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Genetic selection for bovine chromosome 18 haplotypes associated with divergent somatic cell score affects postpartum reproductive and metabolic performance

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ABSTRACT

The susceptibility of animals to periparturient diseases has a great effect on the economic efficiency of dairy industries, on the frequency of antibiotic treatment, and on animal welfare. The use of selection for breeding cows with reduced susceptibility to diseases offers a sustainable tool to improve dairy cattle farming. Several studies have focused on the association of distinct bovine chromosome 18 genotypes or haplotypes with performance traits. The aim of this study was to test whether selection of Holstein Friesian heifers via SNP genotyping for alternative paternal chromosome 18 haplotypes associated with favorable (Q) or unfavorable (q) somatic cell scores influences postpartum reproductive and metabolic diseases. Thirty-six heifers (18 Q and 18 q) were monitored from 3 wk before calving until necropsy on d 39 (± 4 d) after calving. Health status and rectal temperature were measured daily, and body condition score and body weight were assessed once per week. Blood samples were drawn twice weekly, and levels of insulin, nonesterified fatty acids, insulin-like growth factor-I, growth hormone, and β-hydroxybutyrate were measured. Comparisons between the groups were performed using Fisher's exact test, chi-squared test, and the GLIMMIX procedure in SAS. Results showed that Q-heifers had reduced incidence of metritis compared with q-heifers and were less likely to develop fever. Serum concentrations of β-hydroxybutyrate were lower and insulin-like growth factor-I plasma concentrations were higher in Q- compared with q-heifers. However, the body condition score and withers height were comparable between haplotypes, but weight loss tended to be lower in Q-heifers compared with q-heifers. No differences between the groups were detected concerning retained fetal membranes, uterine involution, or onset of cyclicity. In conclusion, selection of chromosome 18 haplotypes associated with a reduced somatic cell score resulted in a decreased incidence of postpartum reproductive and metabolic diseases in this study. The presented data add to the existing knowledge aimed at avoiding negative consequences of genetic selection strategies in dairy cattle farming. The underlying causal mechanisms modulated by haplotypes in the targeted genomic region and immune competence necessitate further investigation.

Key words: BTA18, somatic cell score, metritis, insulin-like growth factor-I

INTRODUCTION

Puerperal infectious diseases, such as metritis and mastitis, have a tremendous economic effect on the dairy industry due to the use of antibiotic treatment, resulting withdrawal periods, delayed onset of cyclicity after calving, extended intervals between calving, and subfertility (Pryce et al., 2004; Roche, 2006; Halasa et al., 2007; Walsh et al., 2011; Mahnani et al., 2015; Aghamohammadi et al., 2018). Most high-yielding dairy cows struggling with reproductive postpartum

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diseases also show metabolic disorders (Raboisson et al., 2014; Vercouteren et al., 2015; Mostert et al., 2018), and several authors have demonstrated the connection between the capacity of immunological defense strategies and metabolic regulatory mechanisms (Overton and Waldron, 2004; Roche et al., 2017; Wankhade et al., 2017). In the future, it will be important to reconcile economic efficiency and ethical requirements by breeding cows that are high-yielding as well as healthy and long-lived.

Growth hormone (**GH**) releasing hormone and growth-hormone inhibiting hormone are released from the hypothalamus to control GH release from the pituitary (Frago and Chowen, 2005). In the liver, GH binds to the GH receptor (**GHR**) to induce IGF-I release into the bloodstream (Scarth, 2006). A negative feedback mechanism reduces this release in the case of high IGF-I concentrations. The whole endocrine axis is referred to as the hypothalamic–pituitary–somatotropic axis, in which GH and IGF-I represent the somatotropic part. Past studies have shown that GH and IGF-I control distribution of nutrients (Lucy et al., 2009) because GH facilitates lipolysis, as during early lactation (Etherton and Bauman, 1998). Throughout the transition from late pregnancy to early lactation, an "uncoupling of the somatotropic axis" is observed in modern dairy cows (Lucy, 2008). Systemic GH levels are high, but due to inadequate expression of GHR, systemic IGF-I concentrations remain low. As a consequence of excessive GH levels, fat is mobilized to increase lipolysis. If the hepatic metabolic capacity is maxed out, ketone production exceeds ketone utilization, resulting in clinical or subclinical ketosis. The degree of uncoupling has been found to depend on feed allowance and genetic predisposition (Lucy et al., 2009). Lucy and colleagues (2009) compared 3 different genetic strains of dairy cows: North American Holstein cows with genetics typical of the mid-1990s (NA90), New Zealand Holstein cows with genetics typical of the mid-1990s (NZ90), and New Zealand Holstein cows with genetics typical of the early 1970s (NZ70). Whereas NA90 were not able to recouple their somatotropic axis, NZ90 cows showed reduced uncoupling throughout the additional feed allowance, and NZ70 cows even retained a coupled somatotropic axis during the study (Lucy et al., 2009). Reducing the incidence of metabolic and infectious diseases facilitates reduction of antibiotic treatment in bovine medicine, which simultaneously increases animal welfare and economic efficiency.

Genetic selection offers a sustainable tool to improve dairy cattle farming via the selection of animals with reduced susceptibility toward diseases, which in turn results in improved periparturient health. Until now, association and linkage studies have revealed promising results concerning bovine chromosome 18 (BTA18) and performance traits (Mao et al., 2016; Abo-Ismail et al., 2017; Müller et al., 2017; Wu et al., 2017). Concerning udder health, Kühn et al. (2008) showed that the prediction of half-sib heifers with a high or low SCS was possible through marker-assisted selection regarding a confirmed QTL for SCS in the telomeric region of BTA18. The expression profiles of the primary mammary gland epithelial cells of these animals displayed a faster and stronger response after pathogen challenge in vitro if they were obtained from heifers that had inherited the favorable QTL allele (Brand et al., 2011). Detailed knowledge about causal genomic variants as well as physiological mechanisms leading to disease resistance would improve selection strategies and is essential to avoid potential negative consequences of selection strategies.

