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# Analysis of epistatic interactions in Fleckvieh cattle

**Master Thesis**

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## Statutory declaration

I declare that I have prepared, developed and written this thesis independently and I have not used any sources, thoughts or literature of others than clearly stated in the text. The master thesis was not used to award an academic degree at any other university.

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## Abstract

The main interest for each cattle breeder is to select animals with the best genetic value for production. In addition to production the animal health should be also considered, because of its importance in length of productive life. Therefore, functional traits such as calving ease or stillbirth were attributed to an important position in the selection process. The age of genomics provides opportunities of better understanding the genetic mechanisms causing differences in traits. Epistasis describes the interaction of different genes in the expression of a single trait.

In this thesis, medium and high-density SNP chip (54,001 or 777,000 SNPs) were used to investigate the genomes of 7,327 Fleckvieh sires to identify chromosomal regions associated with epistatic interactions of four functional traits: fertility, calving ease, longevity and stillbirth. De-regressed breeding values from the German-Austrian Fleckvieh population were used as phenotypes. The epistatic effects were identified as significant interaction for pairwise linear regressions performed for each trait after correction for multiple testing.

In fertility traits, BTA3, 4, 13 and 29 showed epistatic interactions between SNPs. Especially five genomic regions containing CDKN1C, PHLDA2, PRDM10, BSND and PROKR2 genes appeared to have major influence on traits of interest. A genomic region with especially high impact on calving ease was found on BTA14, contained NSMCE2, KIAA0196, PLAG1, CHCHD7, MOS, RPS20, LYN, PENK and CYP7B1 genes associated with dwarfism, height and stature or growth retardation. Furthermore, epistatic interactions on BTA21 identified MAGEL2, MKRN3, NDN, UBE3A and ATP10A genes, with apparent connection to Prader-Willi syndrome. BTA1, 2, 3, 5, 9, 10, 14, 17, 19, 21 and 24 were involved in trait formation of longevity. The biggest effect regarding epistatic interactions for stillbirth could be observed on BTA14. Nine significant regions containing PRKDC, EFCAB1, SNTG1, ST18, OPRK1, ATP6V1H, RGS20, XKRN4, PENK protein coding genes, showed a relationship to stature expressions like paediatric spinal deformation, bone formation, body size and height. Besides, the protein coding genes LYPLA1, TMEM68 and XKR4 on BTA10 showed influence on the regulation of prolactin secretion. In calving traits, especially stature formations like height or body size increased calving difficulties, potentially led to stillbirth. Longevity and fertility could be impaired trough diseases or metabolic dysfunctions.

This study contributes to a better understanding of interactions between different regions for functional traits in the bovine genome and provides useful information for breeding organisations and for future work on the topic epistasis in cattle.

## Zusammenfassung

Das Hauptinteresse jedes Viehzüchters besteht darin, Tiere mit dem besten genetischen Wert für die Produktion auszuwählen. Neben der Produktion sollte auch die Tiergesundheit wegen ihrer Bedeutung für die Lebensdauer berücksichtigt werden. Funktionelle Merkmale wie der Kalbeverlauf oder Totgeburtenrate wurden daher einer wichtigen Position im Auswahlprozess zugeschrieben. Das Zeitalter der Genetik eröffnet Möglichkeiten, die genetischen Prozesse, welche zur Ausprägung solcher Merkmale führen, besser zu verstehen. Epistasie beschreibt die Interaktion mehrere Gene beim Entstehen eines einzelnen Merkmals.

In dieser Arbeit wurden SNP-Chips mittlerer und hoher Dichte (54.001 oder 777.000 SNPs) verwendet, um die Genome von 7.327 Fleckvieh Stieren zu untersuchen, um chromosomale Regionen zu identifizieren, welche mit epistatischen Interaktionen von vier funktionellen Merkmalen assoziiert sind: Fruchtbarkeit, Kalbeverlauf, Nutzungsdauer und Totgeburtenrate. Als Phänotypen wurden de-regredierte Zuchtwerte aus der deutsch-österreichischen Fleckvieh-Population herangezogen. Die epistatischen Effekte wurden als signifikante Interaktion für paarweise lineare Regressionen identifiziert, die für jedes Merkmal nach Korrektur für mehrere Tests durchgeführt wurden.

Für Fruchtbarkeitsmerkmale zeigten Chromosom 3, 4, 13 und 29 epistatische Interaktionen zwischen SNPs. Insbesondere fünf genomische Regionen, welche die CDKN1C, PHLDA2, PRDM10, BSND und PROKR2 Gene enthielten, schienen einen wesentlichen Einfluss auf Fruchtbarkeitseigenschaften zu haben. Eine genomische Region mit besonders hoher Auswirkung auf den Kalbeverlauf wurde auf Chromosom 14 gefunden, Diese enthielt die Gene NSMCE2, KIAA0196, PLAG1, CHCHD7, MOS, RPS20, LYN, PENK und CYP7B1, welche mit Zwergwuchs, Größe und Statur von Rindern oder Wachstumsverzögerungen assoziiert sind. Darüber hinaus identifizierten epistatische Interaktionen auf Chromosom 21 die Gene MAGEL2, MKRN3, NDN, UBE3A und ATP10A mit einer offensichtlichen Verbindung zum Prader-Willi-Syndrom. Chromosom 1, 2, 3, 5, 9, 10, 14, 17, 19, 21 und 24 waren an der Merkmalsbildung der Nutzungsdauer beteiligt. Der größte Effekt in Bezug auf epistatische Interaktionen bei der Totgeburtenrate konnte für Chromosom 14 beobachtet werden. Neun signifikante Regionen, die die proteinkodierenden Gene PRKDC, EFCAB1, SNTG1, ST18, OPRK1, ATP6V1H, RGS20, XKRN4 und PENK enthielten, zeigten eine Beziehung zu Staturausprägungen wie pädiatrische Wirbelsäulenverformung, Knochenbildung, Körpergröße und -höhe. Darüber

hinaus zeigten die proteinkodierenden Gene LYPLA1, TMEM68 und XKR4 auf Chromosom 10 einen Einfluss auf die Regulation der Prolaktinsekretion. Für Abkalbungsmerkmale, wie beispielsweise eine zunehmende Körperhöhe oder -größe, traten Schwierigkeiten bei der Abkalbung auf, welche möglicherweise zur Totgeburt führten. Langlebigkeit und Fruchtbarkeit könnten durch Krankheiten oder Stoffwechselstörungen beeinträchtigt werden.

Diese Studie trägt zu einem besseren Verständnis der Wechselwirkungen zwischen verschiedenen Regionen für funktionelle Merkmale im Rindergenom bei und liefert nützliche Informationen für Zuchtorganisationen und für zukünftige Arbeiten zum Thema Epistasie bei Rindern.

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My biggest thanks go to my family and friends, especially to my parents and my husband Andreas. They have awakened in me the love of Fleckvieh breeding, supported me unconditionally during my entire study time, always cheered me up and motivated me. This master thesis is dedicated to you.

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# List of abbreviations

bp ..... *base pair position*
BTA ..... *Bos taurus autosome*
CE ..... *calving ease*
FER ..... *fertility*
GWAS ..... *Genome-wide association study*
LPL ..... *longevity/ lenght of productive life*
Mb ..... *Megabase*
SB ..... *stillbirth*
SNP ..... *Single Nucleotide Polymorphism*

# 1 Introduction

The systematic animal breeding began at the end of the 19th century. The genetic rules discovered by Gregor Mendel via his experiments with peas were transferred to animal breeding. The animals were selected to be optimally adapted to their environment. Initially cattle were used for work, but also for milk and meat. From 1970s and 1980s the breeding started to focus solely on production. In dairy cows, milk yield, udder health and protein and fat content were defined as distinctive breeding criteria. Phenotypic traits were of great importance when selecting animals for further breeding.

During the last decades, milk yield increased tremendously in most dairy cattle populations worldwide. In Austrian Fleckvieh cattle, on average a genetic trend of +643 kg milk and +48 kg fat-protein per lactation could be observed during the last 10 years (Egger-Danner et al. 2017), with simultaneous improvement of functional traits. Functional traits for Fleckvieh cattle include fertility, functional longevity, persistency, fertility, calving ease, stillbirth and somatic cell count (Willam et al. 2002), as well as other health traits. Generally, functional traits have low heritability's ranging from 0.01 to 0.15. Hence selection for genetic improvement for functional traits was considered to be difficult (Boettcher 2005).

For example, in longevity a low, but important increase of +0.26 years was noticed in the last 10 years. Longevity is notoriously hard to evaluate, as many management decisions like stricter selection and external factors influence the life length of dairy cows. Another important functional trait is stillbirth. Although the stillbirth rate decreased in the last decade, the annual report showed a small increase in stillborn calves for the last few years in Fleckvieh cattle (Egger-Danner et al. 2017).

In practice, the key interest for each cattle breeder is in selection of the animals with the best genetic value for beef or milk production. In the past, the selection based only on pedigree and phenotype data, but currently these approaches are being expanded with the use of genomic data. Predictions of the genetic value for complex traits simplified (Kemper and Goddard 2012). This enabled functional traits such as calving ease or stillbirth to be correctly evaluated and included in the selection process (Daetwyler et al. 2014). With the availability and broad use of genotyping the animal breeding industry has changed, and the genomic selection became a standard method for estimating breeding values for both production and functional traits.

The cattle genome *(Bos taurus and Bos indicus)* contains of 30 chromosome pairs: 29 autosomes and a sex chromosome pair. Sequencing and assembly processes of the bovine genome was completed in 2009 (Garrick and Ruvinsky 2015). For genomic analysis in livestock, microsatellites were the most used DNA markers in the past. In the last 10 years however, a new class of genetic markers named Single Nucleotide Polymorphisms (SNPs) now replaced microsatellites as the most popular markers. SNPs are single base changes in DNA sequence (Orrù et al. 2009). Any of the four possible nucleotide bases (adenine, thymine, guanine and cytosine) could be present at each position of the sequence. In practice, the SNPs on the commercial chips were biallelic however (Vignal et al. 2002). Compared with other types of DNA markers, SNPs were attractive because they were abundant, genetically stable, and amenable to high-throughput automated analysis (Heaton et al. 2002).

Although SNPs were only biallelic, their large number allowed tracking the inheritance of short chromosomal segments (Garrick and Ruvinsky 2015). Low-, medium- and high-density SNP-Chips became available for relatively cheap price and this new technology revolutionized dairy cattle breeding and was a major source of innovation in research (Weng et al. 2012).

The study of cattle chromosomes developed from low resolution mapping of microsatellites and genes to genome-wide association studies involving thousands of genes and millions of polymorphisms. This improved our understanding of the genetic basis of economically important traits in cattle and successfully connected many phenotypes to chromosomal regions. So, the underlying genetic architecture of important traits like growth, disease resistance, milk production, meat and carcass quality became more available for research (Garrick and Ruvinsky 2015).

Genomic prediction was proven to help to select breeding animals more efficiently. Building such models to estimate genomic breeding values could be very challenging though, especially when non-additive effects like epistasis are involved. Particularly linear methods normally ignore gene by gene interactions. To take possible non-additive genetic effects into account, there has to be a growing interest in exploring gene interactions in animal breeding (Ehret et al. 2015).

## *1.1 Literature review and background*

Up to now epistasis was often ignored in studies, because of its hidden complexity in the regulation of complex traits (Carlborg and Haley 2004) or because most of the genetic variance still expected to be an additive variance (Hill, Goddard and Visscher 2008). In general, epistatic interactions distinguished between complex and mendelian traits, due to factors such as an increased number of involved loci or the susceptibility of the alleles, incomplete penetrance or involved environmental effects (Cordell 2002; Carlborg and Haley 2004). Complex traits, which often have huge influence on health and fertility traits in humans, livestock and plants are typically affected by several genes and by environmental factors. Interactions between these different effects are also possible (Carlborg and Haley 2004; W. G. Hill, Goddard, and Visscher 2008; Wei, Hemani and Haley 2014). The analysis of complex traits in this thesis might identify genes responsible for such differences between individuals in a population.

