Polymorphisms in the bovine leptin gene associated with perinatal mortality in Holstein-Friesian heifers

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ABSTRACT

Leptin is an important regulator of fetal and placental growth. This study evaluated the association of single nucleotide polymorphisms (SNP) in the leptin gene with perinatal mortality (stillbirths and mortality within 24 h of parturition) in 385 Holstein-Friesian heifers on 18 dairy farms in the United Kingdom. The 3 SNP evaluated were exon 2FB, UASMS1, and UASMS2. The mean age at first calving was 27.0 ± 0.2 mo. Associations between each SNP and perinatal mortality (calf alive or dead) were tested using a generalized linear model that included herd-year-season, calf sex, age at first calving, and age and pedigree of the dam. The overall level of perinatal mortality in the population was 16.9%, with significant allelic substitution effects for exon 2FB and UASMS1. These 2 SNP were in close linkage disequilibrium with each other ($r^2 = 0.98$) but not with UASMS2 ($r^2 = 0.10$). For exon 2FB, perinatal mortality was similar between heifers carrying the CT and TT alleles (20%), but was higher than in heifers carrying the CC allele (11%). For UASMS1, mortality was 21% with the CC and CT alleles but only 10% with the TT allele. No associations of perinatal mortality with SNP were found in the UASMS2 data set, possibly influenced by the low frequency (2%) of the TT genotype. No significant effects of herd-year-season, age at first calving, or calf sex were found. In conclusion, polymorphisms in the leptin gene were associated with 2-fold differences in perinatal mortality in dairy heifers.

Key words: leptin gene, perinatal mortality, heifer

INTRODUCTION

Leptin, a protein hormone synthesized and secreted primarily by white adipocytes, has been found to be an important physiological marker of BW, feed intake, energy expenditure, and reproductive function in hu-

mans and other mammals (reviewed by Ingvarsen and Boisclair, 2001). Other tissues also produce leptin, including the stomach, muscle, placenta, and fetal tissues (reviewed by Forhead and Fowden, 2009). There is now substantial evidence implicating leptin as a key regulator of fetal development (Ashworth et al., 2000; Henson and Castracane, 2000; Grisaru-Granovsky et al., 2008; Forhead and Fowden, 2009). During pregnancy, leptin can act as a placental and fetal growth factor, signaling nutritional status between the mother and fetus. In humans and mice, there is an increase in maternal plasma leptin concentration toward the end of pregnancy; the localization of both leptin and its receptor in the placenta suggests that placental leptin is a major source of this increase (Masuzaki et al., 1997; Hoggard et al., 2001). In humans, dysregulation in leptin function during pregnancy has been associated with several pathological conditions, including recurrent miscarriage, gestational diabetes, preeclampsia, and fetal growth retardation (Bajoria et al., 2002; Sagawa et al., 2002). Leptin levels in human cord blood at term are also correlated with birth weight, length, head circumference, and ponderal index (Ong et al., 1999).

In cattle, plasma leptin concentration is high during late pregnancy and decreases at parturition (Block et al., 2001; Liefers et al., 2003). Unlike in humans and mice, the ruminant placenta has negligible expression of leptin mRNA throughout gestation, indicating that the placenta is not a major source of circulating leptin during late pregnancy (Block et al., 2001; Ehrhardt et al., 2001; Thomas et al., 2001). The leptin receptor is, however, highly expressed both in the bovine endometrium (Thorn et al., 2007) and at the maternal-fetal interface in the ovine placenta (Thomas et al., 2001). The possibility remains that circulating leptin may also influence placental development in ruminants, as well as alter the quantity of specific nutrients supplied to the fetus by the placenta, which is vital for supporting fetal survival. Placental dysfunction has previously been suggested as a risk factor for stillbirth in calves (Berglund et al., 2003; Kornmatitsuk et al., 2004).

Perinatal mortality (defined as stillbirths and mortality within 24 h of parturition) is a major contributing

Received June 5, 2009.

Accepted September 29, 2009.