The aim of the present study was to compare the postpartum performance of 2 Holstein Friesian half-sib heifer groups genetically selected via SNP genotyping for alternative paternal chromosome 18 haplotypes associated with favorable (**Q**) or unfavorable (**q**) effects on udder health, respectively. In this context, we examined whether this selection influences the incidence of postpartum reproductive and metabolic diseases, as well as the concentrations of selected blood parameters in the respective heifer groups.

MATERIALS AND METHODS

Setup of Animal Model

To investigate the clinical, immunological, pathophysiological, and molecular genetic effects modulated by the targeted BTA18 chromosomal interval, a selection process was established to identify heifers with a particular genetic makeup in this genomic region, essentially as described by Meyerholz et al. (2018). The selection process was based on previous studies reporting a genomic region on BTA18 associated with SCS, mastitis, and calving ease (Kühn et al., 2003; Brand et al., 2009, 2010; Cole et al., 2011; Wang et al., 2017). For the selection process, we took advantage of the genotype database for German Holstein sires at the Vereinigte Informationssysteme Tierhaltung w.V. (VIT) Verden (www.vit.de), containing approximately 11,503 50K genotypes. Using this data set, haplotyping was performed for all individuals according to Segelke et al. (2014). The SNP allele effects for all SNP were calculated in the routine genomic evaluation, run in August 2015. This process applies a SNP BLUP model (Liu et al., 2011) for the target trait SCS, which serves

as a surrogate for mastitis incidence due to its high correlations with mastitis in the German Holstein population (Miglior et al., 2005).

For 3 genomic target regions on BTA18 (43.0 to 59.0, 43.0 to 48.0, and 53.0 to 59.0 Mb), SNP effects were summarized for each of the BTA18 haplotypes for all sires in the data set. The margins of the subregions were established by transferring the microsatellite coordinates from a previous successful BTA18 mastitis model (Kühn et al., 2008; Brand et al., 2011) to the UMD 3.1.1 bovine genome assembly (Merchant et al., 2014).

To identify sires segregating for the QTL for udder health in the genomic target region, we calculated contrasts between SNP effects for alternative paternal BTA18 subregion haplotypes for all 11,503 sires in the data set born between 1999 and 2012. In a previous study (Kühn et al., 2008), alternate BTA18 haplotype effects on SCS were confirmed. Via alignment of favorable and unfavorable haplotypes of the sires involved in that study, common haplotypes were discovered and, in combination with existing knowledge (Brand et al., 2009; Cole et al., 2009, 2011), margins for the genomic regions to be investigated in the present study were set: rs41880634 (BTA18: 43,098,071) to rs109689271 (BTA18: 47,983,685) and rs29021987 (BTA18: 53,013,208) to rs43072554 (BTA18: 58,696,066). For all individual sires in the present data set and within each haplotype, summary for SNP allele effects was performed for the defined target genomic intervals. Moreover, this procedure was fulfilled for the region spanning from rs41880634 (BTA18:43,098,071) to the telomeric end of the chromosome as well. The threshold for sire selection was a difference between alternative summarized BTA18 subregion haplotype SNP effects of at least 2 standard deviations (SD) more than the mean haplotype difference of all sires for the 43 to 59 Mb interval. To avoid opposite effects for the 2 subregions 43 to 48 Mb and 53 to 59 Mb, we further excluded sires with inverse phasing (Q – q or q – Q) regarding the direction of the haplotype differences between those 2 subregions. Furthermore, sires were required to have at least a difference larger than 2 SD from the mean in at least one of the subregions 43 to 48 or 53 to 59 Mb. Finally, we excluded sires with extreme breeding values for SCS and milk performance traits that were potentially correlated with mastitis susceptibility. We required sires to have an index for milk yield of at least 100, an index for milk somatic cell count of 88 to 112 (for daughter-proven sires) or 94 to 118 (for sires with exclusively genomic information), and an index for milk flow and milkability of 88 to 112. Of the 156 remaining sires, daughters were selected for age at the start of the experiments (at least 18 mo of age) and anticipated day of parturition according to insemination records.

Finally, 282 heifers were genotyped with the 50K Illumina SNP chip (BovineSNP50 v2.0, Illumina, San Diego, CA), haplotyped according to the same protocol as described above, and allocated to groups according to inherited paternal haplotype. The favorable haplotype associated with low SCS was referred to as Q and the unfavorable haplotype associated with high SCS as q. Furthermore, we required the damsires' index for milk somatic cell count to be below 100 for the q-heifer cohort and above 112 for the Q-heifer cohort. The cohorts were balanced for the number of daughters per sire by creating half-sib groups of similar numbers within Q- and q-cohorts. One of the selected sires was heterozygous for the cholesterol deficiency defect (CD; Kipp et al., 2016). Thus, when setting up the groups, we paid further attention to including an equal proportion of CD carriers in the Q- and q-groups to avoid potential bias of the results due to differences in lipid metabolism of the selection candidates. In total, 6 sires were represented in the selected heifers, with each sire having a similar number of daughters in the Q- and q-cohorts.

Clinical Setup

The experiment was approved by the ethics committee of the Lower Saxony Federal State Office for Consumer Protection and Food Safety (reference number 33.12–42502–04–15/2024) and performed from January to September 2016. The selected Holstein Friesian heifers, originating from different German farms, were transported to the Clinic for Cattle, University of Veterinary Medicine Hannover. On the day of arrival, a detailed general examination was performed to evaluate health status, including udder health and the presence of late-term pregnancy. Each animal's withers height was measured. Blood samples were collected for photometric determination of glutathione peroxidase activity in erythrocytes and calculation of selenium content in the blood (Counotte and Hartmans, 1989). In the case of selenium concentrations <50.0 μg/L, selenium (1.5 g of all-rac-α-tocopherol acetate and 11 mg of sodium selenite/animal, Vitamin-E-Selen, aniMedica GmbH, Senden-Bösensell, Germany) was supplemented subcutaneously. The glutaraldehyde test was performed to rule out potential hypergammaglobulinemia caused by existing severe inflammatory processes (Sandholm, 1974; Metzner et al., 2007). During the first week after arrival, all animals underwent routine hoof-trimming procedures and were checked for and, if necessary, treated for hoof diseases. The heifers were housed in