Bateson (1909) defined about 100 years ago the term epistasis, when he described, as one of the first authors, the overlapping of a disease-causing mutation with the transmission of a mutation from another locus. In general, epistasis means that the phenotype of an individual cannot be described with the sum of effects of individual loci (Carlborg and Haley 2004). So it could be described as an interrelationship between genes or as gene-gene interaction (Rose and Bell 2012). Wei, Hemani and Haley (2014) defined epistasis as a statistical interaction between loci with effects on a trait, which meant that the influence of a genotype of a single locus depends on a genotype of another locus.

**Table 1: Example for hair colour obtained from different genotypes at two epistatic loci interacting under Bateson's definition according to CORDELL (2002)**

| | Genotype at locus G | | |
|---|---|---|---|
| Genotype at locus B | *g/g* | *g/G* | *G/G* |
| *b/b* | white | grey | grey |
| *b/B* | black | grey | grey |
| *B/B* | black | grey | grey |

For example, two loci (*B* and *G*) influenced the hair colour in mice, each locus with two possible alleles, *B* or *b* and *G* or *g*. The possible phenotypic outcomes (white, black or grey hair) given genotype are shown in Table 1. Generally, allele *G* and *B* were dominant against

allele *g* and *b*. Nevertheless, individuals with any copies of the *G* allele have grey hair regardless of genotype at locus *B*, i.e. the effect at locus *B* is masked by that of locus *G*. In summary, allele *G* at locus *G* is epistatic to allele *B* at locus *B*.

Figure 1 shows a classical example of epistasis using flower colour in sweet peas. Combinations of mutations at two loci encoded enzymes that are responsible for processing anthocyanin operate within a single biochemical pathway.

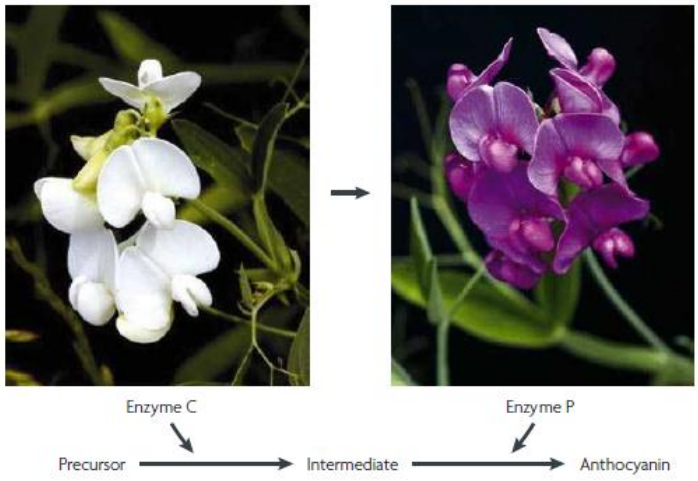


**Figure 1: Reconstructing genetic pathways using epistasis analysis according to PHILLIPS (2008)**

Literature characterizes three main categories of epistasis:

*Functional epistasis* operated directly at interfaces between proteins, whereby these proteins interact within the intracellular signalling pathways or directly with one another (Phillips 2008). This is a strictly biological description without a direct genetic link. A better expression would be protein-protein interaction (Hansen and Wagner 2001). This type of epistasis used in studies of immune functions and their interactions like cytokines and their receptors, or antigen receptors and their binding partners (Rose and Bell 2012).

*Compositional epistasis* is a new term that describes the traditional meaning by blocking of the phenotype of an allele by an allele at another locus. Therefore, it describes the way that a specific genotype was constituted and how this specific genetic background influences a set of given alleles (Phillips 2008).

The concept of *statistical epistasis* used in quantitative genetics was modified according to FISHER'S (Fisher 1919) definition, where the genetic variations in a population broke down to its additive, dominance and epistatic components (Phillips 2008). Statistical epistasis is

equally crucial, because the accuracy of predictive models of disease risk derived from population-level studies depends on understanding the estimation and magnitude of statistical epistasis (Sackton and Hartl 2016).

Unfortunately, there was no connection found between functional and statistical epistasis and it is not permissible to implement a statistical test and interpret the outcome in a biological way (Cordell 2002). Statistical interaction did not necessarily imply interaction on the biological or mechanistic level. The reason is, that we have to differentiate between functional epistasis that affects the expression of particular genotypes in individuals and statistical epistasis that describes genetic variation in populations (Sackton and Hartl 2016). The basic problem was that any given data or statistical model could usually be achieved from a number of completely different underlying mechanisms or models. Only if the base biological model can be postulated in some detail, it is likely that the statistical model allowed insights into the underlying biological mechanisms (Cordell 2002).

Literature research for other species than cattle in context of epistasis showed a wide range of results. The presented studies refer to fertility and reproduction traits in humans.

In neuropsychiatric diseases like Alzheimer's disease are genetically complex, with combined influences from multiple interacting genes and therefore a good example for epistasis. Bullock et al. (2013) verified an epistatic interaction between a SNP on the HHEX/IDE/KIF11 locus on chromosome 10 and a SNP on GSTM3 gene. This interaction was associated with Alzheimer's disease risk. In detail, HHEX gene related to risk of type 2 diabetes, IDE played a role in degradation of β-amyloid and KIF11 was involved in microtubule axonal transport of amyloid precursor protein. The epistatic SNP on GSTM3 was related to the prevention of oxidative stress in the brain (Bullock et al. 2013).

In addition, Hohman et al. (2016) emphasised the importance of epistatic interactions between SIRT1 and ABCB1 through alterations in amyloid clearance in Alzheimer's disease risk. SIRT1 showed to suppress amyloid-beta production and ABCB1 played a key role in amyloid-beta clearance. Multiple sclerosis is one of the most common neurological diseases in young adults and classified as an autoimmune disease. It expressed an inflammatory disease of the central nervous system characterized by myelin loss, axonal pathology, and progressive neurological dysfunction. Epistatic interactions between alleles

HLA-DRB1, HLA-DQA1, and HLA-DQB1 appeared to play central role in multiple sclerosis susceptibility (Lincoln et al. 2009).

Human height examined for epistatic interactions between chromosomes 2 and 6. For the SNP position on chromosome 6 genes like COL11A2, CCD, RUNX2 and RXRB associated to longitudinal growth or skeletal development were found. In detail, mutations of COL11A2 caused short stature, CCD gene was responsible for skeletal dysplasia and growth retardation. Further, RUNX2 gene regulated osteoblast differentiation and RXRB gene encodes retinoid X receptor-β, which was important for longitudinal growth. On the exact position for SNP on chromosome 2 was no gene located and the searched region contained no genes, which knew to be directly involved in skeletal growth (Liu et al. 2006).

In rats, quantitative trait loci (QTL) on chromosome 10 investigated to influence blood pressure. Charron et al. (2005) demonstrated that QTL4, QTL1 and QTL3 are epistatic to one another in their effects to blood pressure, and by adding QTL2 from chromosome 10 to the previous QTL combination, blood pressure increased proportionately. As the increase was proportional, rather than different for each combination it could concluded that there was no epistatic interaction between QTL2 and the other 3 QTLs on chromosome 10.

Preeclampsia is a leading cause of perinatal morbidity and mortality and is characterized by hypertension and proteinuria after 20 weeks of gestation. Two important genes, COMT and MTHFR, are associated with preeclampsia. Variation in COMT associated with changes in enzyme activity levels. The COMT enzyme involved in a wide variety of physiological processes, such as prefrontal cortex function and lipid metabolism and implicated in diseases. Variations in the MTHFR gene associated with elevated homocysteine, a risk factor for endothelial dysfunction, vascular disease, and preeclampsia. Epistatic interactions between foetal COMT and MTHFR resulted in significantly increased risk for preeclampsia in human population (Hill et al. 2011). Furthermore, the effect of genes EGR2, CTNNA3, LRRTM3, DDX50, HK1, TACR2 on chromosome 10 was in an epistatic interaction with the STOX1 gene in humans. The results showed a three times higher transactivation potential for preeclampsia. Especially, the Y153H variant of STOX1 carried the highest risk of maternal and foetal morbidity and mortality (Oudejans and van Dijk 2008).

Premature ovarian failure (POF) defined as the development of amenorrhea secondary to loss of ovarian function in women younger than 40. CYP19A1 gene encoded the enzyme

aromatase, which was responsible for the final step in the biosynthesis of oestrogen. Follicle-stimulating hormone induced maturation of ovarian follicles by acting on the FSH receptor expressed on granulosa cells. A significant epistatic interaction between SNPs on CYP19A1 gene and FSHR gene influenced the risk of POF (Kim et al. 2011). A further study by Pyun et al. (2013), showed epistatic interaction between SNPs on IGF2R and ADAMTS19 genes for POF. IGFs showed key functions in ovarian feolliculogenesis in mammals, especially IGF2R played an important role in the regulation of steroidogenesis. ADAMTS gene family members suggested to be involved in follicular growth and ovulation, but the function of ADAMTS19 itself is still poorly studied.

The FOXO transcription factors were essential in human development and adult physiology. In roundworms *(C. elegans)*, FOXO showed to regulate life span. In humans, genetic variations FOXO1a and FOXO3a have been associated with longevity. Tan et al. (2013) concluded in their study, that an epistatic interaction between SNP on FOXO3a gene and SNP on FOXO1a gene contributed to human longevity.

For development of obesity an epistatic interaction between SNPs on PRKD1 and FTO was investigated. Both loci indicated an interaction influencing body mass index during growth. FTO is also expressed in hypothalamus and was responsible for feeding behaviour and associated with emotional and uncontrolled eating. PRKD1 played a role in glucose regulation by influencing insulin signalling (Young et al. 2016).

Only a few studies of epistasis in functional traits were conducted in cattle. In dairy cattle the epistatic regions influencing stillbirth and calving ease were found from 2.1-2.5Mb, 36.6Mb and 44.7Mb on BTA21. The two known genes in the region from 2.1-2.5Mb are UBE3A and ATP10A, which were associated to Angelman and Prader-Willi syndrome. The epistatic interactions from BTA21 were connected to BTA6 (4.2 Mb), BTA14 (23.5 Mb), BTA17 (64.8 Mb), BTA21 (36.6 Mb) and BTA21 (44.7 Mb). These regions were related to craniofacial development, growth and size related genes and other protein coding genes which had to be studied more precisely (Mészáros, Taferner and Sölkner 2016). Further epistatic studies for stillbirth in Fleckvieh cattle showed main epistatic interactions on BTA 14 from 9 to 31Mb. In particular the region around 25Mb contained genes connected to height and body size such as PLAG1, CHCHD7, LYN, RDHE2 (SDR16C5) and PENK. Even if body size is not directly related to stillbirth, a correlation between difficulties during calving

caused by big calves and stillbirth could be observed. Additionally, an interesting region at 50.5Mb contained the TRPS1 gene influencing bone malformations (Mészáros and Sölkner 2016; Ackert-Bicknell et al. 2012). A number of significant epistatic effects for milk yield at the beginning on BTA14 and near DGAT1 gene were investigated for Holstein and Jersey cows. Further research found an association with the mutation in the DGAT1 gene, but the inclusion of the additive effect from DGAT1 into the epistatic model minimized the significant effect. Thus, the results concluded that individual non-additive effects only made a small input to the genetic variation of milk yield and fertility (Aliloo et al. 2015). Significant evidence of epistasis was observed in Holstein cows for fat content between LEPR and DGAT1 and for protein content between LEPR and BTN1A1. Physiological mechanisms for the epistatic interaction between LEPR and DGAT1 were not studied in detail so far. There is evidence, however that leptin modulates some DGAT1 functions. Leptin might be involved in several pathways of milk lipid synthesis, e.g. if prolactin was present, leptin enhanced the fatty acid synthesis in the mammary gland. A lack of DGAT1 led to an increased sensitivity of leptin production in mice (Suchocki, Komisarek and Szyda 2010).

