um 24.145 Mb auf dem Chromosom 14 und die Region 2.461 Mb auf dem Chromosom 21 einen sehr wichtigen Einfluss auf Kalbemerkmale im Generellen zu haben scheint.

Außerdem konnte gezeigt werden, dass die beiden Methoden der Genomweiten Assoziationsstudie basierend auf zwei verschiedenen Datengrundlagen, SNPs und Haplotypen, hauptsächlich die gleichen Resultate zeigen und so jeweils von der anderen Methode unterstützt werden.

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