Table 1. Composition of 3 component rations fed to Holstein Friesian heifers according to status relative to calving[1]

Item	Ration 1	Ration 2a	Ration 2b	Ration 3a	Ration 3b[2]
Ingredient (kg wet weight)					
Concentrate Prima 18 III pe[3]		0.5	2.0	2.0	8.0[4]
Mineral TR 40[4]	0.15	0.15	0.15		
Mineral Makro[4]				0.15	0.15
Hay	14.0	5.0	5.0		
Grass silage		10.0	10.0	20.0	20.0
Corn silage		3.0	3.0	20.0	20.0
Rapeseed extraction meal				1.0	1.0
Soy extraction meal				1.0	1.0
Composition					
DM (g/kg wet weight)	861	538	564	437	549
NE_L (MJ/kg of DM)	5.83	6.18	6.35	6.81	7.12
MP (g/kg of DM)	131	136	141	151	164
Crude fiber (g/kg of DM)	257	236	221	196	158

[1]Heifers were fed 3 component rations, starting with Ration 1 until d 270 post-insemination (p.i.), followed by the transition Ration 2 from d 270 p.i. until calving, and Ration 3 after calving. The amount of concentrate in Ration 2 was increased daily by 0.5 kg starting from 0.5 (Ration 2a) up to 2.0 kg (Ration 2b). After calving, the concentrate was increased in a merit-based manner from 2 kg (Ration 3a) by 0.5 kg per d up to a maximum of 8 kg (Ration 3b). The energy content of Ration 3a was calculated for a daily milk yield of 28 L. DM, NE_L, protein, and crude fiber refer to the calculated base ration with 2 kg concentrates.

[2]The maximum of the added concentrate resulted in staple feed supersession of approximately 5.7 kg of DM, which is reflected in the composition calculation.

[3]ForFarmers Langförden GmbH, Vechta-Langförden, Germany; NE_L = 7.61 MJ/kg of DM, MP = 178 g/kg of DM.

[4]Salvana, Klein Offenseth-Sparrieshoop, Germany.

individual loose stall pens on straw. After an adaptation period of at least 2 wk, the heifers were assigned to experimental groups. Every morning, the health status of the animals was evaluated using an abbreviated general examination method. Rectal temperature was measured twice daily, followed by a detailed general examination, including udder and vaginal examination if body temperature exceeded 39.5°C. Pens were cleaned twice daily, and heifers were fed one of 3 component rations according to status relative to calving (Table 1). After parturition, the animals were milked twice daily, and the milk yield was recorded. One animal was transported to the clinic after calving and entered the clinical setup on d 12 after calving.

Determination of BW, BCS, and rectal examination including ultrasound were performed weekly. The Medi-Test Ketone (Machery-Nagel, Düren, Germany) served as a cow-side test for immediate detection of hyperketonuria and decisions regarding treatment. The presence of ketone bodies in urine was scored once weekly (score 0 to 3) and repeated daily in the case of hyperketonuria (score 1 to 3). In moderate cases (score 1 or 2), animals received 200 mL of propylene glycol twice per day, whereas in severe cases (score 3), the animals were treated with glucose solution (400 g/L). Retrospectively, serum samples were analyzed for BHB concentration as described below. Starting from the first day after calving, all heifers received treatment with enrofloxacin for 5 d (2.5 g enrofloxacin/animal per day; Enrotron 100, aniMedica) to clear any pre-existing microbial infection. The treatment was prolonged in the case of infectious diseases. Weekly quarter milk samples were collected for analysis of milk components, SCC, and microbiological examination. In cases of disease, the animals were evaluated based on a standardized score sheet and treated according to standard veterinary practices. For comparisons between the 2 groups, only diagnoses that required treatment were included in the statistical analyses. Due to their high frequency and relevance, the following diseases were evaluated separately: retained fetal membranes (>12 h after calving), metritis grade I and II (Sheldon et al., 2009), clinical mastitis (altered milk secretion and local or systemic signs of inflammation in combination with positive bacteriological examination), subclinical mastitis (SCC of at least one quarter >100,000/mL after d 6 postpartum and positive bacteriological examination, no signs of inflammation), bronchopneumonia (respiratory frequency >40/min in combination with rectal temperature >39.5°C and inspiratory or expiratory exacerbated breathing and lung sound), intertrigo (hairlessness, wound, or inflammation of the skin between udder quarters or udder and thigh), hyperketonuria (Medi-Test Ketone, Machery-Nagel), and hyperketonemia (serum BHB concentration >0.7 mmol/L before calving or plasma BHB concentration