For growth, carcass and fertility traits of beef cattle a number of epistatic effects have been detected. The trait ‘live weight measured post weaning’ shows epistatic effects on BTA2, 5, 8, 9, 19 and 29 and could identify interactions with the PLAG1 gene. As interacting candidate genes could be found MBNL1 on BTA1, FAT/CD36 on BTA4, GRN, FASN and ITG on BTA19 and INS and IGF2 on BTA29. Although these results were not significant, an interaction between PLAG1 and IGF2 is biologically plausible, because PLAG1 regulates many genes and pathways, including the IGF2 pathway (Bolormaa et al. 2015). Ali et al. (2015) identified epistatic loci located in 19 potential genes that affect the serum concentration of IGF-1 (insulin-like growth factor-1) and ultrasound-scanned fat rump depth traits in Australian Brahman cattle population. The strongest significant epistatic interaction for IGF-1 was detected between 86.5Mb on BTA10 and 7.8Mb on BTA16 as well as between 44.3Mb and 18.8Mb on BTA17.

Epistatic interactions also play a role in expressions of diseases. A study by Knaust et al. (2016) deals with the "rat-tail" syndrome (RTS) in cattle and is characterized by misshaped, curly and sparse hair and by missing hairs at the tail switch. The RTS phenotype results from an epistatic interaction between three independent loci, which is necessary for the

expression of the RTS phenotype. More precisely, an interaction among the PMEL gene at 55Mb on BTA5, the MC1R gene on BTA18 and the RTS locus in the region between 14-22Mb on BTA5 caused this specific disease in cattle.

Fürst and Fürst-Waltl (2006), van Pelt et al. (2015) and the Federation of Austrian Fleckvieh cattle breeders (2013) defined the phenotypes used in this study as follows:
For the trait **fertility**, the characteristics non-return rate, calving interval, fertility disorders and cyst formation were included into the breeding value for fertility.
**Calving ease** is based on the 5-level ADR-scale self-assessed by the farmer:

1. easy (without help or help not necessary, calving at night)
2. medium (1 helper or easy use of mechanical pulling aid)
3. difficult (several helpers, mechanical pulling aid and/or veterinarian)
4. Caesarean
5. Embryectomy

**Longevity**, or length of productive life is defined as the time from first calving to the day of last official milk yield testing, before the animal died or was culled for slaughter (including dry periods).
The trait **stillbirth** counts all stillborn calves and those, who die within 48 hours after birth. The reason is that from a veterinary point of view, an infection immediately after birth cannot lead to the calf's death so quickly.

The effects of difficult calving's are several, like possible losses in milk, fat, and protein yields in the next lactation, a poorer fertility, increased cow and calf morbidity and increased veterinary costs. Furthermore, calving difficulties impaired reproductive performance that resulted in more days in between two calving and more unsuccessful inseminations per pregnancy (Dematawewa and Berger 1997). Factors like dystocia, size of the calf and sex of the calf were associated with stillbirth (Olsen et al. 2010). Also gestation length and season of calving reported to affect the rate of stillborn calves (Johanson and Berger 2003). Calving ease and stillbirth are reproductive traits of economic importance in dairy cattle, but both traits with low heritability. Positive correlations between calving ease and stillbirth could be found (Heringstad et al. 2007).

Female fertility is important for the maintenance of the production in a dairy cattle herd. In Nordic Holsteins it became evident, that BTA4 and BTA13 showed the most significant genetic influence for fertility traits like number of inseminations per conception, 56-day non-return rate, days from first to last insemination and the length in days of the interval from calving to first insemination. Five candidate genes (SEMA3C, CD36, GNAT3, 5SrRNA and U6) on BTA4 were found in the region from 38.7-40.9Mb. For BTA13 no candidate genes were identified for the candidate region of 33.2-34.4Mb (Höglund et al. 2014).

Longevity is a highly required trait in the dairy cattle breeding, because it affects overall profitability. Nayeri et al. (2017) identified several significant regions on BTA5, 6, 7, 14, 18, 20 and 21 for direct herd life and on BTA5, 6 and 18 for indirect herd life. The most significant SNP was located on BTA18 at 42-65Mb related to a QTL associated with direct calving at 57.12Mb.

Results from genome-wide association studies (GWAS) showed that BTA2,4,5,17,19 and 27 associated with fertility traits, in particular with non-return rate (Frischknecht et al. 2017; Minozzi et al. 2013; Schulman et al. 2011; Höglund et al. 2014). For calving ease, Saatchi et al. (2014) identified that BTA6, 14 and 20 influenced the direct and maternal calving ease in Fleckvieh cattle. Further GWAS showed relationships for BTA6 and 21 with calving ease (Chen et al. 2010; Pereira et al. 2016; Kanber et al. 2009; Pausch et al. 2011; Bongiorni et al. 2012; Matic et al. 2016; Szewczuk et al. 2013). Zhang., L. et al. (2014) associated bone mineral density with BTA3, 6 and 21. As a consequence, decreased bone density will affect the length of productive life of livestock. Olsen et al. (2010) and Cole et al. (2011) found signals for stillbirth in and BTA6, 9, 18, 20 and 23 in GWAS analysis.

Fertility and health traits are complex and high priority traits in dairy cattle from a breeding perspective. It is interesting to explore them further, with special focus on possible epistatic effects.

## *1.2 Aim of the thesis*

The main goal of the thesis is to find genomic regions responsible for epistatic interactions for the traits longevity, fertility, calving ease and stillbirth in Fleckvieh cattle using SNP chip data.

# 2 Material and methods

## *2.1 The data*

### 2.1.1 The Fleckvieh breed

The Fleckvieh has the largest population in Austria with 1.5 million animals and a distribution area from the alpine mountain regions to the lowlands in the east. The breed is used as dairy or dual-purpose breed as a crossbreeding partner in suckler cows for meat production. A high proportion of the young cattle grazed on alpine pastures, which has a positive effect on the health and longevity. Under appropriate management the milk yield is comparable to specialized milk breeds. Fitness features such as fertility, longevity, calving ease and persistence are given special attention. The exterior type and udder traits are further priorities in breeding (Association of Austrian Cattle Breeders 2014). Currently, 256,392 herd book cows achieved 7,370kg milk, 4.16% fat, 3.41% protein and 558kg fat and protein (Federation of Austrian Fleckvieh cattle breeders 2016).

The breeding goal is a sustainable improvement in profitability in milk production, taking into account meat and especially the fitness traits. This objective is achieved most efficiently by selection on the basis of the economic value (Federation of Austrian Fleckvieh cattle breeders 2012).

With the new genomic breeding program, the breeding progress can be significantly increased. Currently the annual breeding progress of +3.2 total merit index points, +2.6 milk value points (+99 milk/kg, +0.001 fat% and -0.005 protein%), +0.6 meat value points and +1.8 fitness value points was recorded on average among bulls of the birth cohorts from 2010 to 2015 (Kalcher et al. 2017).

The new weighting in the breeding program in April 2016, increased the relative importance of beef from 38:16:46 for milk:meat:fitness to 38:18:44. This adaptation is a response to the changed economic reality after the loss of the milk quota and further to strengthen the dual-purpose use of the breed. Within the fitness block, the most important changes are the doubling of the weight of fertility and the inclusion of the new vitality value. Since the breeding value estimation in August 2013, the health traits have also been integrated into the total merit index (TMI). The fitness breeding value include data about

longevity, fertility, somatic cell count, calving ease, stillbirth and persistence of Fleckvieh cattle (Federation of Austrian Fleckvieh cattle breeders 2012).

### 2.1.2 Genotyping data and phenotypes

The Illumina BovineSNP50 BeadChip contains 54,609 highly informative SNPs. This high density SNP data is widely used in animal breeding and genetics, for example for genome-wide enabled selection, identification of quantitative trait loci, evaluation of genetic merit of individuals and comparative genetic studies (Illumina Inc. 2017). In order to investigate epistatic interactions in complex traits of Fleckvieh, genotypes from both versions of Illumina BovineSNP50 BeadChips and the Illumina BovineHD chip from the German-Austrian genotype pool were considered.

For the current study, de-regressed breeding values of 7,327 Fleckvieh sires were provided by ZuchtData EDV-Dienstleistungen GmbH. The number of available de-regressed breeding values (EBVde) varied from trait to trait, as shown in Table 2.

**Table 2: Number of available de-regressed breeding values for each trait**

| Trait | Number of EBV.de per trait |
|---|---|
| Fertility (FER) | 6,658 |
| Calving ease (CE) | 7,279 |
| Longevity/ Length of productive life (LPL) | 5,592 |
| Stillbirth (SB) | 7,261 |

### 2.1.3 Quality control

The management of the genotype data and its quality control were done using PLINK1.9 (Chang et al. 2015). As first step, only autosomal SNPs were kept, i.e. sex chromosomes and unplaced SNPs were excluded. To delete SNPs below commonly used quality control thresholds (Mészáros and Sölkner 2016; Mészáros, Taferner and Sölkner 2016; Knaust et al. 2016) following commands have been used:

*--mind 0.1* was the missing rate per animal and excluded genotypes with more than 10% missing rate.

*--maf 0.05* was the minor allele frequency (MAF) which referred to the frequency of the second most common allele occurred in a given population and excluded SNPs below 5% (=monomorphic SNPs).

*--geno 0.1* was the missing rate per SNP and excluded SNPs with genotyping rate below 90%.

*--hwe 0.000000001* filtered out all variants which had the Hardy–Weinberg equilibrium Fishers exact test with p-value below $10^{-9}$.

The final number of SNPs and individuals included on each set is shown in Table 3.

**Table 3: Overview of steps in quality control with remaining genomic data after each analysing step**

| Data size at the start of the step | Plink command | Number of excluded SNPs or animals |
|---|---|---|
| 780,146 SNP; 7456 cattle | --autosome | 39,494 SNP |
| 740,652 SNP | --geno 0.1 | 690,439 SNP* |
| 50,213 SNP | --hwe 0.000000001 | 388 SNP |
| 49,825 SNP | --mind 0.1 | 40 cattle |
| 49,825 SNP; 7416 cattle | --maf 0.05 | 11,568 SNP |
| 38,257 SNP; 7416 cattle | | |

*large number of excluded SNPs, because approx. 90% of them appear only in the HD SNP chip, but not in the BovineSNP50 BeadChip

## *2.2 Software used*

### 2.2.1 GEMMA

GEMMA is a software we used for GWAS, with the Genome-wide Efficient Mixed Model Association algorithm for a standard linear mixed model. Marker association tests with the univariate linear mixed model (LMM) and a single phenotype, took population stratification and sample structure into account (Zhou and Stephens 2012).

As input data PLINK binary ped format files with no missing phenotypes used. Before running GEMMA, an estimated relatedness matrix file from genotypes was created with the command *-gk 1*, which calculated the centered relatedness matrix. The associations test with LMM performed with a likelihood ratio test. The results were used as the basis for the SNP restriction for further epistasis analysis.

### 2.2.2 PLINK1.9

PLINK is an open-source C/C++ toolset, where all pairwise epistatic combinations of SNPs can be tested. The software was able to analyse large data sets including hundreds of thousands of SNPs genotyped for thousands of individuals. The output consisted only of pairwise epistatic results above a certain significance value. Also, for each SNP, a summary of all the pairwise epistatic tests was given (Purcell et al. 2007; Chang et al. 2015).

In this work, the PLINK program was used to estimate the epistatic interactions (Chang et al. 2015; Schüpbach et al. 2010), with the command *--epistasis.* The test used for quantitative traits a linear regression model following in the form of

$$Y = \beta_0 + \beta_1 g_A + \beta_2 g_B + \beta_3 g_A g_B$$

for each allele pair (A and B), where $g_A$ and $g_B$ were allele counted and then the $\beta_3$ coefficients tested for significance (Purcell and Chang 2015). Further analyses for the most interesting regions, as well as statistical evaluations or plotting graphs were done with the program R® (RStudio Team 2016).