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factor to heifer loss in the dairy industry. Perinatal mortality in Holstein-Friesian animals has previously been estimated at 6 to 8%; levels are often twice as high in heifers at first calving compared with multiparous cows (12–13 vs. 6%; Johanson and Berger, 2003; Lombard et al., 2007; Brickell et al., 2009). There is some evidence for an increasing prevalence of perinatal mortality in Holstein populations (Harbers et al., 2000; Meyer et al., 2001; Steinbock et al., 2003); an increased proportion of this loss does not appear to be associated with the traditional risk factors (Mee et al., 2008). Several authors have associated perinatal mortality with genetic factors (Steinbock et al., 2003; Hansen et al., 2004; Jamrozik et al., 2005). Thomasen et al. (2008) identified several quantitative trait loci affecting both direct and maternal calving traits (stillbirth, calving difficulty, and calf size) in the Danish Holstein population. Determination of which genotypes may be associated with calf mortality at parturition offers the possibility of future genetic selection against this adverse trait.

In cattle, the leptin gene is located on chromosome 4 (Pomp et al., 1997); several SNP have been reported for exonic, intronic, and promoter regions of the gene (Buchanan et al., 2002; Nkrumah et al., 2005). Exon 2FB is located at the exon 2 region, at position 305 (C to T base change), resulting in an AA change from Arg to Cys (Buchanan et al., 2002). Two other SNP, UASMS1 and UASMS2, are located at positions 207 (C to T substitution) and 528 (C to T substitution), respectively, in the promoter region of the bovine leptin gene (Nkrumah et al., 2005). These polymorphisms have previously been linked to important production traits in both beef and dairy cattle, including milk production (Banos et al., 2008; Chebel et al., 2008), carcass composition (Buchanan et al., 2002; Schenkel et al., 2005), and growth parameters (Nkrumah et al., 2005; Lusk, 2007). In addition, polymorphisms in both the leptin and leptin receptor gene have been associated with differences in serum leptin concentrations during pregnancy (Liefers et al., 2003; Nkrumah et al., 2005).

Based on the evidence available, leptin was selected as a candidate gene to influence perinatal mortality. The objective of this study was to evaluate the association of 3 previously identified SNP in the bovine leptin gene with perinatal mortality in Holstein-Friesian heifers at first calving.

MATERIALS AND METHODS

Animals and Phenotypic Data

The study was based on 385 heifers with known pedigree, born between August 2003 and October 2004, on 17 commercial dairy farms and 1 primarily research

farm across southern England. These farms provided a range of management practices representative of those commonly encountered on dairy farms in the United Kingdom, with a median herd size of 228 (range = 105–540) Holstein-Friesian adult cows. The research farm provided 3 groups of heifers, making a total of 20 cohorts of heifers (mean cohort size = 19; range = 12–26 heifers per cohort).

Heifers were bred for the first time at a mean age of 16.4 ± 0.2 mo (range = 12–28 mo). All heifers calved for the first time between June 2005 and November 2007 at a mean age of 27.0 ± 0.2 mo (range = 21–40 mo). Calving records were collected, including calf birth date, calf status [alive vs. dead (born dead or died within 24 h of parturition)], and calf sex (male vs. female). Date of calving was subsequently used to assign a season of calving: winter (December–February), spring (March–May), summer (June–August), and autumn (September–November). For each cow, pedigree information for the preceding 3 generations was collected from the Holstein UK web site (www.ukcows.com; accessed June 2008). This created a total pedigree containing 2,251 animals.

Blood Sampling and DNA Extraction

Blood sample collection was performed under the UK Animals (Scientific Procedures) Act of 1986. Blood samples from each animal were collected from the jugular vein (at 6 mo) or from the coccygeal vein or artery (at 15 mo), spotted onto Whatman FTA cards (Whatman International Ltd., Maidstone, UK), and allowed to air dry. Three 2.0-mm punches per sample were subsequently taken from each card, placed into a mixture of 189 μ L of FTA purification reagent (Whatman) and 1 μ L of 20 mg/mL proteinase K (Qiagen, Crawley, UK), and incubated at 56°C for 1 h. After incubation, the punches were washed twice with Tris-EDTA buffer (10 mM Tris-HCl, 0.1 mM EDTA; pH 8.0). Once the washing steps were complete, 50 μ L of H₂O was added to the punches and DNA was released from the FTA card by incubation at 96°C. The DNA extracts were then either used immediately for PCR or stored at –20°C.