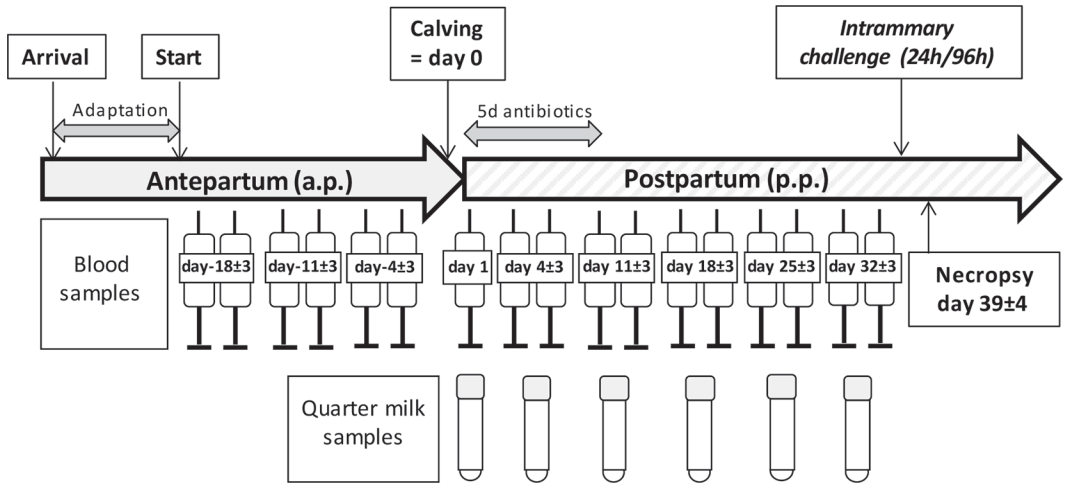


Figure 1. Graphical illustration of the clinical setup. Heifers (n = 36) were examined every day from the day of arrival in the Clinic for Cattle in Hannover, Germany, until necropsy on d 39 (SD = 4) after calving. Blood samples were collected 2 times per week starting on d 259 ± 1 after insemination (within the week of 21 d before calving until d 15 before calving). Retrospectively, samples were grouped in days relative to calving. On d 1, an additional blood sample was obtained from every animal. After calving, quarter milk samples were collected once per week. All heifers received an intramammary challenge 24 h or 96 h before necropsy.

>1.2 mmol/L after calving; Roberts et al., 2012). The remaining diseases were grouped into orthopedics (bursitis tarsalis lateralis or praecarpalis purulent or nonpurulent, phlegmon, limax, dermatitis digitalis), digestive problems (left displaced abomasum, dilatation of cecum, dilatation of colon), and others (postoperative wound healing disturbance, teat injury, hematoma, conjunctivitis, selenium deficiency, abscess, thrombophlebitis). At the end of the experimental setup, at 36 ± 3 d after calving, heifers received an intramammary challenge with mastitis pathogens and were killed either 24 or 96 h after the challenge, on d 39 ± 4. The heifers were stunned with a penetrating captive bolt pistol, immediately followed by exsanguination via longitudinal section of the jugular veins and carotid arteries. During necropsy, the weight of the uterus and ovaries was measured, and the ovaries were examined for follicles and functional corpora lutea. The clinical setup is summarized in Figure 1.

Blood Samples

Blood samples were collected from the left jugular between 0700 and 0900 h, according to the sampling protocol indicated in Figure 1. Serum and EDTA plasma samples were collected in 10-mL tubes (Sarstedt, Nümbrecht, Germany). The samples were left at room temperature for 2 h, centrifuged for 10 min (3,000 × *g*; Megafuge 40R, Thermo Fisher Scientific Inc., Waltham, MA), pipetted into 2-mL Eppendorf tubes, and stored at −20 °C until further processing. Sodium-heparinized blood was collected with the Vacutainer system (10 mL, Heparin 170 IU; Becton Dickinson, Heidelberg, Germany) and assessed immediately for total leukocyte numbers (10^6/mL) using a hemocytometer. The blood serum concentration of BHB (mmol/L) and nonesterified fatty acids (**NEFA**; μmol/L) were determined using the kits Ranbut D-3-Hydroxybutyrate (Randox Laboratories, Crumlin, UK) and NEFA-HR(2) (Wako Chemicals GmbH, Neuss, Germany). Blood plasma concentrations of insulin (μU/mL) and IGF-I (ng/mL) were determined using the commercial radioimmunoassay IM3210 Insulin IRMA Kit and A15729 IGF-I IRMA (Beckman Coulter, Brea, CA). Plasma concentrations of GH (ng/mL) were determined using an enzyme-linked immunosorbent assay, as described by Roh et al. (1997), modified by Kawashima et al. (2007), and adapted to bovine plasma by Meyerholz et al. (2015).

Statistical Analyses

Statistical analyses were performed using SAS 9.4.1 (SAS Institute Inc., Cary, NC). Differences between the 2 groups of heifers (Q versus q) regarding incidence of detected diseases were evaluated using Fisher's exact test or the chi-squared test, if the classification groups contained more than 5 animals. Data were analyzed for normal distribution using the Shapiro-Wilk test. In the case of single comparisons between the 2 groups, the Student's *t*-test was used for normally distributed data and the Mann-Whitney U test for non-normally distributed data. The GLIMMIX procedure was ap-

Table 2. Incidence of diagnosed diseases requiring treatment, compared between Holstein Friesian heifers selected for paternal chromosome 18 haplotypes associated with favorable (Q) and unfavorable (q) SCS

Disease	Q (no.)	q (no.)	*P*-value[1]
Retained fetal membrane (>12 h p.p.)[2]	1	3	0.603
Metritis (grade I or II; Sheldon et al., 2009)	3	11	0.015*
Clinical mastitis (altered milk secretion and local or systemic signs of inflammation in combination with a positive bacteriological examination)	1	5	0.177
Subclinical mastitis (one quarter SCC >100,000/mL after day 6 p.p. and a positive bacteriological examination, no signs of inflammation)	4	6	0.711
Bronchopneumonia (respiratory frequency >40/min and rectal temperature >39.5°C and inspiratory or expiratory exacerbated breathing and lung sound)	2	3	1.000
Hyperketonuria (Medi-Test Ketone, Machery-Nagel, Düren, Germany, score 1 to 3)	12	14	0.248
Hyperketonemia a.p.[2] (serum BHB concentration >0.7 mmol/L)	4	3	1.000
Hyperketonemia p.p. (plasma BHB concentration >1.2 mmol/L)	4	10	0.086†
Intertrigo (hairlessness, wound, or inflammation of the skin between udder quarters or udder and thigh)	10	9	1.000
Orthopedic diseases (bursitis tarsalis lateralis/praecarpalis purulent/nonpurulent, phlegmon, limax, dermatitis digitalis)	7	3	0.246
Digestive problems (left displaced abomasum, dilatation of cecum, dilatation of colon)	1	4	0.338
Other, severe (postoperative wound healing disturbance, teat injury, hematoma, conjunctivitis)	2	2	1.000
Other, mild (selenium deficiency, abscess, thrombophlebitis)	6	4	0.711

[1]Comparison was performed using Fisher's exact test if no. <5 in at least one subgroup or chi-squared test if no. ≥5 in every subgroup.