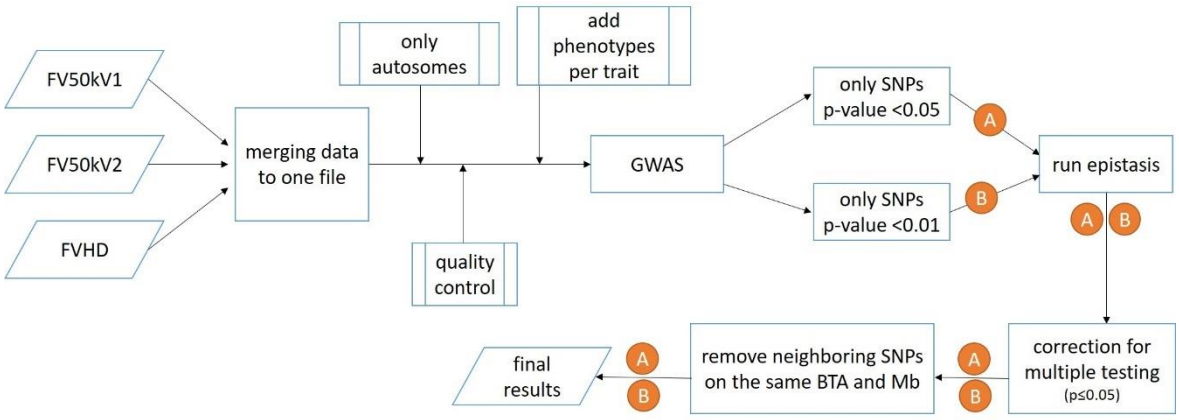


**Figure 2: Diagram workflow of the study design from raw data to final results**

As first step in the analysis PLINK1.9 was used to prepare the genotype data by merging the different files to one file. Next, only autosomal chromosomes had been kept and quality control was performed (see **Fehler! Verweisquelle konnte nicht gefunden werden. Fehler! Verweisquelle konnte nicht gefunden werden.**). To establish binary files with phenotypic data, we created with the commands *--pheno [PHENOLIST]* and *--pheno-name [TRAIT NAME]* for

each trait one file with the specific phenotypes. Afterwards the GWAS with GEMMA was implemented (see 2.2.1 GEMMA).

To reduce the dimension of SNP combinations after GWAS, only SNPs using the p-value below the thresholds of 0.01 and 0.05 have been kept (Ali et al. 2015) for epistatic analysis with *--epistasis* in PLINK1.9.

Finally, for taking care of multiple testing, adjusted p-values for the epistasis results following Bonferroni-Holm procedure (Holm 1979) were generated and only pairwise tests below the significant threshold of p≤0.05 selected, as shown in Table 4. For that, $\frac{N*(N-1)}{2}$ pairwise interactions were tested where N is the number of SNPs before running epistasis analysis (Aliloo et al. 2015).

The results for calving ease and stillbirth showed because of the pairwise combination SNPs with significant interactions with neighbouring SNPs. Finally, to remove duplicate results we implemented another filtering step, removing SNPs that likely pointed to the same interactions, i.e. both associated SNPs were on the same BTA and Mb.

**Table 4: Overview of remaining SNP-pairs after running epistasis and after a correction for multiple testing**

| Trait | p < 0.05 | | p < 0.01 | |
|---|---|---|---|---|
| | sig. epistatic interactions after --epistasis | sig. epistatic interactions after multiple testing | sig. epistatic interactions after --epistasis | sig. epistatic interactions after multiple testing |
| FER | 1,420 | 3 | 52 | 1 |
| CE | 10,082 | 1,566 | 2,421 | 1,162 |
| LPL | 2,177 | 6 | 85 | 0 |
| SB | 3,392 | 788 | 1,425 | 715 |

**Post processing**

The genes and their positions were taken from the National Center for Biotechnology Information (NCBI 1988) database. We defined our regions of interest as follows: The exact base pair location of the significant SNPs what we found through our analysis and with a range of ± 0.5Mb around the SNPs.

If there was no gene found in the exact searched position or in the vicinity of ± 0.5Mb, larger regions were examined to find interesting genes for the specific trait.

# 3 Results and discussion

## *3.1 Fertility*

After GWAS a filtering step was implemented, which included SNPs at p<0.01 level (FER1) and p<0.05 level (FER5) for running the epistasis analysis only 1 SNP pair (FER1) and 3 SNP pairs (FER5) showed significant interactions at the p<0.05 level, after correction for multiple testing. The following section described how epistatic interactions on BTA3, 4, 13 and 29, which suggested the importance of those BTA regions for fertility indicators like embryo development or premature birth.

**Table 5: Significant SNP pair for fertility p<0.01**

| CHR1 | SNP1 | SNP1.bp | CHR2 | SNP2 | SNP2.bp |
|---|---|---|---|---|---|
| 29 | Hapmap24835-BTA-140780 | 48123622 | 29 | ARS-BFGL-NGS-117323 | 49357135 |

**Table 6: Significant SNP pairs for fertility p<0.05**

| CHR1 | SNP1 | SNP1.bp | CHR2 | SNP2 | SNP2.bp |
|---|---|---|---|---|---|
| 3 | BTB-00143481 | 92015484 | 13 | BTB-00525367 | 47915618 |
| 4 | BTB-00203567 | 95388890 | 4 | ARS-BFGL-NGS-40249 | 105169576 |
| 13 | ARS-BFGL-NGS-9922 | 69970083 | 29 | ARS-BFGL-NGS-29834 | 36803768 |

The significant SNP on BTA29 located on the PPFIA1 gene, which was identified, but not described in cattle. Other protein coding genes like FGF4, FGF3, ANO1, FADD, CTTN and SHANK2 located in the region 47.65-48.78Mb. In humans, the PPFIA1 gene encoded a tyrosine phosphatase of the liprin family. PPFIA1 was an important gene regulating cell edge dynamics during cell motility and required for migration and invasion of some breast cancer cells (Astro et al. 2011; Dancau et al. 2010; Yang et al. 2017; Chiaretti et al. 2015). Further, Choi et al. (2014) showed that the combined expression of TMEM16A, PPFIA1 and FADD had significant association with disease-free survival in invasive ductal breast carcinoma.

The other SNP from the epistatic pair also located on BTA29, but on this exact position there was no coding gene. In the region ±0.5Mb around this SNP the CDKN1C and PHLDA2 genes were located, which were major contributors to embryo growth and placental development in cattle (Driver et al. 2013; Dória et al. 2010). Especially CDKN1C attracted attention as a key gene in Beckwith-Wiedemann syndrome (BWS) and cancer. For cattle,

BWS referred to as large offspring syndrome caused overgrowth disorders such as excessive birth weight, large tongue, umbilical hernia, hypoglycaemia and visceromegaly (Z. Chen et al. 2013; Hori et al. 2010).

Twelve protein coding genes from region 91.7-92.6Mb were identified for BTA3 in cattle, but not further researched. In the human genome the protein coding gene BSND connected to the Bartter syndrome Type IV. This is a phenotype of neonatal Bartter syndrome due to defects in chloride channels, which led to deafness and facial features like a triangular face, large eyes and protruding ears. Patients with Bartter syndrome type IV also manifested for example an excess of amniotic fluid in the amniotic sac, premature birth and postnatal polyuria (Kitanaka et al. 2006). In cattle, especially these prenatal anomalies may influence the development of the calf.

The epistatic SNP on BTA13 located on the protein coding gene PROKR2, called prokineticin receptor 2 belongs to the family of G-protein-coupled receptors. PROKR2 was a common receptor form in the central nervous system and also expressed in the corpus luteum, beside PROKR1. In the central nervous system, PROKR2 abundantly expressed in major target nuclei of the suprachiasmatic nucleus (SCN) output pathway, which controlled the circadian rhythm of physiological and behavioural processes in mammals (= Master-Clock) (Cheng et al. 2002; Kisliouk et al. 2007). Further studies in humans and mice showed, that a mutation in PROKR2 had influence on fertility by affecting foetal testis differentiation and also was associated with the loss of smell (=Kallmann syndrome) (Dodé and Rondard 2013).

On BTA4 no known gene on this exact location for the significant SNP was found. In the searched region from 94.3-95.9Mb eight genes in cattle had been detected: CPA5, CPA1, CEP41, MEST, COPG2, TSGA13, KLF14 and MKLN1, but those were not further researched in cattle. The MEST and KLF14 genes showed in humans effects on obesity and higher metabolic disease risk such as type 2 diabetes or regulation of fat gene expression if a epigenetic malprogramming occurred (Consortium et al. 2011; Hajj et al. 2013).

The epistatic SNP was located on the same BTA on gene TMEM178B, within only 9.8Mb. TMEM178B was identified, but not further researched gene for transmembrane proteins neither in cattle nor in humans or mice. Further genes in the searched region ± 0.5Mb were identified, but not researched in detail.

The last significant epistatic interaction had been found between BTA13 and BTA29. Three genes (MAFB, TOP1 PLCG1) in region from 69.5-70.5Mb detected on BTA13, but none of them on the exact position of the researched SNP and none with any context to fertility issues.

Likewise, on BTA29 no gene found on the exact SNP position of 36.80Mb. Close to the SNP, the PRDM10 gene coding for transcription factor was located. PRDM10 was a target gene for tissue differentiation and bone formation defects during mouse embryogenesis and involved in the pathogenesis of arthritis in human (Park and Kim 2010; Park et al. 2013). Further genes (APLP2, ST14, ADAMTS8, ADAMTS15) found in the region showed connections to diseases like Alzheimer and different human cancers and tumours'.

## *3.2 Calving ease*

After GWAS a filtering step was implemented, which included SNPs at $p<0.01$ level (CE1) and $p<0.05$ level (CE5) for running the epistasis analysis. For the trait calving ease 1,162 SNP pairs (CE1) and 1,566 SNP pairs (CE5) showed significant interactions at the $p<0.05$ level, after correction for multiple testing. For further calculations only results from CE1 had been used.

In SNP pairs from CE1 some duplicated results of neighbouring SNPs were shown. Therefore, another filtering step in R® removed such cases and only 250 SNP pairs were pictured in the final result. For the trait calving ease, 214 epistatic interactions within the same chromosome and 36 epistatic interactions between different chromosomes occurred.

The main region for genes affecting calving ease was found on BTA14, but also other BTAs played an important role. In general, calving ease associated with the birth weight of calves (Johanson and Berger 2003). It seems logical that large calves would determine more birth difficulties than small calves, compared between cows with the same stature.

### 3.2.1 Interactions between different chromosomes

The most active region was BTA14 with 26 interactions between various SNPs located between 1.80Mb and 34.45Mb. As showed in Figure 3, one part of epistatic interaction (first SNP) was located on BTA1 to 14, while the other part of epistatic interaction (second

SNP) is located on BTA14 to 27. Due to this separation on BTA14 like a notch the data showed some SNPs which affected two or more SNPs simultaneously (Table 7).