SNP Genotyping

Genotyping of 3 previously identified SNP was carried out by Orchid Cellmark (Abingdon, UK): exon 2FB (identified by Buchanan et al., 2002) and UASMS1 and UASMS2 (identified by Nkrumah et al., 2005). A single PCR was used to generate 12 amplicons. Each 10- μ L reaction contained 5 μ L of DNA extract, 100 μ M of each deoxynucleotriphosphate (dNTP), 1 $\times$ Qiagen

PCR buffer containing 1.5 mM MgCl₂, 1× primer mix, 1 U of HotStar Taq (Qiagen), 1.5 mM MgCl₂, and 0.1 µg/µL of BSA. Thermocycling conditions consisted of 15 min at 95°C followed by 32 thermocycles of 30 s at 94°C, 1 min at 67°C, and 1 min at 72°C. Following amplification, 4 µL of a solution containing 4 U of Exonuclease I (New England Biolabs, Hitchin, UK), 1.4 µL of 10× Antarctic phosphatase buffer (New England Biolabs), and 2 U of Antarctic phosphatase (New England Biolabs) were added to the PCR products. The samples were incubated for 60 min at 37°C followed by 15 min at 72°C.

Snapshot reactions (Applied Biosystems, Warrington, UK) contained 5 µL of the treated PCR product treated with Antarctic phosphatase–Exonuclease I, 2 µL of Snapshot Multiplex Ready Reaction mix (Applied Biosystems), 2 µL of H₂O, and 1 µL of probe mix. The samples underwent 30 thermocycles of 30 s at 94°C and 20 s at 67°C. After single-base extension, 4 µL of calf intestinal alkaline phosphatase solution (CIP) containing 0.4 µL of 10× NEBuffer 3 buffer and 2 U of CIP was added to each sample. The samples were incubated for 60 min at 37°C followed by 20 min at 85°C.

Five microliters of the Snapshot–CIP product was added to 10 µL of Hi-Di formamide (Applied Biosystems) and the samples were electrophoresed on a 3100 Genetic Analyzer (Applied Biosystems). GeneMapper software (version 4.0, Applied Biosystems) was used to interpret the genetic profiles.

Statistical Analysis

The genotype frequencies of each polymorphism were examined for deviations from Hardy-Weinberg equilibrium within the population. The extent of linkage disequilibrium (LD) between pairwise genotype combinations was determined by analysis with Haploview software (Barrett et al., 2005). Single-marker association analyses were carried out to evaluate the relationship between different genotypes of each SNP and perinatal mortality. The dependent variable in these analyses was an indicator of perinatal mortality, coded as 0 (calf alive at 24 h after parturition) or 1 (calf born dead or died within 24 h of parturition) at an individual heifer calving. Because this dependent variable was a class rather than a numerical value describing mortality, the data were analyzed using a binary model. Several other recorded factors may also have affected perinatal mortality: farm, year and season of birth, sex of the calf, and age of the heifer at calving, as well as the particular polymorphisms carried at the loci of interest and the pedigree structure of the data set in the form of the additive genetic relationship matrix. All these factors were also included in the model to see whether

they affected the level of perinatal mortality in the data set. Age of heifer at calving was fitted as a third-order polynomial to see if there was a curvilinear relationship between age at first calving and perinatal mortality. The following generalized linear model was fitted to the data:

$$y_{ijkl} = \mu + \text{HYS}_i + A + A^2 + A^3 + \text{sex}_j \\ + \text{SNP}_k + g_l + e_{ijkl}, \quad [1]$$

where y_{ijkl} was perinatal mortality (0, 1) of the l th heifer ($l = 1\text{--}385$), calving in the i th herd-year-season combination (HYS), giving birth to a calf of the j th sex (male, female), possessing the k th SNP, and being A days old at calving. This animal model included g_l as a random genetic effect of the l th heifer. The data had an overall mean of μ . The residual term was e_{ijkl} . The number of calvings varied for the 3 SNP investigated. Calvings did not occur in all of the HYS combinations (20 herds, 3 yr, and 4 seasons), so there were a total of 60 HYS groups.

Observations on the calving outcome scale were related to the linear predictor using a logit link function of the form $\mu/(1 - \mu)$. The generalized linear model [1] was fitted to the data using ASREML (Gilmour et al., 2006). Means testing was carried out on the underlying scale and predicted means were calculated using the inverse link function $1/[1 + \exp(-p)]$, where p was the linear predictor on the underlying scale. Standard errors of differences were calculated for each pair of means tested on the underlying scale as outlined by Gilmour et al. (2006).

RESULTS

Gene frequencies for the 3 genotypes are summarized in Table 1. The genotype frequencies of the 3 SNP were distributed according to Hardy-Weinberg equilibrium proportions in the population. The SNP UASMS1 and exon 2FB were shown to be in close LD, with an r^2 value of 0.98. The r^2 values between UASMS1 and UASMS2 were, however, low (0.10), as were values between UASMS2 and exon 2FB (0.11).