[2]p.p. = postpartum; a.p. = antepartum.

* indicates significance ($P < 0.05$); † indicates statistical tendency ($P < 0.1$).

plied to test for differences between groups for traits with repeated measurements. Before using the GLIMMIX procedure, NEFA, IGF-I, BHB, GH, and insulin were log-transformed to base 10. The GLIMMIX model included the fixed effects day and group, the interaction of day and group ($y = \beta0 + \beta1 \cdot day + \beta2 \cdot group + \beta3 \cdot day \cdot group$; day = {−18 ± 3; −11 ± 3; −4 ± 3; 1; 4 ± 3; 11 ± 3; 18 ± 3; 25 ± 3, 32 ± 3}; group = {Q; q}), and the random effect sire (G-side effect). Furthermore, subject cow was repeated using compound symmetry as covariance structure (R-side effect). In the case of milk yield, data were calculated per day from d 0 to 35. If data were available antepartum (**a.p.**) and postpartum (**p.p.**), 2 separate GLIMMIX procedures were performed. Data in the text are presented as the mean ± SD, and graphic illustrations include the mean ± standard error of the mean. Energy-corrected milk was calculated via the following formula (Kirchgessner, 1997; Kühn et al., 2008):

$$\text{ECM} = \text{milk yield (kg)} \times (0.37 \times \text{milk fat percentage} + 0.21 \times \text{milk protein percentage} + 0.95)/3.1.$$

Differences at $P < 0.05$ were considered significant. Differences at $P < 0.1$ were denoted as trends.

RESULTS

Postpartum Reproductive and Metabolic Diseases

The incidence of diagnosed diseases requiring treatment is presented in Table 2. Significantly fewer Q-heifers suffered from metritis compared with q-heifers, although the incidence of retained fetal membranes did not differ between the 2 groups. The duration of treatment for metritis was comparable between the 2 groups (Q: 7 ± 5 d, vs. q: 7 ± 5 d; $P = 1.0$). Cases of hyperketonuria did not differ significantly between the 2 haplotypes, but Q-heifers required fewer days of treatment compared with q-heifers (Q: 11 ± 9, vs. q: 20 ± 9 d; $P = 0.008$). A retrospective analysis revealed a trend of fewer Q-heifers with plasma BHB concentrations >1.2 mmol/L in at least one of the analyzed blood samples ($P = 0.086$). Incidence of fever (rectal temperature >39.5°C) in at least one of the measured time points was significantly lower in Q-heifers compared with q-heifers (a.p.: Q n = 4, vs. q n = 10, $P = 0.086$; p.p.: Q n = 11, vs. q = 17, $P = 0.041$).

Calving Ease, Uterine Involution, and Milk Yield

At the start of the experiment, 3 wk before the calculated calving date, all heifers were in good health, including udder health and hoof condition. Age at insemination (Q: 521 ± 62, vs. q: 501 ± 93 d), withers height (Q: 1.43 ± 0.04, vs. q: 1.42 ± 0.05 m), selenium concentration (Q: 87.3 ± 41.7, vs. q: 88.1 ± 30.5 μg/L), and glutaraldehyde test (all animals >10 min) were comparable between Q-heifers and q-heifers ($P > 0.1$).

We did not detect differences between groups concerning either the day of calving in relation to insemination (Q: 279 ± 3, vs. q: 276 ± 8), preterm (less than 270 d post-insemination; Q: n = 0, vs. q: n = 2) or delayed calving (more than 280 d post-insemination; Q:

n = 4, vs. q: n = 4), or the requirement for obstetric interventions as easy extractions (Q: n = 2, vs. q n = 5), difficult extractions (Q: n = 1, vs. q: n = 0), or caesarean sections (Q: n = 0, vs. q: n = 1; $P > 0.1$). Calves of Q-heifers tended to be heavier than those of q-heifers (Q: 39.4 ± 3.8 kg, vs. 36.6 ± 4.7 kg; $P = 0.06$); however, the sex of the calves was distributed equally (Q: 11 female, 6 male, vs. q: 12 female, 6 male; $P > 0.1$).

We found no differences in the number of days after calving until the uterus was palpable in the pelvic cavity (Q: 19 ± 7, vs. q: 21 ± 4 d p.p.; $P > 0.1$). Neither uterine weight (Q: 0.7 ± 0.1 kg, vs. q: 0.6 ± 0.1 kg; $P > 0.1$) nor ovarian weight at necropsy differed between groups (left ovary, Q: 7.4 ± 3.0 g, vs. q: 7.0 ± 1.9 g; right ovary, Q: 8.1 ± 3.2 g, vs. q: 8.0 ± 3.18 g; $P > 0.1$). We detected functional corpora lutea in one-third of Q-heifers and one-sixth of q-heifers via weekly transrectal ultrasonography and confirmed the findings at necropsy (Q: n = 6, vs. q: n = 3; $P > 0.1$).

We did not observe significant differences concerning daily milk yield between the 2 groups during the postpartum period (Figure 2), and ECM yields between d 7 and 35 after calving did not differ between the groups (Q: 24.3 ± 5.6, vs. q: 25.2 ± 5.3; $P > 0.1$). Furthermore, we did not observe significant differences between the 2 groups concerning the total number of leukocytes during the experiment (data not shown).

IGF-I and BHB Blood Concentrations

No significant sire effect was observed concerning insulin, NEFA, IGF-I, GH, BHB, milk yield, BW, weight loss, or BCS. Weight loss postpartum in relation to the last documented BW before calving tended to be less in Q-heifers compared with q-heifers ($P = 0.096$). Body weight and BCS were comparable between the 2 groups throughout the experiment (Figure 2; $P > 0.1$). Plasma insulin and serum NEFA concentrations did not differ significantly between the 2 groups ($P > 0.1$). But the changes in NEFA levels before calving tended to differ between Q- and q-heifers (day × group interaction effect; $P = 0.078$). In Q-heifers the reduction of serum NEFA concentrations was observed in the last week before calving, whereas serum NEFA levels of q-heifers were already descending between wk 3 and 2 before calving. Plasma concentrations of IGF-I tended to be higher before calving and were significantly higher after calving in Q-heifers compared with q-heifers (a.p.: $P = 0.056$; p.p.: $P = 0.002$). During the postpartum period, plasma GH tended to be lower ($P = 0.081$), and serum concentrations of BHB were significantly lower ($P = 0.026$) in Q- than in q-heifers (Figure 3).