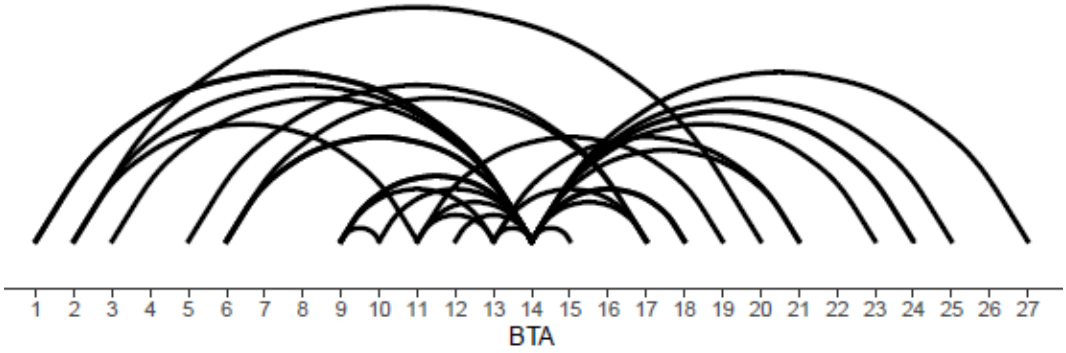


**Figure 3: Epistatic interactions for calving ease between different chromosomes**

On BTA6 for the region from 38-39.1Mb several genes such as ABCG2, PKD2, SPP1, IBSP, MEPE, LAP3, MED28, FAM184B, DCAF16, NCAPG and LCORL had been found. The gene LAP3 encoded for a leucine aminopeptidase and involved in the oxytocin metabolism and in protein maturation and degradation (Zheng et al. 2011). The second gene, NCAPG associated in cattle with foetal growth and carcass size. LCORL related to height in humans and controlled stature in cattle. LAP3, NCAPG, LCORL showed a direct association with calving ease, which had been examined in Piedmontese cattle breed (Bongiorni et al. 2012). The most common epistatic interactions appeared on BTA14. From 15.7-16.7Mb two genes (NSMCE2, KIAA0196) related to height and physiological features in humans. A mutation in NSMCE and KIAA0196 was associated with dwarfism and insulin resistance (Payne et al. 2014) or neurodegenerative gait disorders (Jahic et al. 2015). Within the 22.8-23.8MB, 24.9-25.9Mb and 25.1-26.1Mb regions, several interesting protein coding genes connected to calving ease like SOX17, PLAG1, SDR16C5, PENK, IMPAD1, MOS, LYN, RPS20 and CHCHD7 were found. The genes PLAG1, CHCHD7, MOS, RPS20, LYN and PENK found to influence human and cattle height. Especially PLAG1, which interacted with growth factors or lead to growth retardation or associate with peripubertal body weight, was one of the most important genes in connection with calving ease (Utsunomiya et al. 2013).

On BTA10 the region from 7.7-8.7Mb studied for protein coding genes e.g. AGGF1, CRHBP, F2RL1, F2R and IQGAP2. AGGF1 was an angiogenic factor and promote varicose veins, capillary malformation and hypertrophy of bone tissue (= Klippel–Trenaunay syndrome). The Klippel-Trenaunay syndrome mainly affected the legs in humans and leaded to an

increased girth and length of the legs and arms (Kihiczak et al. 2006; Timur, Driscoll and Wang 2005). Another interesting gene was IQGAP2, which encoded a protein that interacted with components of the cytoskeleton to regulate cell morphology and motility. IQGAP2 described to be associated with preterm birth (Uzun et al. 2016).

Epistatic interactions for calving ease were also present on BTA24 and BTA25. On BTA24 the DYM (dymeclin) gene, which was described in humans, occupied a central position to bone formation and brain development during early pregnancy. Mutations in DYM led to the Dyggve-Melchior-Clausen syndrome, which was characterized by dysplasia and mental retardation (Khalifa et al. 2011). The epistatic SNP on BTA25 located on the TRAF7 gene, which was not further researched in cattle. The region of interest between 1.2-2.2Mb included the gene CRAMP1L, which showed to be related with direct calving ease (Frischknecht et al. 2017), was located on 1.24Mb.

**Table 7: Epistatic interactions between more than two SNPs on different chromosomes for calving ease**

| CHR1 | SNP1 | SNP1.bp | CHR2 | SNP2 | SNP2.bp |
|---|---|---|---|---|---|
| 1 | ARS-BFGL-NGS-115371 | 24835526 | 14 | ARS-BFGL-NGS-112623 | 20635979 |
| 1 | ARS-BFGL-NGS-115371 | 24835526 | 14 | ARS-BFGL-NGS-607 | 18460103 |
| 6 | Hapmap28104-BTA-156698 | 12261839 | 14 | ARS-BFGL-NGS-33755 | 16185315 |
| 14 | ARS-BFGL-NGS-33755 | 16185315 | 24 | BTA-42867-no-rs | 49418132 |
| 6 | ARS-BFGL-NGS-112812 | 38627070 | 14 | BTB-01119513 | 34448087 |
| 6 | Hapmap30134-BTC-034283 | 38464203 | 14 | BTB-01119513 | 34448087 |
| 9 | Hapmap25907-BTA-159799 | 51473366 | 14 | ARS-BFGL-NGS-4939 | 1801116 |
| 9 | Hapmap25907-BTA-159799 | 51473366 | 14 | ARS-BFGL-NGS-76907 | 13384870 |
| 9 | Hapmap25907-BTA-159799 | 51473366 | 14 | Hapmap54618-rs29021334 | 25612510 |
| 10 | Hapmap35793-SCAFFOLD311748_23298 | 8204943 | 14 | Hapmap35443-SCAFFOLD20068_27016 | 20921507 |
| 14 | Hapmap35443-SCAFFOLD20068_27016 | 20921507 | 27 | ARS-BFGL-NGS-113900 | 10417461 |
| 14 | ARS-BFGL-NGS-607 | 18460103 | 25 | ARS-BFGL-NGS-15062 | 1712523 |
| 14 | ARS-BFGL-NGS-607 | 18460103 | 21 | ARS-BFGL-NGS-54399 | 3245487 |
| 14 | BTB-00556813 | 23384687 | 18 | ARS-BFGL-NGS-17826 | 64286141 |
| 14 | Hapmap46735-BTA-86653 | 25401722 | 18 | Hapmap42446-BTA-118372 | 15199711 |
| 14 | Hapmap46735-BTA-86653 | 25401722 | 18 | ARS-BFGL-NGS-17826 | 64286141 |
| 14 | UA-IFASA-7696 | 13998894 | 17 | ARS-BFGL-BAC-34029 | 16003681 |
| 14 | UA-IFASA-7696 | 13998894 | 15 | BTB-01561193 | 59891308 |

### 3.2.2 Interactions within the same chromosome

For the trait calving ease, BTA5, 10, 14 and 21 showed epistatic interactions within the same chromosome. From a total of 214 epistatic interactions on the same chromosome, 208 SNP pairs were found for BTA14. This again emphasised the importance of BTA14 for the trait. The results on BTA14 showed 63 different SNPs for the first position and 64 different SNPs for the second position for the epistatic interactions.

As showed in **Fehler! Verweisquelle konnte nicht gefunden werden.**, the main epistatic interactions on BTA14 took place between 20-25Mb. As described above, genes like PLAG1, CHCHD7, MOS, RPS20, LYN and PENK could be found in this region and influenced human and cattle height (Utsunomiya et al. 2013). (Pausch et al. 2011) investigated further SNPs on the BTA14 and also found an association between those six located SNPs in 20-25Mb region and calving ease and growth characteristics. These results were further confirmed by (Utsunomiya et al. 2013).

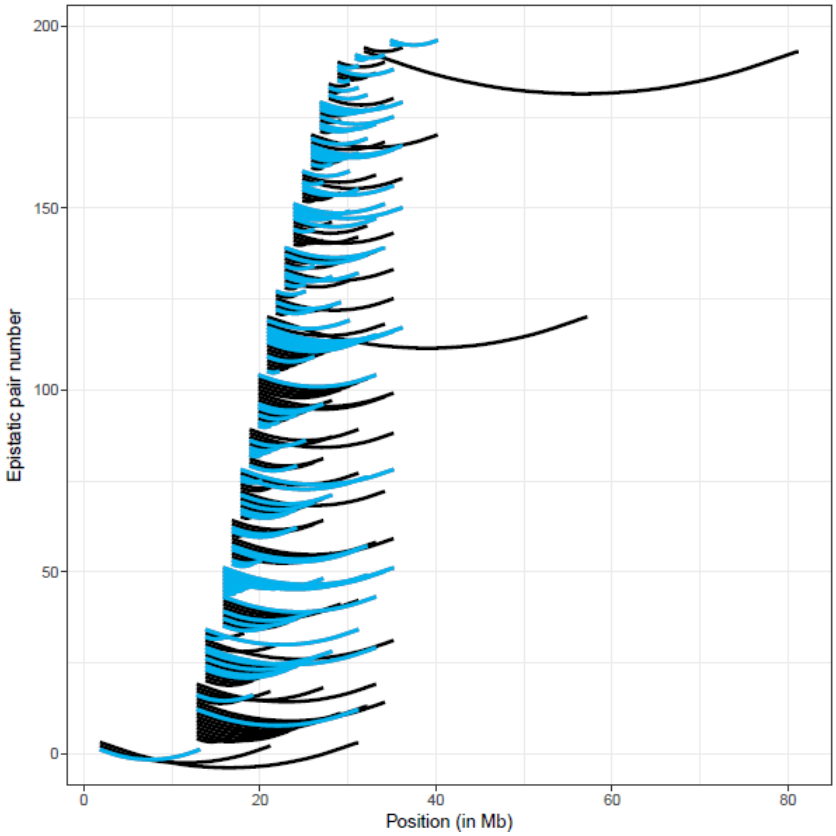


**Figure 4: Significant epistatic effects for calving ease on BTA14 (p ≤ 0.05 in black, p ≤ 0.001 in blue)**

Additionally, five SNPs showed long distances (>19.5Mb) to the second SNP for their epistatic interactions. Three of them were located at 1.80Mb on BTA14, which was the exact position of DGAT1 gene. DGAT1 is a protein coding gene and played the key role in synthesis of major milk lipids. Although the effect of DGAT1 on fertility traits was not intensively examined so far, mutation of DGAT1 could affect fertility through the combined signalling of various metabolites and hormones. This could reduce the gonadotropin release, which is necessary for the development of ovarian follicles and oocytes. In this regard, the ovulation rate could increase and the non-return rate would be reduced (Komisarek and Michalak 2008). One of these three epistatic SNPs is located at 31.22Mb, where the gene CYP7B1 was found in the vicinity ± 0.5Mb. CYP7B1 encoded proteins, which catalysed reactions like the synthesis of cholesterol, steroids and other lipids. Furthermore, a connection to reproduction abnormalities can be established by a study in mice from Stiles et al. (2009), which showed that loss of CYP7B1 leads to early ovarian failure.

The highest distance between epistatic SNP pairs was between SNPs on 31.97 and 80.75Mb. For the first SNP, there was not any gene coding on this exact position. In the searched region the gene ARMC1, MTFR1, PDE7A, DNAJC5B, TRIM55 and CRH were found, but had not showed any connection to reproduction traits or calving ease. The epistatic SNP, located on the position of the RALYL gene, was identified, but not further researched in cattle or any other species.

Besides BTA14, BTA5, 10 and 21 also showed several epistatic interactions between SNPs on the same chromosome (Table 8).

**Table 8: Epistatic interactions within the same chromosome at BTA5, 10 and 21 for calving ease**

| CHR1 | SNP1 | SNP1.bp | CHR2 | SNP2 | SNP2.bp |
|---|---|---|---|---|---|
| 5 | ARS-BFGL-NGS-7850 | 42775818 | 5 | ARS-BFGL-NGS-40753 | 48460111 |
| 10 | BTB-00408453 | 10955343 | 10 | BTB-00412151 | 12020216 |
| 21 | BTB-01171128 | 802673 | 21 | ARS-BFGL-NGS-73082 | 2642904 |
| 21 | ARS-BFGL-NGS-70221 | 2749974 | 21 | ARS-BFGL-NGS-112210 | 4193189 |
| 21 | ARS-BFGL-NGS-54399 | 3245487 | 21 | ARS-BFGL-NGS-113595 | 3498796 |
| 21 | ARS-BFGL-NGS-18711 | 4638691 | 21 | Hapmap52397-rs29025170 | 7694470 |

For the first part of each interaction, three SNPs on BTA21 were located near each other, pointing to the same genes. Five protein coding genes had been found in these regions. MAGEL2, MKRN3, NDN and UBE3A, ATP10A associated with the Prader-Willi and Angelman syndrome in humans. The Prader–Willi syndrome was a neurogenetic disorder characterised for example by neonatal muscular hypotonia, obesity starting in early childhood, hypogonadism, short stature and small hands and feet (Kanber et al. 2009). The lack of a functional paternal genes (MAGEL2, MKRN3, NDN) caused the Prader–Willi syndrome, whereas the lack of UBE3A resulted in the Angelman syndrome (Pausch et al. 2011). Kanber et al. (2009) suggested that Prader-Willi syndrome was not caused by a single-locus defect, but by a deficiency of several genes.