Overall perinatal mortality in this data set for 385 heifers at first calving was 16.9%. The incidence of each of the 3 leptin SNP investigated is summarized in Table 2. For exon 2FB, higher levels of perinatal mortality were associated with both the CT (0.202 ± 0.0281) and the TT (0.194 ± 0.0438) alleles. These values were both 2-fold higher than the mortality rate found in heifers carrying the CC allele (0.111 ± 0.0243). For UASMS1, the TT allele was associated with lower levels of perinatal mortality (0.099 ± 0.0231), whereas both the CC

Table 1. Distribution of leptin genotypes and allelic frequencies in the study population of Holstein-Friesian heifers

SNP	n	Genotypic frequency			Allelic frequency		SE
		TT	CT	CC	T	C	
Exon 2FB	385	0.17	0.48	0.35	0.41	0.59	0.018
UASMS1	380	0.35	0.47	0.18	0.585	0.415	0.018
UASMS2	381	0.02	0.24	0.74	0.14	0.86	0.013

(0.206 ± 0.0448) and CT (0.206 ± 0.0285) alleles were higher and indistinguishable from each other. No differences in the level of perinatal mortality were found in the UASMS2 data set. However, the population had a skewed allele frequency with only 9 TT heifers present, so the number was too small for meaningful analysis.

Differences between alleles were investigated using the generalized linear model [1]. There was no effect of HYS, age of dam at calving, or sex of calf on the level of perinatal mortality for any of the 3 SNP ($P > 0.05$), although the random genetic term did significantly increase the log-likelihood compared with the model that excluded it ($P < 0.05$) in all 3 SNP. Perinatal mortality varied by allele for both exon 2FB and UASMS1 ($P < 0.05$; Table 3). Table 3 also shows the results of testing the differences between the alleles for both these SNP. Using the standard errors of differences on the underlying scale, for exon 2FB, the CC allele had a lower level of mortality than both the CT and TT alleles, and these latter 2 alleles had similar levels of perinatal mortality. In the case of UASMS1, the TT allele had a lower level of mortality than both the CC and CT alleles, which themselves were not different from each other.

DISCUSSION

These data show that 2 polymorphisms in the leptin gene (exon 2FB and UASMS1) are both associated with differences in perinatal mortality in a British population of Holstein-Friesian heifers. At the locus of both

exon 2FB and UASMS1, the studied mutation involved a cytosine to thymine base substitution. The exon 2FB SNP results in an AA change from Arg (encoded by the C allele) to Cys (encoded by the T allele). It has been suggested that the addition of an unpaired Cys to the leptin protein may affect its tertiary structure and disrupt the binding of leptin to its receptor (Buchanan et al., 2002; Liefers et al., 2003). Mutations in the promoter of leptin may also play an important role in differences in leptin expression during pregnancy. For exon 2FB, the mortality levels were similar between the TT and CT genotypes, suggesting dominance of base T over C, whereas for UASMS1, base C was dominant. These 2 polymorphisms were shown to be in close LD, with an r^2 value of 0.98. In support of this, LD between these 2 SNP loci has previously been reported as >0.99 (Schenkel et al., 2005; Banos et al., 2008). Therefore, the exon 2FB and UASMS1 SNP are considered together. In contrast, the r^2 values between UASMS1 and UASMS2 were low (0.10), as were values between UASMS2 and exon 2FB (0.11). This indicates a low level of LD between these SNP.

The distribution of heifers with CC, CT, and TT genotypes for exon 2FB was identical to that reported previously for North American Holsteins (Chebel et al., 2008). Heifers bearing the CC genotype had approximately half as many dead calves (15/135; 11%) compared with those bearing CT and TT genotypes (50/250; 20%). This is in contrast to the study by Chebel et al. (2008), in which no association between

Table 2. Incidence of perinatal mortality (born dead or died within 24 h of parturition) for 385 heifers with different genotypes for the exon 2FB, UASMS1, and UASMS2 SNP in the leptin gene

SNP	Allele of dam	Calf status		Proportion of calves born dead or died within 24 h
		Alive	Dead	
Exon 2FB	CC	120	15	0.11
	CT	146	37	0.20
	TT	54	13	0.19
UASMS1	CC	54	14	0.21
	CT	143	37	0.21
	TT	119	13	0.10
UASMS2	CC	233	48	0.17
	CT	75	16	0.16
	TT	8	1	0.11