DISCUSSION

This project aimed to establish a clinical model with half-sib heifers inheriting alternative paternal chromosome 18 haplotypes, which have been associated with effects on SCS in former studies (Kühn et al., 2008; Brand et al., 2011). Differences in clinical parameters between the animals with divergent haplotypes in the postpartum period were successfully identified and can add to existing knowledge about SCS from previous studies.

We did not detect striking differences between the 2 groups concerning calving ease, retained fetal membranes, uterine involution, or onset of cyclicity. However, Q-heifers showed lower incidence of metritis compared with q-heifers and were less likely to develop fever. Furthermore, Q-heifers tended to lose less BW after calving, although milk yields were comparable, and Q-heifers were less susceptible to and easier to treat for ketosis. Blood analyses revealed less intense uncoupling of the somatotropic axis in Q-heifers compared with q-heifers. From the antepartum perspective, IGF-I can be used as a biomarker for postpartum diseases. It has been shown that animals with clinical ketosis postpartum have significantly lower antepartum IGF-I blood levels compared with healthy cows (sensitivity 0.87; Piechotta et al., 2015).

We hypothesize that the observed less-severe uncoupling of the somatotropic axis in Q- compared with q-heifers is indicative of a better metabolic capacity of animals with the Q-haplotype. This phenomenon might result in a more adequate systemic energy distribution, in which less mobilization of adipose tissue is necessary, and which results in less BW loss. This difference could originate in the differing feed intake capacities of the 2 haplotypes. For technical reasons, it was not possible to record the DMI of the animals in the present study, but a parallel long-term experiment with 6 animals (3 Q and 3 q) showed significantly higher feed intake in Q-heifers (Heimes et al., 2019).

Several authors have shown that cows suffering from ketosis are more likely to develop infectious diseases such as metritis and mastitis (Holtenius et al., 2004; Hammon et al., 2006; Suthar et al., 2013), which can be explained by the increased cellular energy demands during lower levels of DMI, resulting in reduced immune cell function, as reviewed by Esposito et al. (2014). Recently, Zhang and coworkers (2018) have shown that ketotic cows with low glucose and high NEFA concentrations have higher blood concentrations of inflammatory cytokines. Those authors related the phenomenon to an overactivation of toll-like receptor 2/4-mediated NF-κB pathway signaling in polymorphonuclear neutrophils as

a cause for increasing inflammation in cows suffering from ketosis (Zhang et al., 2018). In the present study, ketosis was treated as soon as ketone bodies were detected in the urine, and mean BHB levels were under the cutoff value for ketosis (p.p.: 1.2 mmol/L) during the whole period, which might explain why NEFA levels did not differ between the 2 groups, although before parturition a trend for a stronger decrease in the NEFA concentration of q-heifers compared with Q-heifers was observed. In addition, the animals had a

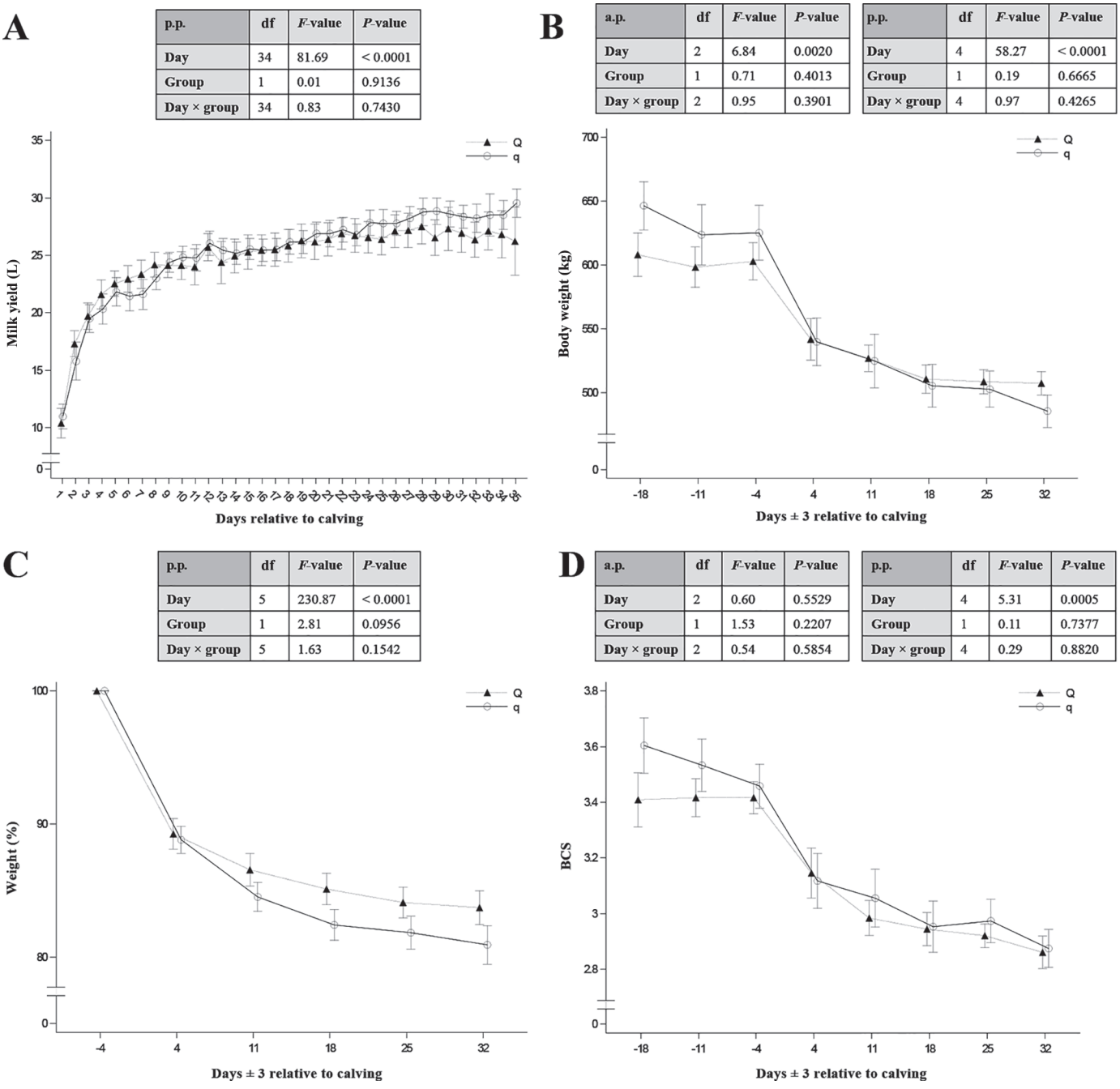
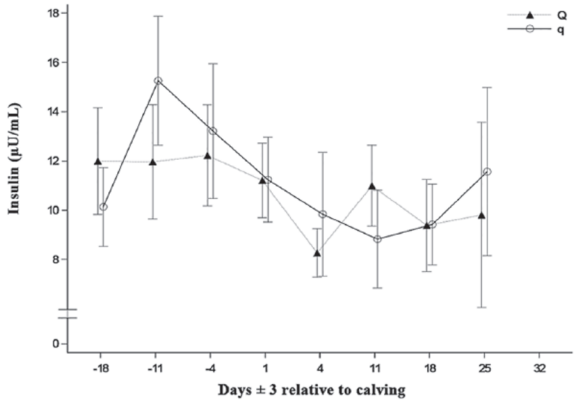