The last epistatic interaction on BTA21 established a connection between Prader-Willi syndrome (GBRG3 gene) and growth factors in humans. MEF2A, SYNM and IGF1R were related to growth and physique traits for example body size, atherosclerosis or regulation of muscle specific genes (F. Chen et al. 2010; Pereira et al. 2016; Szewczuk et al. 2013; Matic et al. 2016).

This led again to the conclusion, that the growth-related traits e.g. body-size or birth weight of calves were associated with calving ease in cattle (Johanson and Berger 2003).

## *3.3 Longevity*

After GWAS a filtering step was implemented, which included SNPs at p<0.01 level (LPL1) and p<0.05 level (LPL5) for running the epistasis analysis. For the trait longevity, 6 SNP pairs (LPL5) showed significant interactions at the p<0.05 level, after correction for multiple testing. For LPL1 no epistatic interactions had reached the threshold as defined by multiple testing, shown in Table 4.

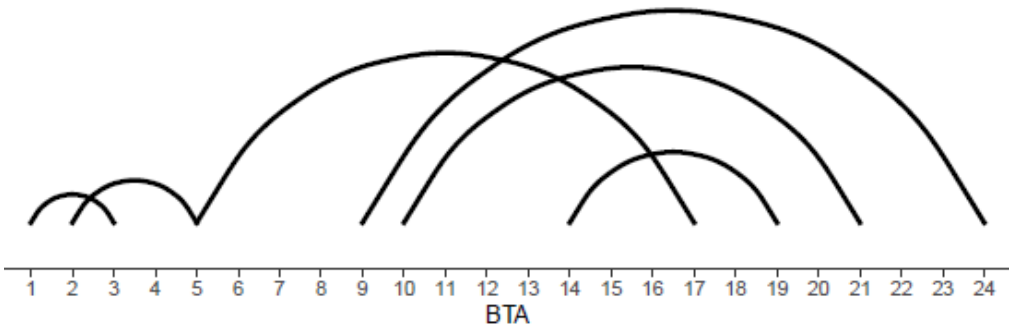


**Figure 5: Epistatic interactions for longevity between different chromosomes**

As described below, the identified BTAs showed connections to cattle longevity because of their influence on metabolic processes or diseases, which would lead to an earlier culling.

**Table 9: Significant SNP pairs for longevity p<0.05**

| CHR1 | SNP1 | SNP1.bp | CHR2 | SNP2 | SNP2.bp |
|---|---|---|---|---|---|
| 1 | ARS-BFGL-NGS-42159 | 150459773 | 3 | ARS-BFGL-NGS-103874 | 33307217 |
| 2 | Hapmap56915-rs29026677 | 23379654 | 5 | Hapmap25652-BTA-73867 | 65074617 |
| 5 | ARS-BFGL-NGS-32908 | 110320607 | 17 | ARS-BFGL-NGS-112404 | 59749528 |
| 9 | ARS-BFGL-NGS-74851 | 23322608 | 24 | ARS-BFGL-NGS-44158 | 46027109 |
| 10 | Hapmap25793-BTA-125304 | 29787321 | 21 | ARS-BFGL-NGS-5739 | 4825612 |
| 14 | Hapmap52798-ss46526455 | 1923292 | 19 | Hapmap34814-BES8_Contig361_961 | 20945594 |

First epistatic interaction for longevity showed up between SNPs on BTA1 and BTA3. For BTA1 the gene CLDN14, a member of the claudin family, had been found at the exact position of the significant SNP, but no further researches for cattle were done so far. The protein encoded by this gene is an integral membrane protein and a component of tight junction strands. In human, CLDN14 was associated with kidney stones and reduced bone mineral density. The CLDN14 gene was related with levels of urinary calcium and serum parathyroid (Tang et al. 2016; Thorleifsson et al. 2009; L. Zhang et al. 2014). Therefore, through its regulation of bone metabolism, diseases like osteoporosis could be associated with disorders in CLDN14 (L. Zhang et al. 2014). Eight further genes had been found in the searched region, but they had not been described in cattle. Two of these protein coding genes, SIM2 and HLCS, had also been reviewed in humans. These genes might show a connection to the trait longevity, because of their contribution to diseases e.g. Down-Syndrome, skin rash, delayed development or seizures (Chatterjee et al. 2013; Suzuki et al. 2005).

The epistatic SNP on the second locus was on the exact position of the protein coding gene KCNC4 at 33.30Mb on BTA3. KCNC4 encoded components of voltage-gated $K^+$-channels. The protein encoded by this gene was important for neuronal excitability. Leblanc (2010) described, that $K^+$-channels were recognized as key factors in the complex series of events controlling the cell cycle and growth. In the searched region ± 0.5Mb around the SNP, three further genes encoded members of the K+-channel family had appeared. Furthermore, the gene CSF1, which meant colony-stimulating factor 1, appeared in the searched region at position 33.60Mb. This protein coding gene was a hematopoietic cytokine and was

responsible for growth and differentiation of hematopoietic progenitor cells. Haematopoiesis is the process by which all mature blood cells and the immune system were set up. Additional CSF1 played an important role in placental physiology, because of its expression in the uterus. The high level of CSF1 expression during bovine pregnancy in uteroplacental tissues emphasized the importance during reproduction and pregnancy (R. S. F. Lee et al. 2003).

The second epistatic interaction was found between BTA2 and 5. The significant SNP was located exactly at position 23.38Mb on the CDCA7 gene. The CDCA7 (=cell division cycle associated 7) gene had not been further researched in cattle. In humans, mutations in this gene resulted to the life-threatening Immunodeficiency Centromeric Instability and Facial Anomalies (ICF) syndrome, which led e.g. to fatal respiratory and gastrointestinal infections or distinct facial anomalies. Thijssen et al. (2015) concluded that for the ICF syndrome a genetic heterogeneity had to be emphasized.

The epistatic SNP on BTA5 was on the position of the gene ANO4, which was not studied so far in cattle, human or any other species. Five more genes appeared in the searched region. SLC5A8 was a tumor suppressor and was first identified in the colon. Further, the transporter had been silenced in ten different cancers of other organs. In particular for treatment of cancer, a reactivation of this tumor suppressor gene might improve the success rate of medical cancer treatments. SLC5A8 gene showed a context to the health status and to another consequence to the trait longevity (Elangovan et al. 2013).

In the region from 109.8-110.8Mb on BTA5, 33 protein coding genes found on the cattle genome, but not studied in detail so far. On the exact position of the SNP was not any gene located, but the gene SOX10 was close. The gene SOX10 was a key transcription factor of neural-crest development. Mutations in SOX10 caused several types of the Waardenburg syndrome resulting in e.g. deafness, broad nasal root, stenoses of the colon associated with depigmented skin/hair patches and disparity of both iris skins of the eyes (Bondurand et al. 2007). The Waardenburg syndrome caused by this gene had also been identified in white or white-spotted animals (Reissmann and Ludwig 2013).

The epistatic SNP on BTA17 was located on gene KSR2, which was called kinase suppressor of ras 2 and was an intracellular scaffolding protein. This gene played a role in energy

homeostasis. A targeted deletion of KSR2 was associated with obesity, insulin resistance and impaired cellular fuel oxidation, studied for mice and humans (Pearce et al. 2013).

Another significant epistatic interaction was found between the SNPs on the genes DOPEY1 (BTA9) and SIGLEC15 (BTA24), but both genes had not been researched in cattle or humans yet. The same situation could be found between SNPs on the protein coding gene FMN1 on BTA10 and GABRG3 on BTA21.

The last significant interaction between SNPs for longevity was found between the SNP on the MAF1 gene on BTA14 and a SNP on BTA19, which was not located on a gene. In both searched regions more than 30 genes showed up. The gene MAF1 was described in humans as a regulator of glucose metabolism and lipid homeostasis (Mierzejewska and Chreptowicz 2016), but no further studies about health issues were done so far. The epistatic SNP on BTA19 was located near the SEZ6 gene, member of the family of seizure-related genes, which played a role at epilepsy and seizure activity (Wakana et al. 2000; Yu et al. 2007).

## *3.4 Stillbirth*

After GWAS a filtering step was implemented, which included SNPs at $p<0.01$ level (SB1) and $p<0.05$ level (SB5) for running the epistasis analysis. For the trait stillbirth 715 SNP pairs (SB1) and 788 SNP pairs (SB5) showed significant interactions at the $p<0.05$ level, after correction for multiple testing. The follow up text described only for the results from SB1.
The results after correction for multiple testing showed all pairwise combinations, SNPs which showed significant interactions with neighbouring SNPs were filtered out. The filtering step in R® removed such cases and 169 SNP pairs had been kept. For stillbirth, 165 epistatic interactions within the same chromosome and 4 epistatic interactions between different chromosomes occurred.
In contrast to the results of calving ease, in stillbirth, interactions within the same chromosome could only be found for BTA14.

### 3.4.1 Interactions between different chromosomes

When considered the results, it was noticeable that any interaction between different chromosomes was bound to BTA14. Again, the influence of BTA14 on stillbirth trait could be clearly recognized.

**Table 10: Epistatic interactions between different chromosomes for stillbirth**

| CHR1 | SNP1 | SNP1.bp | CHR2 | SNP2 | SNP2.bp |
|---|---|---|---|---|---|
| 5 | ARS-BFGL-NGS-37989 | 47906844 | 14 | UA-IFASA-7112 | 16109986 |
| 5 | ARS-BFGL-NGS-37989 | 47906844 | 14 | ARS-BFGL-NGS-28867 | 20323857 |
| 10 | ARS-BFGL-NGS-95091 | 13758980 | 14 | ARS-BFGL-NGS-102351 | 24407125 |
| 14 | UA-IFASA-7112 | 16109986 | 18 | ARS-BFGL-NGS-25758 | 62512168 |

As showed in Table 10, the significant SNP on BTA5 affects two different SNPs on BTA14. On BTA5, this specific SNP was located in a non-coding gene region, but four genes (HELB, IRAK3, TMBIM4, LLPH) had been found ± 0.5Mb around. LLPH and TMBIM4 had been identified as important genes for blood pressure in humans. Especially, they made an impact on hypertension, in combination with further genes (Liang et al. 2017). Ananth and Basso (2010) studied the relationship between pregnancy-induced hypertension (PIH), stillbirth and neonatal death and figured out, that PIH was associated with higher risks of stillbirth and neonatal mortality, especially for multiparous women.

The searched region at epistatic SNP on BTA14 showed two interesting genes (CEBPD, TRIB1), which were associated with oocyte maturation, cell turnover and proliferation (Becker et al. 2011; Brisard et al. 2014). Growth of foetus requires high rates of cellular turnover and differentiation. Particularly in-vitro fertilized cattle could be affected by compromised placental functions, foetal stress, abnormalities in development and abortion (Facciotti et al. 2009). Although abortion had to be distinguished from stillbirth, the connection of these genes to fertility traits had to be considered.

BTA10 is mainly recognized for body conformation traits (Cole et al. 2011), but in this case the SMAD3 and SMAD6 genes in the searched region for the significant SNP on BTA10 were presented in bovine oocytes and showed the functional role of these genes in bovine early embryonic development (K. Zhang et al. 2015; Kirkpatrick and Morris 2015). The epistatic SNP was located in the middle of XKR4 gene at 24.4Mb on BTA14. In the searched region

± 0.5Mb further genes e.g. LYPLA1, TMEM68 were found. XKR4, LYPLA1 and TMEM68 played a role in the regulation of prolactin secretion, feed intake and gain in cattle (Bastin et al. 2014; Lindholm-Perry et al. 2012).

Müller, Rothammer and Seichter (2017) investigated calving ease and stillbirth in Holstein cattle with special focus on BTA18. The epistatic SNP at 62.51Mb on BTA18 was located on the SHISA7 gene, which was identified, but not further researched in cattle or any other species. Compared to the results from (Müller, Rothammer and Seichter 2017), an identified QTL from 62.62-64.52Mb matched with the observed region for the epistatic SNP and was associated to calving ease and stillbirth.