Table 3. Association of exon 2FB and UASMS1 genotypes [predicted means $\pm$ standard error of differences (SED)] with perinatal mortality in 385 heifers

SNP	ANOVA <i>F</i> -value	Allele	Predicted mean	Logit value	SED	
					CC allele	CT allele
Exon 2FB	3.06*	CC	0.104 ^b	−2.155		
		CT	0.187 ^a	−1.472	0.350	
		TT	0.177 ^{ab}	−1.538	0.442	0.387
UASMS1	4.27*	CC	0.189 ^{ab}	−1.458		
		CT	0.189 ^a	−1.456	0.380	
		TT	0.091 ^b	−2.298	0.449	0.366

^{a,b}Means within a SNP with the same superscript were not significantly different ($P > 0.05$).

* $P < 0.05$.

genotype and the proportion of cows experiencing stillbirth was found. This earlier study, however, included more multiparous than primiparous cows, and these were not separated by parity in the analysis. Perinatal mortality is considered a different trait in younger and older cows (Mee et al., 2008). Because the present study was based exclusively on primiparous heifers, it can be speculated that the negative association of the T allele in exon 2FB with calf survival occurs only in heifers. Alternatively, or in addition, animals suffering a dead calf at first calving may be less likely to survive in the herd and reach a second lactation.

Heifers suffer a higher rate of perinatal mortality compared with multiparous cows (12 vs. 6%, Johanson and Berger, 2003; 13 vs. 6%, Lombard et al., 2007; 12 vs. 6%, Brickell et al., 2009). This higher mortality rate is in part thought to be a result of fetopelvic incompatibility causing increased calving difficulties (Hansen et al., 2004). Several studies in both Europe and North America indicate that the incidence of bovine perinatal mortality is increasing (Harbers et al., 2000; Meyer et al., 2001; Steinbock et al., 2003). Furthermore, an increased proportion of this loss does not appear to be associated with the traditional risk factors for perinatal mortality, which include dystocia, age at first calving, twinning, fetal gender, gestation length, and season (Mee et al., 2008). In support of this, there was no significant effect of HYS, age of the heifer at calving, or sex of the calf born on perinatal mortality in this study. Berglund et al. (2003) reported that approximately one third of dead calves were born clinically normal at full term with no difficulties at parturition, although they tended to be slightly smaller. Furthermore, about one-tenth of the stillborn calves were classified as dead in the uterus; the cause of death was speculated to be dysfunction of the placenta or hormonal changes (Berglund et al., 2003).

Heifers are still growing significantly during their first gestation (Coffey et al., 2006). Competition for nutrients exists between the heifers' own growth and

that of the developing fetus. In many mammals that carry singleton pregnancies (e.g., humans, Ong et al., 2002; horses, Wilsher and Allen, 2003; and dairy cows, Swali and Wathes, 2007), the firstborn offspring therefore has a lower birth weight than later siblings. Maternal nutritional status can alter the subsequent fetal growth trajectory via alterations to placental growth (McCrabb et al., 1991; Osgerby et al., 2003). An adequate placental size is vital to ensure adequate transfer of nutrients from the dam to the fetus (Fowden et al., 2008). A previous study found that the number of cotyledons and the placental weight in one Swedish Holstein heifer with stillbirth was low in comparison with other heifers, and it was postulated that the deviating placental characteristics affect the health status of the calf at term (Kornmatitsuk et al., 2004). Leptin has a functional role during pregnancy, acting as a placental growth factor (Masuzaki et al., 1997; Forhead and Fowden, 2009). Leptin concentrations in human cord blood are correlated with placenta size, suggesting a possible mechanism whereby the placenta can regulate its own growth (Hoggard et al., 2001). Furthermore, concentrations of leptin in cord blood are positively correlated with fetal birth weight (Ong et al., 1999). There is a substantial body of evidence from the human literature linking dysregulation in leptin function during pregnancy with a variety of pathological conditions, including recurrent miscarriage, gestational diabetes, preeclampsia, and fetal growth retardation (Bajoria et al., 2002; Sagawa et al., 2002; Meller et al., 2006; Toth et al., 2008). Leptin expression was decreased in the syncytiotrophoblast of miscarried placentas, whereas expression of leptin and its receptor in glandular epithelial cells was increased in miscarried tissue (Toth et al., 2008, 2009). Similarly, the expression level of leptin was significantly higher in the placental tissue from preeclamptic patients than that from normal pregnancies (Nishizawa et al., 2007). One study also reported that a SNP in the human leptin gene promoter was associated with gestational diabetes mellitus (Vaskú et