Figure 2. (A) Milk yield, (B) BW, (C) percent change in weight postpartum, and (D) BCS compared between Holstein Friesian heifers selected for paternal chromosome 18 haplotypes associated with favorable (Q) and unfavorable (q) SCS. Data are presented as mean ± SEM. Milk yield was compared between Q-heifers and q-heifers from d 1 to 35 after calving. If data were available antepartum (a.p.) and postpartum (p.p.), 2 separate GLIMMIX procedures were performed; results are shown in the tables above each graph. $P < 0.05$ was considered significant, and $P < 0.1$ was considered a statistical tendency.

A

a.p.	df	*F*-value	*P*-value
Day	2	0.86	0.4282
Group	1	0.02	0.9027
Day × group	2	1.55	0.2189

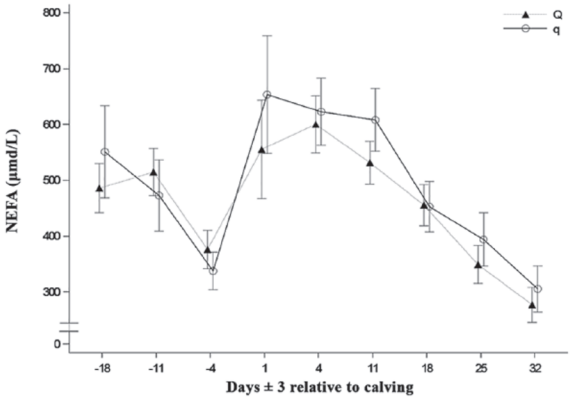
p.p.	df	*F*-value	*P*-value
Day	4	1.02	0.4023
Group	1	0.00	0.9833
Day × group	4	0.57	0.6838



B

a.p.	df	*F*-value	*P*-value
Day	2	15.37	< 0.0001
Group	1	0.00	0.9956
Day × group	2	2.60	0.0775

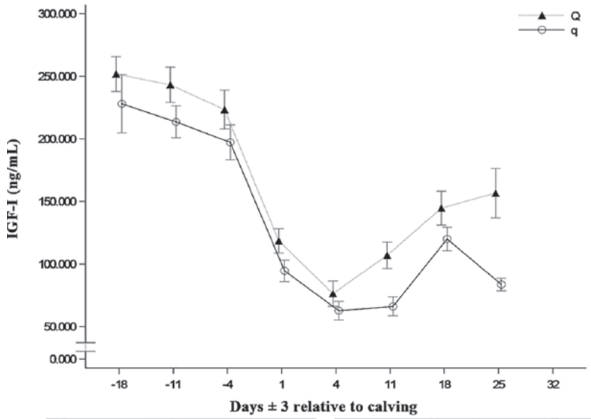
p.p.	df	*F*-value	*P*-value
Day	5	36.06	< 0.0001
Group	1	0.27	0.6049
Day × group	5	0.21	0.9588



C

a.p.	df	*F*-value	*P*-value
Day	2	2.95	0.0587
Group	1	3.76	0.0564
Day × group	2	0.02	0.9801

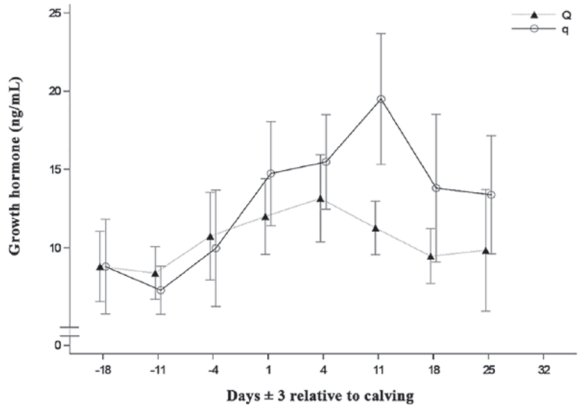
p.p.	df	*F*-value	*P*-value
Day	4	24.62	< 0.0001
Group	1	10.07	0.0019
Day × group	4	1.19	0.3200