### 3.4.2 Interactions within the same chromosome

Epistatic interactions within the same chromosome for the trait stillbirth had been found only for BTA14. As mentioned before, 165 significant epistatic interactions were identified. Significant SNPs were found in a range between 9.81 and 35.47Mb for the most part. Only four epistatic interactions showed a longer distance between both SNPs (Figure 6). The results showed 47 different SNPs on the first position and 52 different SNPs on the second position for the epistatic interactions.

A broader genome region from 19.5-26.5Mb on BTA14 was investigated, within this region most significant epistatic interactions were involved. Nine SNPs located exactly on protein coding genes were found in the searched region: PRKDC, EFCAB1, SNTG1, ST18, OPRK1, ATP6V1H, RGS20, XKR4, PENK.

PRKDC knew as protein kinase, DNA-activated, catalytic polypeptide showed association to body size in sheep (Kominakis et al. 2017). EFCAB1, named EF-hand calcium binding domain 1 coded from 21.44-21.46Mb was primarily involved in blood pressure (Takeuchi et al. 2010). Yamawaki et al. (2018) considered, that EFCAB1 might play an important role in regulation of microglial function and thus generated an antidepressant-like effect of sodium butyrate. The third gene is SNTG1 (syntrophin gamma 1), coded from 21.93-22.35Mb region was member of the syntrophin family. SNTG1 was expressed in areas of the brain (cerebellum, hippocampus and cortex) that had been suggested to affect body balance and implication of idiopathic scoliosis. Idiopathic scoliosis was the most common

paediatric spinal deformity (Bashiardes et al. 2004). The gene ATP6V1H encoded of vascular ATPase and regulated bone formation. A lack of ATP6V1H led to loss of bone mass, and exhibit increased MMP9 and MMP13 levels, thus result in osteoporosis (Y. Zhang et al. 2017). XKR4 ranged from 24.29-26.61Mb on BTA14, played a role in the regulation of prolactin secretion. Prolactin disorders led to spontaneous abortion in humans, but without accompanying clinical abnormalities like ovarian dysfunction (Hirahara et al. 1998). PENK (proenkephalin) coded from 25.21-25.22Mb and had been identified as a candidate gene for carcass weight in cattle and affected height in humans and cattle (S. H. Lee et al. 2013; Utsunomiya et al. 2013). The protein coding genes ST18, OPRK1 and RGS20 had been identified, but not further researched in cattle. In other species, these genes did not show any connection to stillbirth.

Utsunomiya et al. (2013) investigated genes on BTA14 in context of birth weight in Nellore cattle and presented seven protein coding genes PLAG1, CHCHD7, MOS, RPS20, LYN, RDHE2 (SDR16C5) and PENK, related to height. All of these genes were found in the region of our observed epistatic SNPs from 24-25Mb. Body size and height could be related to stillbirth. But also other risk factors like anomalies in extremities or lethal genetic defects had to be considered for stillbirth (Fürst and Fürst-Waltl 2006; Zindove and Chimonyo 2015).

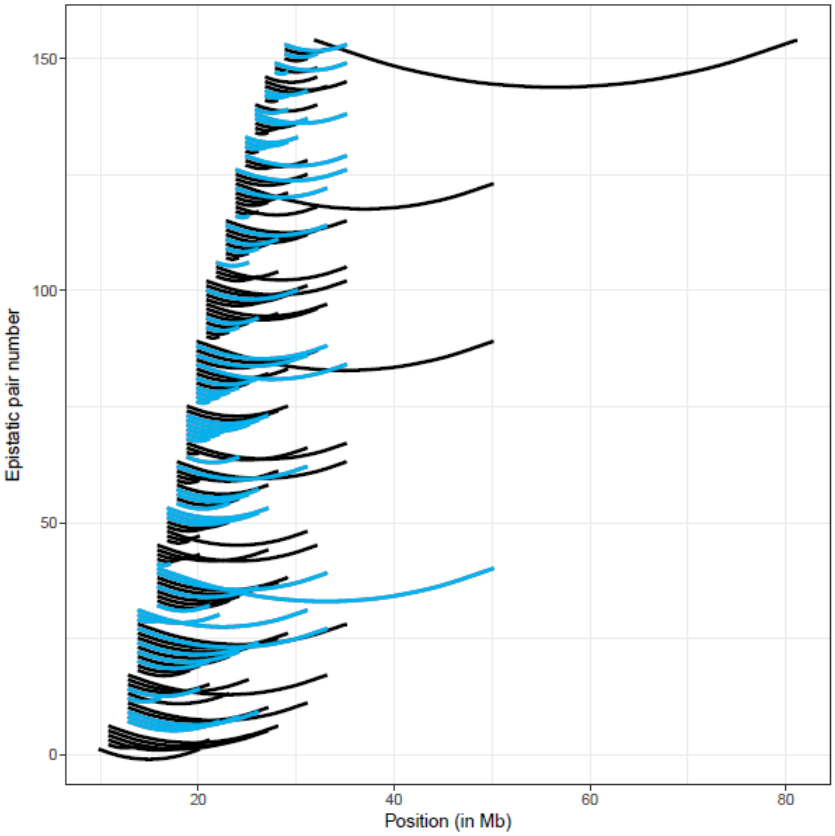


**Figure 6: Significant epistatic effects for stillbirth on BTA14 (p ≤ 0.05 in black, p ≤ 0.001 in blue)**

Three SNPs showed longer distances between epistatic SNPs. For each of these three interactions, the second SNP could be found at position 50.41Mb. No gene encoded at this position, but in the searched region ± 0.5Mb the gene TRPS1 (50.84-51.07Mb) was found. Mutations in TRPS1, called transcriptional repressor GATA binding 1 are associated with skeletal and craniofacial malformations in humans (Ackert-Bicknell et al. 2012). Skeletal abnormalities would lead to higher risk in stillbirth, due to difficulties during calving.

The highest distance in epistatic interactions was shown between SNPs on 31.97Mb and 80.75Mb. For the first SNP was no gene on the exact position found, but the PDE7A gene in searched vicinity ± 0.5Mb showed an impact to the expression in skeletal muscle (Han, Zhu, and Michaeli 1997). The exact position of the second SNP was located on RALYL gene, which encoded a RNA binding-protein, but was not researched in detail at any species so far.

## *3.5 Comparison of results from PLINK and GEMMA*

The Genome-Wide Association Study (GWAS) was implemented to identify major loci that showed significant associations with fertility, calving ease, longevity and stillbirth and their effect. Although it was primarily used to limit the number of potential SNP pair combinations for epistasis, the most important peaks were also described in the followed text.

The Manhattan plots mapped the SNP -$\log_{10}$ p-values for each chromosome. The Bonferroni threshold was -$\log_{10}$ value of 5.9 (red line) and our accepted lower indicative threshold was -$\log_{10}$ value of 5 (blue line). This indicative threshold was fixed for all traits in this GWAS analysis. The lower indicative threshold was considered, because the Bonferroni threshold was known to be strict.

### 3.5.1 Fertility

The Manhattan plot for fertility (Figure 7) showed, that only two SNP on BTA19 exceeded the threshold line and one SNP on BTA25 nearly reached the threshold -$\log_{10}$ of 5. The genes TEN1 and UNK on BTA19 and C16orf72 on BTA25 had been identified as coding region for these three SNPs but were not examined in detail for cattle.

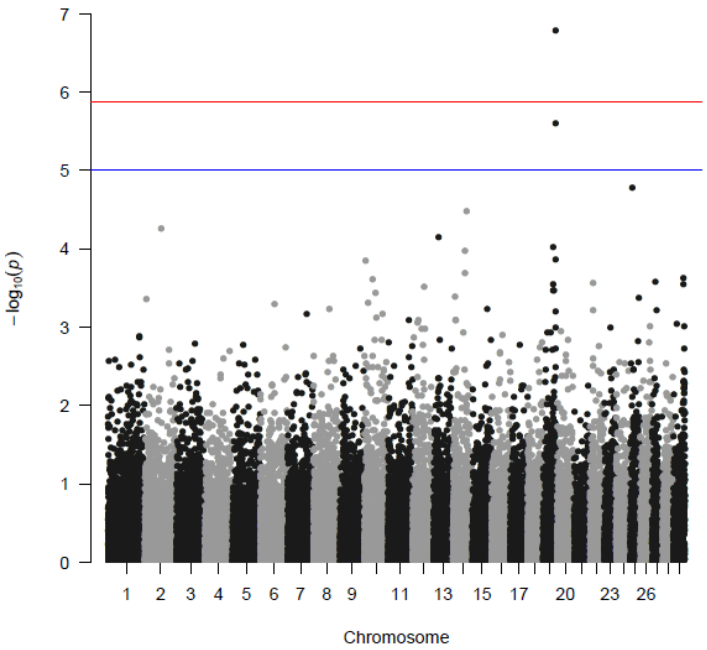


**Figure 7: Manhattan plot for fertility - Bonferroni threshold at 5.9 (red), indicative threshold at 5.0 (blue)**

Frischknecht et al. (2017) identified a QTL on BTA17, which was associated with 56-day non-return rate and with interval from first to last insemination. Minozzi et al. (2013) associated BTA5, 14 and 19 with non-return rate 56, Schulman et al. (2011) showed significant SNPs on BTA4 and 27 for non-return rate in heifers and SNPs on BTA2 and 27 for non-return rate in cows and Höglund et al. (2014) confirmed the relationship between BTA4 and non-return rates for dairy cows. Even for the same trait a wide range of involved chromosomes was shown. In comparison to the results from PLINK for fertility, a correspondence to the literature regarded BTA4, but not to the results of GWAS, had been found.

### 3.5.2 Calving ease

Strong peaks for calving ease had been identified in BTA6,14 and 21 and significant SNPs above the threshold line for BTA5 and 17. Furthermore, SNPs on BTA10, 18 and 23 were located near the threshold -$\log_{10}$ of 5. This result showed an important connection of BTA14 to calving ease (Figure 8).

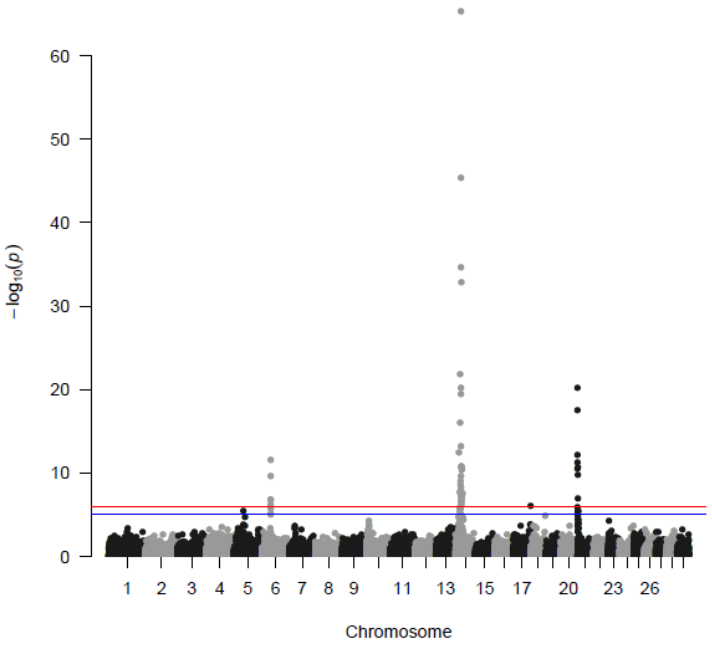


**Figure 8: Manhattan plot for calving ease - Bonferroni threshold at 5.9 (red), indicative threshold at 5.0 (blue)**

Calving ease was one of the most important functional traits in cattle breeding. For the most significant SNPs on BTA14 no related genes had been found. But one of the SNPs from BTA21 on 0.83Mb, was located on the protein coding gene MKRN3. Furthermore, for BTA6

three SNPs had been found, which were located on the LAP3 and LCORL genes. MKRN3 was associated with the Prader-Willi syndrome in humans (Pausch et al. 2011; Kanber et al. 2009) and LAP3 and LCORL showed direct association with calving ease in cattle and were related to height in humans (Bongiorni et al. 2012). These three genes had been defined as positional candidate genes (Saatchi et al. 2014) and had high influence in calving ease, because of the relationship between calving ease and stature/body weight of calves (Johanson and Berger 2003).

