

Prepubertal growth and single nucleotide polymorphism analysis of the growth hormone gene of low birth weight Holstein calves

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Holstein calves weighing less than 20 kg at birth have been noted in Korea. Due to insufficient information, we raised small calves with age-matched normal birth weight Holstein calves and determined body weights before puberty. In addition, 3 single nucleotide polymorphisms (SNPs) of the growth hormone (GH) gene were analyzed. Up to 10 months of age, low birth weight calves were smaller than normal weight calves. In exon 5 of the GH gene, SNP genotype variation was detected in some small calves; however, this did not appear to be the only factor inducing low birth weight and slow growth.

Keywords: Holstein calf, growth hormone, low birth weight, prepubertal growth, single nucleotide polymorphism

Introduction

Normal body weight of Holstein calves is approximately 40 kg at birth [6], but calves weighing less than 20 kg at birth have been reported by local veterinarians in Korea since 2013. Epidemiological surveys have revealed that this phenomenon occurs in some calves born from cows that were artificially inseminated with one specific source of frozen semen. To our knowledge, there have been no previous studies on low birth weight Holstein calves in Korea, and there is insufficient information on the causes, growth patterns, productivity, and physiological characteristics of low birth weight Holstein calves. In Japanese black cattle, single nucleotide polymorphisms (SNPs) within the growth hormone (GH) gene have been associated with low body weight at birth [5]. Therefore, to assess productivity, we evaluated prepubertal growth and examined SNPs of in the GH gene of small Holstein calves to elucidate the causes of low birth weight.

Low birth weight calves (n = 14), which were considered to be smaller than normal Holstein calves by farmers, were transported to the University Animal Farm at Seoul National University, Korea (UAF) and their body weights determined (Table 1). Subsequently, those small calves were raised under the same conditions as age-matched normal birth weight Holstein calves (CONT, n = 7). To compare growth patterns, calf body weight was measured on a movable scale (DeLaval, Sweden) at 50-day intervals until 10 months of age. Furthermore, SNP genotyping of the GH gene was performed for nucleotides 2141, 2277, and 2283, which were previously reported to be located in the gene's exon 5 region [5,8]. For SNP genotyping of the GH gene, genomic DNA was extracted from blood collected from small Holstein calves born at local farms to cows artificially inseminated with semen from one sire (SCTS, n = 11), from normal calves born at local farms to cows artificially inseminated by semen from the same sire (NCTS, n = 5), and from normal calves born at the UAF to cows fertilized by a

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different source of semen (NCOS, n = 16). Three calves among 14 small calves were excluded in SNP genotyping due to death before the examination. Genomic DNA was extracted from hairs from the Holstein sire that was the source of the frozen semen. Primer sets used for sequencing of exon 5 of the GH gene were 5'-CCCTTTTGAAACCTCCTTCC-3' and 5'-GCGATGCAATTCCTCATT T-3' with a size of 425 bp. The sequences were genotyped by using Sequencher software (ver. 5.2; Gene Codes, USA).

Table 1. Characteristics of low birth weight calves after transportation to the University Animal Farm

ID	Sex	Transportation	
		Day after birth	Body weight (kg)
701	Male	1	14.6
502	Female	1	15.7
703	Male	4	20.7
704	Male	2	17.2
705	Male	1	20.2
706	Male	2	19.9
504	Female	2	13.9
505	Female	11	24.5
506	Female	9	31.8
507	Female	40	23.2
509	Female	23	35.6
510	Female	66	40.2
707	Male	47	29.6
511	Female	45	31.4

All numerical data are expressed as mean ± standard deviation. After conducting normality tests, significant differences among groups were analyzed by using one-way analysis of variance with Tukey's multiple comparison tests. Significance was set at $p < 0.05$. All statistical analyses were performed by using GraphPad Prism software (ver. 5.02; GraphPad Software, USA).

