INTRODUCTION

Selection for better reproductive performance is a time-consuming process. As reproduction is a complex trait (i.e., controlled by many genes and environmental factors), some genomic locations might account for large amounts of genetic variation, but this is not well understood. Molecular markers in or around genes may be involved directly or indirectly in reproduction. Therefore, selection programs using specific genetic markers could be a good strategy for precise and improved genetic changes of these traits. Many studies reported that leptin protein may affect the hypothalamo–pituitary–gonadal axis through specific hypothalamic receptors (e.g., Williams et al., 2002). Leptin (LEP/Sau3AI) and leptin receptor (LEPR/T945M) have known association with milk production traits, calving interval (CI), and age at first calving (AgeFC) in Slovak spotted and Pinzgauer cows (Trakovicka et al., 2013). Almeida et al. (2003) also found that the CI and weight at first calving increased when considering different markers (LEP/Sau3AI and IDVGA-51) in LEP for Bos indicus × Bos taurus cattle. In addition, Clempson et al. (2011) further supported the role of LEP genotype in reproductive traits by finding association of fertility traits (e.g., age at first service, total number of artificial insemination services, days to conception, and CI) with LEP single nucleotide polymorphism markers in Holstein heifers and cows. Therefore, it has been established that selection using the LEP marker can be performed in cattle. However, there is limited information on the association of the LEP genotype with reproductive characteristics such as gestation length, pregnancy status, weaning success, and reproductive success over time in commercial beef cows. In addition, little is known on the effect of the LEP genotype and circulating leptin hormone (LEPH) concentration on antral follicle count, reproductive tract score, and ovary measurements in forage-fed developing beef heifers. Thus, this study was conducted to determine the association of the LEP genotype and circulating LEPH concentrations with reproductive characteristics in commercial beef cows and developing heifers.

MATERIALS AND METHODS

Animals and Phenotypic Data

All procedures were approved by the Institutional Animal Care and Use Committee of North Dakota State University. Data were generated in part by 1) the original cow herd (base herd;
completed (n = 218) at Dickinson Research Extension Center (Dickinson, ND) and 2) daughters of the base herd that became part of a long-term study. The base herd consisted of cows influenced by Angus, Red Angus, American Aberdeen, Hereford, Limousin, Simmental, Shorthorn, or Gelbvieh. Daughters produced from the base herd from 2014 to 2017 (n = 258) are considered Cycle 1 in the long-term study, where daughters of these Cycle 1 females (n = 100) are considered Cycle 2 and were produced specifically from Red Angus or American Aberdeen sires. All females varied in frame size; therefore, frame size was calculated based on age and hip height. Reproductive data on base herd and cycle females included AgeFC, CI (the period between two subsequent calving events), success at pregnancy (yes or no at pregnancy check), weaning (yes or no at weaning time), and overall reproduction (0 to 3; yes or no at pregnancy check), weaning (yes or no at weaning time), and overall reproduction (0 to 3; yes or no). Other reproductive characteristics (gestation length [GL], antral follicle count, uterine horn diameter, and ovary measurements) were collected from Cycle 1 and 2 as heifers during feed trials leading up to their first breeding season.

DNA and LEP Genotyping

Blood samples were collected via jugular venipuncture on all animals (n = 576) for DNA extraction using Qiagen DNeasy kit protocol. DNA quality was checked using Synergy H1 microplate reader by BioTek, then stored at −80 °C until LEP genotyping. Genotyping for the LEP c.73C>T marker (Buchanan et al., 2002) was performed using KASP by Design assay (LGC Genomics, Beverly, MA) with an Applied Biosystems 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA).

LEPH Concentration

Blood samples for 333 heifers were collected the day prior to entering their breeding season (August 1 ± 2 d year). Plasma LEP concentrations were determined in duplicate using the Multi-Species Leptin RIA kit (XL-85K, EMD Millipore Corporation, St. Charles, MO) at the Department of Animal Science, South Dakota State University, Brookings, SD. All values were expressed as ng/mL human equivalent. Cycle 1 and 2 females in the study were grouped into high and low LEPH groups based on the median concentration, respectively. For a given trait, only females with both LEPH and data records determined the median value and were used for analysis.