Saatchi et al. (2014) showed in a GWAS for ten different breeds, that BTA6, 14 and 20 influenced direct and maternal calving ease in Fleckvieh cattle. Compared to the result from epistasis analysis in PLINK, BTA14 showed the most important influence on SNP interactions in calving ease (Pausch et al. 2011; Utsunomiya et al. 2013). Additional involved BTAs in GWAS like BTA6 and 21 had also contributed epistatic results in PLINK and showed a relationship to calving ease (F. Chen et al. 2010; Pereira et al. 2016; Kanber et al. 2009; Pausch et al. 2011; Bongiorni et al. 2012; Matic et al. 2016; Szewczuk et al. 2013).

### 3.5.3 Longevity

The trait longevity showed 5 SNPs above the thresholds.

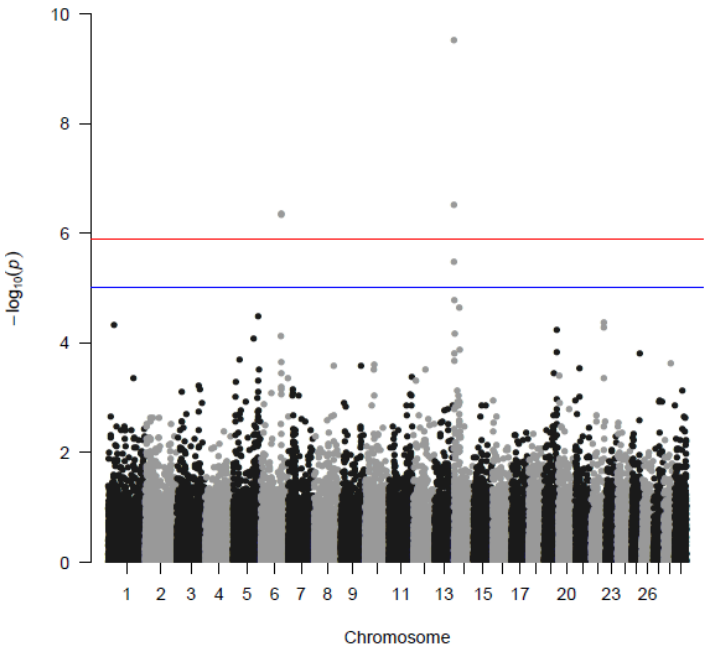


**Figure 9: Manhattan plot for longevity - Bonferroni threshold at 5.9 (red), indicative threshold at 5.0 (blue)**

As showed in Figure 9, two SNPs on BTA14 on 1.8Mb and 1.92Mb and two SNP on BTA6 on 89.1Mb and 89.13Mb had been found above the Bonferroni threshold. One SNP on BTA14 (1.68Mb) exceeded the threshold $-\log_{10}$ of 5. These three most significant SNPs on BTA14 were located on the exact position of following genes: DGAT1, MAF1 and CYHR1.

DGAT1, MAF1 and CYHR1 genes were associated with synthesis of milk lipids, glucose metabolism and effect milk, fat and protein yield (Mierzejewska and Chreptowicz 2016; Calvo et al. 2004; Cardoso et al. 2011).

X. Zhang et al. (2017) associated in a GWAS the relationship between hip height and girth for four age groups (6, 12, 18 and 24 months) for BTA3, 6 and 21. The growth and maturity of cattle body size affected not only feed efficiency, but also productivity and longevity.

The results from X. Zhang et al. (2017) showed a combination of PLINK and GWAS results and the importance of those BTAs for longevity .

### 3.5.4 Stillbirth

For the trait stillbirth, the provided Manhattan plot (Figure 10) showed a very clear peak of SNPs on BTA14. Furthermore, significant SNPs above the threshold line for BTA6 and 21 had been identified. SNPs on BTA3, 7, 10, 17, 18 and 23 nearly reached the threshold $-\log_{10}$ of 5.

As BTA14 clearly played an important role in stillbirth, the highly significant SNPs were studied in detail. These SNPs were located within a region from 24.06-25.40Mb. In literature, Utsunomiya et al. (2013) described the region from 24-25Mb on BTA14 as an essential region for gene with influence on stillbirth. For the ten most significant SNPs on BTA14, only two SNPs were located on the exact position of XKR4 gene. XKR4 played a role in the regulation of prolactin secretion, while an occurring disorder of prolactin led to spontaneous abortion in humans (Hirahara et al. 1998).

The two most significant SNPs (38.58 and 39.94Mb) on BTA6 were found on the position of LAP3 and LCORL gene. LAP3 and LCORL showed direct association to height in humans (Bongiorni et al. 2012). This trait would normally be associated with calving ease, but emphasised the correlation between calving ease and stillbirth (Mészáros, Taferner and Sölkner 2016).

One of the three most significant SNPs (0.83Mb) on BTA21 was located on the exact position of the MKRN3 gene. A mutation in this gene led to the Prader-Willi syndrome in

humans, which was a neurogenetic disorder (Pausch et al. 2011; Kanber et al. 2009). Thus, a connection between mutations in genes which led to diseases, died during the perinatal period due to stillbirth and neonatal death (Watanabe and Nagai 2009).

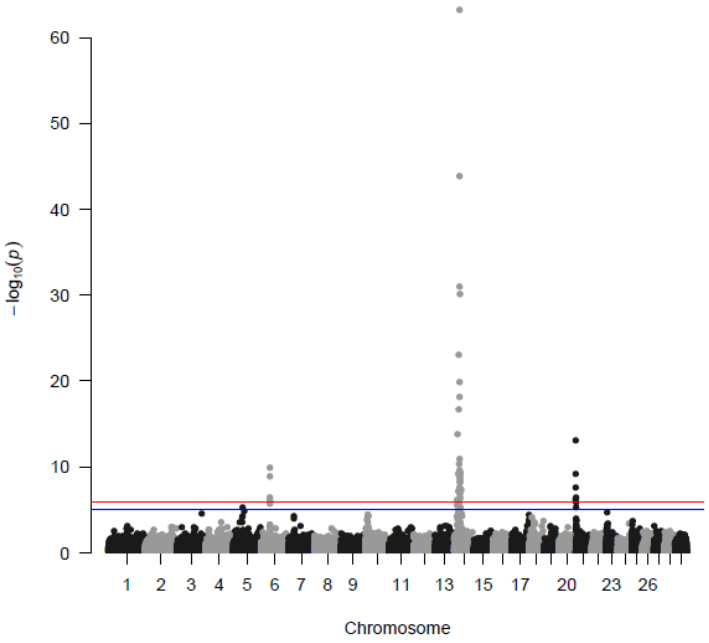


**Figure 10: Manhattan plot for stillbirth - Bonferroni threshold at 5.9 (red), indicative threshold at 5.0 (blue)**

Olsen et al. (2010) showed on the basis of a GWAS, that BTA6, 9 and 20 affected direct and maternal effects of stillbirth. Cole et al. (2011) emphasised the relationship between BTA18 and 23 and service-sire stillbirth or rather daughter stillbirth. Compared results from PLINK with those from GWAS, BTA14 played a key role to stillbirth rate, because of its influence on body size, height and bone malformations.

# 4 Conclusions

This thesis presented genomic regions and possible candidate genes involved in epistatic interactions for the traits fertility, calving ease, longevity and stillbirth in Fleckvieh cattle. These functional traits are of considerable interest in livestock breeding, because of their impact to genetic improvements in populations and economic value of individual animals. They are complex in nature, with low heritability's and quantitative, i.e. influenced by many genes and also to a large degree by environmental factors.

For calving ease, the stature formations like height, body size, abnormalities in extremities or lethal genetic defects influenced calving, potentially leading to stillbirth. Genomic regions of high impact on calving ease were found on BTA14, as well as BTA5, 6, 10 and 21. NSMCE2, KIAA0196, PLAG1, CHCHD7, MOS, RPS20, LYN, PENK and CYP7B1 on BTA14 were associated with dwarfism, height and stature or growth retardation. Epistatic interactions on BTA21 included genes like MAGEL2, MKRN3, NDN, UBE3A and ATP10A. A lack in paternal and maternal copy in these genes was connected to reduced muscle strength, massive weight gain, dwarfism and developmental delay known as Prader-Willi syndrome. A further detected gene was AGGF1 on BTA10. Bone tissue hypertrophy, which is an increase in the volume of bone tissue due to the enlargement of its component cells happened trough a mutation on this gene. The mutation showed gigantism in particular one limb, also known as Klippel-Trenaunay syndrome. Also, the protein coding gene DYM on BTA24 was responsible for bone formation.

The biggest effect regarding epistatic interactions for stillbirth was observed on BTA14, which was reviewed in previous studies Nine significant SNPs (PRKDC, EFCAB1, SNTG1, ST18, OPRK1, ATP6V1H, RGS20, XKRN4, PENK), directly located on protein coding genes, showed a relationship to stature expressions like paediatric spinal deformation, bone formation, body size and height. This work also identified interesting genes on BTA5 and 10. CEBPD and TRIB1 located on BTA5 were implicated in oocyte maturation. A triad of LYPLA1, TMEM68 and XKR4 on BTA10 showed influence on the regulation of prolactin secretion. An occurring disorder of prolactin was known for spontaneous abortion in humans. These similar effects for calving ease and stillbirth regarding to body size and stature abnormalities emphasised the correlation between those traits and had to be considered for breeding decisions regarding to both functional traits.

In fertility, BTA3, 4, 13 and 29 were associated with epistatic interactions between SNPs. Especially five regions identified by significant SNPs from those BTAs showed major influence on fertility: CDKN1C, PHLDA2 and PRDM10 on BTA29, BSND on BTA3 and PROKR2 on BTA13. These genes influenced embryonic growth, tissue differentiation and foetal testis differentiation. Furthermore, a lack of these genes led to large offspring syndrome in cattle, comparable to Bartter syndrome in humans. The syndrome was characterized by development of unusually large offspring, exhibited number of organ defects and production of an above average amount of amniotic fluid of the unborn foetus.

Longevity could be impaired trough diseases or metabolic disfunctions. Eleven different chromosomes were involved in epistatic interactions related to longevity. In particular, five genes on BTA1,3,5,14,17,19 showed high importance to longevity in this thesis. CLDN14 on BTA1 related to reduced bone mineral density and can subsequently lead to osteoporosis. The protein coding genes CSF1, KSR2 and MAF1 were involved in metabolic functions like energy homeostasis, glucose metabolism, lipid homeostasis or development of the blood cells. Further interesting genes are SOX10 and SEZ6, which were responsible for neural-crest development and the onset of epileptic seizures.

This study helps to explain the genetic architecture for different functional traits in Fleckvieh cattle crucial to livestock biology.

To conclude, for almost each researched functional trait BTA14 showed a high influence to epistatic interactions. The thesis confirmed the importance of BTA14 to stillbirth and calving ease and the found genes emphasised the impact of height and stature of calves during calving and rate of stillbirth. Our investigation stressed the importance of epistasis in functional traits and the further considerations in breeding. Surprisingly, only few epistatic interactions had been found for fertility and longevity. This could be traced back to the complexity of the traits and their intricate physiological mechanisms. Mapping epistatic interactions is a challenging experimental, statistical and computational topic. The experimental challenge was the large sample sizes required to detect significant interactions and sample the landscape of possible genetic interactions. On this occasion, the sample size of data, but also the intensity of the SNP chip data (high density data) for further studies could be expanded. The statistical challenge was the development of epistatic analysis and the filtering steps to obtain significantly results. The computational challenge was the large numbers of tests or rather the number of genes showed up.

In a nutshell, the work supposed to contribute to a better understanding of interactions between different regions in the bovine genome and offers a basis for a more precise estimation of (genomic) breeding values for functional traits in cattle.

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