The small Holstein calves, with an average body weight of 230.6 ± 44.8 kg at 10 months of age, were undersized in comparison with the average body weight of CONT calves (352.7 ± 25.1 kg) (Fig. 1). According to the National Institute of Animal Science of Korea [10], average Holstein cattle weigh 276.3 kg at 10 months of age. All but one of the small calves in this study were below the standard growth curve for normal Holstein body weight. In comparison with data from the

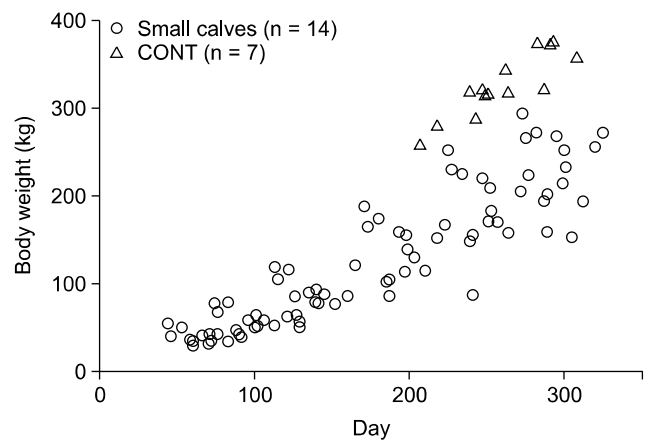


Fig. 1. Changes in body weight of small Holstein calves and normal Holstein calves (CONT) from birth to 10 months of age.

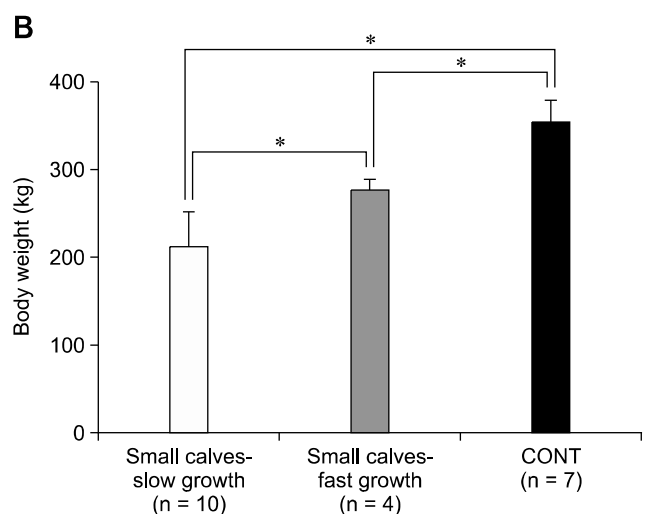
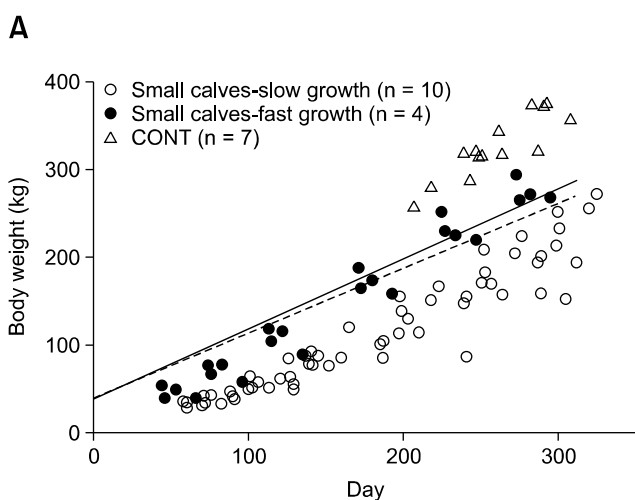


Fig. 2. (A) Comparison of growth between small calves and normal calves. Four small calves gradually exceeded the lower limit (dotted line) of standard body weight reported from the University of Wisconsin [4]. (B) Comparison of body weight in three groups (small calves with slow growth, small calves with fast growth, and normal Holstein calves [CONT]) at 10 months of age. A significant difference between groups is denoted as $*p < 0.01$.

University of Wisconsin-Madison [4], four of the 14 small calves in our study gradually exceeded the lower limit of the Holstein standard growth curve until 10 months of age (panel A in Fig. 2). Mean body weight of four of the small calves (276.5 ± 11.8 kg) exhibited a relatively rapid weight increase and was significantly higher than that of the remaining 10 small calves (212.3 ± 39.4 kg), but lower than that of CONT calves (352.7 ± 25.1 kg) at 10 months of age (panel B in Fig. 2).