D

a.p.	df	*F*-value	*P*-value
Day	2	0.59	0.5563
Group	1	0.07	0.7861
Day × group	2	0.13	0.8819

p.p.	df	*F*-value	*P*-value
Day	4	1.57	0.1857
Group	1	3.11	0.0806
Day × group	4	0.37	0.8304



E

a.p.	df	*F*-value	*P*-value
Day	2	0.65	0.5240
Group	1	0.00	0.9664
Day × group	2	0.60	0.5512

p.p.	df	*F*-value	*P*-value
Day	5	10.05	< 0.0001
Group	1	5.04	0.0255
Day × group	5	1.67	0.1407

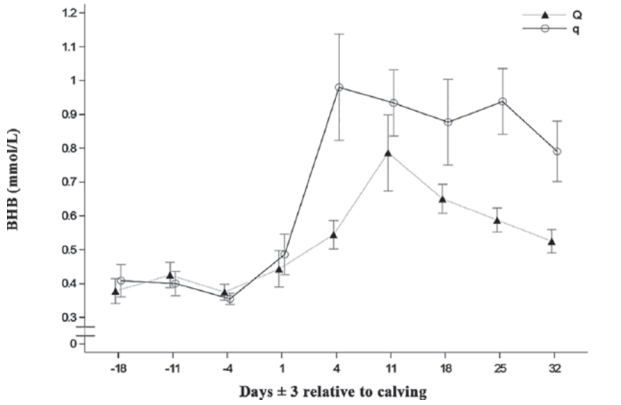


Figure 3. Blood concentrations of (A) insulin, (B) nonesterified fatty acid (NEFA), (C) IGF-I, (D) growth hormone, and (E) BHB in Holstein Friesian heifers selected for paternal chromosome 18 haplotypes associated with favorable (Q) and unfavorable (q) SCS. Data are presented as mean ± SEM. Data antepartum (a.p.) and postpartum (p.p.) were calculated in 2 separate GLIMMIX procedures; results are shown in the tables above each graph. $P < 0.05$ was considered significant, and $P < 0.1$ was considered a statistical tendency.

lower milk yield of up to 7.6 (Q) or 6.7 (q) kg of ECM compared with their sisters housed in a conventional loose stall barn fed a high-energy TMR (Heimes et al., 2019). Based on studies in organic farming, it is known that a reduced-energy diet results in reduced milk yield despite similar genetic merit (Langford et al., 2009).

The lower incidence of metritis and less frequent occurrence of fever in Q-heifers observed in the present study can be explained by the abovementioned associations between hyperketonemia and infectious diseases. These findings suggest superior immunological homeostasis in Q-heifers postpartum, potentially due to improved energy distribution at the cellular level. Over the long term, the observed improved periparturient performance in Q-heifers could also affect fertility, although details concerning the underlying immunologic mechanisms cannot be explained based on the presented data.

Our study established an experimental design that was balanced between the 2 haplotype groups, which were equally distributed over the period of study to prevent season-related effects and which remained in the Clinic for Cattle under highly standardized conditions. Because of this experimental design, fertility could not be evaluated with respect to the AI success rates. The significantly lower incidence of metritis, along with the numerically higher number of cyclic Q-heifers, suggests better reproductive performance compared with q-heifers. In this study, calves of Q-heifers tended to be heavier than those of q-heifers, and the sex of the calves was distributed equally. Thus, potential detrimental effects on the calves of q-heifers attributable to the mother's phenotype (e.g., due to calving problems) can be excluded.

Q-heifers showed less susceptibility toward clinical or subclinical mastitis, but differences between the groups were not statistically significant. Unexpectedly, during wk 5 after parturition, the average SCC in total milking samples (comprising the milk from all 4 quarters for each heifer) was lower in q-heifers compared with Q-heifers (Meyerholz et al., 2018). The parallel long-term experiment, however, confirmed the expected direction of differences between the 2 groups, with significantly lower SCS in the favorable-haplotype Q-heifers, particularly from wk 10 after parturition up to wk 35 (Meyerholz et al., 2018). For the current study, we must consider the fact that all animals received an antimicrobial treatment immediately after parturition, which might have prevented a potential infection. This intervention was due to the final intramammary infection model, which required 2 groups of heifers with differing chromosome 18 haplotypes but no clinical infection at the start of the intramammary challenge (data not shown). This requirement was achieved because, at the start of the challenge experiment, all animals were comparable with respect to health status, particularly udder health, and did not receive any medical treatment thereafter.

In summary, the present study revealed pathophysiological associations involved in susceptibility toward periparturient diseases in dairy cattle selected for chromosome 18 haplotypes associated with divergent SCS. The described selection was consistent with the selection for a more robust metabolism in the respective haplotype group, as Q-heifers showed less intense uncoupling of the somatotropic axis in the postpartum period and were less susceptible to metritis. Further research is needed to elucidate the interrelations between genetic predisposition and metabolic background, as well as the effect on immunological functions. The common management strategies for dairy farmers to reduce the negative energy balance and to improve immunity should be complemented by selection for health traits integrating genomic information and equipped with biological information (e.g., on BTA18 haplotypes).

CONCLUSIONS

The setup of a clinical model including heifers with alternative paternal chromosome 18 haplotypes was successfully established in this study. The animals with divergent haplotypes differed concerning their clinical performance postpartum: Q-heifers tended to have lower BW losses after calving, although they did not produce less milk, and showed less intense uncoupling of the somatotropic axis, resulting in reduced incidence of ketosis and metritis. These results highlight the important interplay of metabolic and immunological mechanisms relative to genetic predisposition. The observed differences reflect a diverse capacity for metabolic adaptation between the 2 groups to the high postpartum energy demand. To date, we cannot conclude whether the observed metabolic differences were the primary cause for the differing periparturient health performance of the 2 haplotypes or secondary to more profound mechanisms. Therefore, the underlying causal relationship between BTA18 haplotypes and immune competence requires further investigation. The presented data complement existing knowledge, which is essential to avoid negative consequences of genomic selection strategies.

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