Statistical Analysis

All animals were classified into five ancestral groups (A: American Aberdeen; B: Angus, Red Angus, Hereford, and F1 British (B) × B cross; C: Gelbvieh, Simmental, Limousin, and F1 Continental (C) × C cross; MIX: animals with unknown parentage, F1 B × C or F1 B × A crosses; and SH: Shorthorn) given their clustering in a population structure analysis (Bhowmik et al., 2019). Some cows were culled from the herd due to health reasons rather than reproductive failure. These incidences were recorded as a potential fixed effect (health cull reason; yes or no). All statistical analyses were performed with SAS v.9.4 (SAS Inst., Cary, NC) using either MIXED or GENMOD procedures based on the trait’s distribution. Base herd cows were included with Cycle 1 and 2 females to see the effects of LEP genotype on reproductive data (CI, AgeFC, and success traits), where fixed effects considered were ancestral group (n = 5), LEP genotype (n = 3), and birth year (as fixed covariate). The effects of LEPH on those traits were analyzed using only Cycle 1 and 2 females, where ancestral group (n = 5), frame size grouping (n = 4), cycle (n = 2), birth year (n = 4), and LEPH hormone (n = 2) were used as fixed effects. In both cases (LEP and LEPH effects), success traits analyses used health cull reason (n = 2) as a fixed effect. Fixed effects evaluated for other reproductive traits included ancestral group (n = 5), frame size grouping (n = 4), cycle (n = 2), birth year (n = 4), or cycle nested within birth year, and either LEP (n = 3) or LEPH (n = 2). Least squares means were generated for significant effects and controlled for experiment-wise error using Tukey–Kramer method. The CORR procedure of SAS was used to obtain Pearson and Spearman correlation coefficients of LEPH with trait records.

RESULTS AND DISCUSSION

In this study, LEP genotypes (CC, CT, and TT) were distributed according to Hardy–Weinberg proportions in total population (base herd cows, Cycle 1 and 2 females). The proportion of heterozygote
animals approximated the total proportion of both homozygote animals (0.487 to 0.511 vs. 0.513 to 0.489, respectively).

Reproductive Characteristics

The effects of \( LEP \) and LEPH on reproductive traits are listed in Table 1. Left ovary length was greater \((P = 0.02)\) in CT heifers compared to CC heifers, but statistical difference of \( TT \) to CC heifers could not be proven due to sample size and variability. The \( T \) allele appears to be dominant to the \( C \) allele for left ovary length (Table 1). Previous studies also reported that the \( T \) allele of \( LEP \ c.73C>T \) was associated with fatter carcasses (Buchanan et al., 2002), faster rate of ultrasound back fat gain (Nkrumah et al., 2004), and increased 12th rib fat thickness (Kononoff et al., 2014) compared to the \( C \) allele. No differences between \( LEP \) genotypes were observed for other reproductive traits measured in this study \((P > 0.10)\). However, sample size was not adequate to prove in these cases due to variability.

As additional data are collected on Cycle 1 and 2 females, this relationship may be clarified. Effects of \( LEP\text{SauAI} \) RFLP and IDVGA-51 STR markers on CI and weight at first calving in composite (Aberdeen Angus × Nelore) beef cattle were also described by Almeida et al. (2003). In our study, small sample size and large standard errors might be an issue to statistically show differences between \( LEP \ c.73C>T \) genotypes for CI and AgeFC. In addition, we did not observe any differences between \( LEP \) genotypes for circulating LEPH concentration \((P > 0.997; \text{data not shown})\). There were significant differences between animals with low LEPH and those with high LEPH for right ovary diameter \((P = 0.05)\) and right ovary length \((P = 0.02)\). No differences between the two LEPH groups were observed for other reproductive traits measured in this study \((P > 0.11)\).

LEPH was positively correlated \((r = 0.146, P = 0.03)\) with gestation length (Table 2). Conversely, LEPH was negatively correlated with antral follicle count \((r = -0.135, P = 0.02)\) and

| Traits\(^1\) | CC | CT | TT | LEPH\(^2\) |
|---------|----|----|----|----------|
| CI, d   | 367.3 ± 6.8 (69) | 374.5 ± 4.6 (176) | 375.1 ± 6.1 (108) | 369.8 ± 11.4 (69) |
| GL, d   | 273.9 ± 1.1 (54) | 275.6 ± 0.8 (117) | 274.1 ± 1.3 (58) | 275.1 ± 0.9 (115) |
| AgeFC, d| 740.5 ± 3.4 (90) | 742.8 ± 2.4 (222) | 745.4 ± 3.2 (132) | 730.0 ± 1.8 (114) |
| Success, % | 0.93 ± 0.02 (100) | 0.93 ± 0.01 (240) | 0.94 ± 0.01 (139) | 0.92 ± 0.02 (129) |
| Preg    | 0.90 ± 0.02 (100) | 0.90 ± 0.01 (240) | 0.90 ± 0.01 (139) | 0.87 ± 0.02 (129) |
| Wean    | 0.94 ± 0.02 (100) | 0.94 ± 0.01 (240) | 0.94 ± 0.01 (139) | 0.92 ± 0.02 (129) |
| Repro   | 14.33 ± 0.26 (71) | 14.75 ± 0.21 (147) | 14.60 ± 0.28 (84) | 14.43 ± 0.21 (147) |
| UHD, mm | 18.70 ± 0.60 (71) | 19.99 ± 0.48 (148) | 19.72 ± 0.64 (84) | 19.98 ± 0.48 (148) |
| Dia, mm | 20.83 ± 0.75 (71) | 22.94 ± 0.60 (148) | 22.03 ± 0.81 (84) | 22.58 ± 0.60 (148) |
| Lh, mm  | 21.21 ± 0.77 (71) | 22.29 ± 0.62 (147) | 22.82 ± 0.84 (84) | 21.60 ± 0.61 (147) |
| Ht, mm  | 16.57 ± 0.68 (71) | 17.03 ± 0.54 (148) | 17.14 ± 0.73 (84) | 17.37 ± 0.55 (148) |
| AFC     | 21.08 ± 1.42 (71) | 24.18 ± 1.14 (147) | 24.02 ± 1.53 (84) | 22.78 ± 1.15 (147) |