Results of SNP genotyping of the GH gene are shown in Table 2. There was no genotype variation detected at the g.2277 and g.2283 nucleotide positions, whereas genotype variation was detected at the g.2141 nucleotide position. At g.2141 C > G, variations in genotypes were observed in the SCTS and NCTS groups, whereas only the CC and CG genotypes were detected in NCOS calves and the sire, respectively.

To the best of our knowledge, this is the first study examining the occurrence of small Holstein calves in Korea. The body weight of normal Holstein calves at birth is approximately 40 kg [6], whereas the small calves in this study were less than 20 kg at birth. Interestingly, the standard growth curve of Holstein calves varies among studies. The median and lower 5th percentile values of body weight at 10 months of age have been reported to be 260.8 kg and 191.4 kg, respectively, by the National Dairy Heifer Evaluation Project in the USA [3]. The Holstein Cattle Association of Japan reported a mean body weight of 276.9 ± 26.6 kg at 10 months of age. In a report by Yan *et al.* [11], average body weight of normal Holstein calves was 167 kg and 299 kg at 6 and 12 months, respectively, whereas the weight of small calves was 126 ± 36 kg and 218 ± 40 kg at 6 and 10 months, respectively. Taken together, the results indicate that Holstein calves with low body weight at birth in this study are notably smaller than normal Holstein calves at 10 months of age.

In previous studies on small cattle, body size was measured using hip or wither height and body weight, and cattle weighing

below 300 kg or 318 kg at maturity were defined as full miniature or mid-size miniature cattle, respectively [2]. Miniature cattle may be produced under several conditions. Cross-breeding is one of the causes of miniaturization because each breed has a specific growth rate for final body height, length, and weight [7,11]; however, the small Holstein calves in this study were not cross-bred. Artificial insemination and embryo transfer may also result in miniature cattle [2], but, in this study, abnormality in artificial fertilization techniques or semen was excluded because the NCTS were born after fertilization with the same semen. Miniature cattle produced due to malnutrition can grow to the normal size range provided they are in an adequate nutritional state, wherein they are excessively fed a concentrated feed [1,2]. In this study, however, nutritional effects could be excluded because feeding conditions were the same for both the small and CONT calves, and they were raised in the same pen.

As the genotype of g.2141 changed from C to G, the amino acid changed from leucine to valine. According to the report by Ishida *et al.* [5], when g.2141 is the GG type and g.2277 is the CC type, body weight at birth and at 30 days of age are significantly lower and the growth rate is slower than those of other genotypes in Japanese black cattle. In the present study, however, there was variation in the genotype for g.2141 in the SCTS group, in which there were no differences in body weight and growth rate based on genotype. Genetic variation at the g.2141 nucleotide position of the GH gene was detected in some small calves, but that variation did not seem to be the only factor inducing small birth weight and slow growth. Therefore, it did not seem that the SNPs of the GH gene that were analyzed in this study were closely associated with body weight at birth in the small Holstein calves.

Among the possible causes of small calves, GH secretion and abnormality in the regulation of GH also need to be considered. The GH-insulin-like growth factor (IGF) axis is involved in growth, reproduction, and milking performance [8], important factors that can significantly affect productivity of adult cattle. Therefore, further studies are required to compare the GH-IGF axis of small Holstein calves with that of normal Holstein calves [9].

In conclusion, low birth weight Holstein calves fertilized with frozen semen of the sire were smaller than normal Holstein calves up to and at 10 months of age. Genetic variation at the g.2141 nucleotide position of the GH gene may not be the main cause of the low birth weight. Further studies should be conducted to monitor growth patterns of low birth weight calves until maturity, productivity, and physiological characteristics.

Table 2. Results of SNP analysis of the growth hormone gene

Nucleotide position	Genotype frequency		
	CC	CG	GG
2141			
Sire (n = 1)	–	1.000	–
SCTS (n = 11)	0.455	0.364	0.181
NCTS (n = 5)	0.400	0.600	–
NCOS (n = 16)	1.000	–	–
2277	CC type in all samples		
2283	CC type in all samples		

SNP, single nucleotide polymorphism; SCTS, small calves born with the sire's semen; NCTS, normal calves born with the sire's semen; NCOS, normal calves born with other semens.

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Conflict of Interest

The authors declare no conflicts of interest.

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