al., 2006), and another study demonstrated that mice with mutations in the leptin receptor gene developed gestational diabetes (Yamashita et al., 2001). Although the ruminant placenta does not appear to be a major source of leptin production (Block et al., 2001; Ehrhardt et al., 2001; Thomas et al., 2001), the presence of leptin receptors in bovine endometrium (Thorn et al., 2007) and at the maternal–fetal interface in the ovine placenta (Thomas et al., 2001) strongly suggests that circulating leptin, derived primarily from adipose tissue, could influence placental growth in the cow. Although the causative mutation was not confirmed in this study, it can be speculated that 1) the disruption of leptin binding to its receptor in the placenta resulting from an AA change, 2) mutations in the leptin promoter resulting in differences in leptin expression during pregnancy, or 3) both could have a detrimental effect on placental growth and fetal survival.

Another interesting possibility relates to the role of leptin in fetal lung development. Fetal lungs express high levels of leptin receptor, which increases in late gestation, and there is also some local production of leptin in lung tissue (Bergen et al., 2002; Henson et al., 2004). Leptin-deficient ob/ob mice experience impaired respiratory function (O'Donnell et al., 2000). Furthermore, studies in rats have demonstrated that antenatal treatment with leptin increases fetal lung weight (Kirwin et al., 2006) and that leptin can stimulate production of surfactant phospholipid in fetal lung explants (Torday et al., 2002). It is therefore possible that deficient leptin action in the lungs of fetal calves may predispose calves to respiratory problems at birth. Future studies should therefore examine the genotypes of the dead calves in addition to those of their dams.

Previous studies have shown significant associations of the exon 2FB SNP with a variety of production traits, such as carcass and meat quality traits in beef cattle (Buchanan et al., 2002; Schenkel et al., 2005). These authors concluded that the presence of the dominant T allele (CT or TT) was related to increased fat deposition and increased DMI. Dairy cows with the CT genotype had a lower BCS in early lactation, associated with higher milk production (Chebel et al., 2008). Sadeghi et al. (2008) found that Iranian Holstein bulls with the TT genotype produced offspring with higher milk, fat, and protein yields, and this polymorphism was also associated with higher milk yield and DMI in a study of British Holsteins (Banos et al., 2008). Increased calving difficulty in dairy heifers has been attributed to a reduction in the elasticity of the pelvis and to accumulation of fat in the pelvic region, which then protrudes into the genital tract (Meijering, 1984). Another possible explanation for the association between the TT or CT genotypes with reduced calf

survival in this study is that the dams were characterized by higher pelvic fat levels.

There were no significant allelic substitution effects for the UASMS2 SNP on perinatal mortality. However, the TT allele of UASMS2 was only present in 2% of the population, so the frequency was too low for meaningful analysis. A similar low genotypic frequency for the TT allele was previously found in several Canadian lines of predominantly beef cattle (range = 0–9%; Nkrumah et al., 2005). Beef cattle with genotype TT have previously been associated with increased serum leptin concentrations and higher measures of body fat compared with those with genotype CC (Nkrumah et al., 2005). In contrast, Schenkel et al. (2005) found no association between UASMS2 and any of the carcass and meat quality traits considered.

CONCLUSIONS

Evidence across several mammalian species suggests that leptin plays a key role in placental and fetal development. This study demonstrates that there are significant differences in the incidence of perinatal mortality across leptin genotypes. Heifers homozygous for the C allele of exon 2FB and the T allele of UASMS1 had a 2-fold lower incidence of calf mortality at first calving. Because the T allele of exon 2FB is associated with higher milk yield, selection for this trait may have contributed to the reported rise in perinatal mortality in the Holstein population. If these findings are validated with additional work using a larger sample from a wider geographical area, the potential exists to use genetic information as a tool to aid in selection, with the aim of reducing perinatal mortality.

ACKNOWLEDGMENTS

We thank Defra (London, UK) and DairyCo (Stoneleigh Park, UK) for funding the collection of the phenotypic data and Merial Animal Health Ltd. (Harlow, UK) for undertaking the SNP analysis. We are also grateful to Nicola Bourne (Royal Veterinary College, Hertfordshire, UK) for assistance with sample collection, and to all the farmers and their veterinary surgeons who participated in the study for their cooperation.

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