\(^1\)Numbers in parentheses are number of observations used.
\(^2\)Reproductive traits included calving interval (CI); gestation length (GL); age at first calving (AgeFC); success at pregnancy (preg), weaning (wean), and overall reproduction (repro); uterine horn diameter (UHD), ovary diameter (Dia), length (Lh), and height (Ht) as well as antral follicle count (AFC).

\(^3\)The median LEPH concentrations used for grouping were 16.71, 15.68, 15.63, and 15.88 ng/mL for CI, GL, AgeFC, and all other reproductive traits, respectively.

\(^4\)Least square means within a row by leptin grouping without a common superscript letter differ \((P < 0.05)\).

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uteroine horn diameter ($r = -0.132$, $P = 0.02$) according to Spearman coefficients. There were also negative correlations between LEPH and right ovary diameter ($r = -0.150$, $P = 0.01$) and right ovary length ($r = -0.121$, $P = 0.04$). We found low negative correlations between circulating levels of LEPH and the majority of the reproductive traits; however, some were not significant. This could be due to environmental aspects rather than genetic aspects. Research has revealed that a threshold level of LEPH is presumably required for maintenance of fertility in animals and humans. As circulating LEPH levels are directly related to body adiposity, Brannian and Hansen (2002) suggested that high LEPH concentrations associated with obesity may have a negative impact on fertility. Increasing serum LEPH concentration during follicle stimulating hormone stimulation also leads to poor ovarian response in terms of number of follicles and retrieved oocytes in women (Bültzow et al., 1999). A negative correlation between LEPH levels and endometrial thickness in humans was reported by Chakrabarti et al. (2012). These outcomes support the negative correlation of LEPH with most of the reproductive traits found in this study. However, Strauch et al. (2013) reported a negative relationship between serum LEPH and the postpartum interval in multiparous Brahman cows.

**Table 2. Phenotypic correlation coefficients of circulating leptin hormone concentration with reproductive traits in beef heifers**

| Traits | $N^2$ | Pearson | Spearman |
|--------|-------|---------|----------|
| CI, d  | 136   | 0.038   | -0.041   |
| GL, d  | 230   | 0.146*  | 0.045    |
| AgeFC, d | 229 | 0.004   | 0.003    |
| UHD, mm | 295  | -0.097*** | -0.132*   |
| Ovary  |       |         |          |
| Dia, mm |       |         |          |
| Left   | 296   | 0.017   | -0.011   |
| Right  | 295   | -0.150**| -0.152** |
| Lh, mm |       |         |          |
| Left   | 296   | 0.047   | 0.031    |
| Right  | 295   | -0.121* | -0.113***|
| Ht, mm |       |         |          |
| Left   | 296   | -0.024  | -0.050   |
| Right  | 295   | -0.068  | -0.058   |
| Follicles |      |         |          |
| AFC    | 295   | -0.094  | -0.135*  |

1Reproductive traits included calving interval (CI), gestation length (GL), age at first calving (AgeFC), uterine horn diameter (UHD), ovary diameter (Dia), length (Lh), and height (Ht) as well as antral follicle count (AFC).

2$N^2$ = number of records used.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.10$.

**IMPLICATIONS**

Animals did not differ between the $LEP$ c.73C>T genotypes for the majority of the reproductive traits. Although these results were not significant, heifers showed improved reproductive characteristics based on the number of T alleles. As additional years of data are acquired for these animals, the relationship of $LEP$ genotypes may be clarified. The negative correlation of circulating LEPH with reproductive indicates that an elevated concentration of LEPH might have negative impacts on reproductive traits. Further research is needed to fully understand LEPH concentration and its role in reproduction; however, it may serve as a viable selection tool early in life.

**ACKNOWLEDGMENTS**

This project was supported by the National Institute of General Medical Sciences of the National Institutes of Health (P20GM103442), North Dakota (ND) State Board of Agricultural Research and Education, ND Agriculture Experiment Station, and North Dakota State University Dickinson Research Extension Center.

**Conflict of interest statement.** None declared.

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