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—Original Article—

Relationships Between the First Ovulation Postpartum and Polymorphism in Genes Relating to Function of Immunity, Metabolism and Reproduction in High-producing Dairy Cows

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Abstract. The decrease in fertility and conception rates of high-producing dairy cows is one of the major negative impacts for today's producers. The recovery of ovarian activity postpartum is affected by the status of immunity, metabolism and reproduction and plays a critical role in subsequent fertility after parturition in the cow. In the present study we investigated the relationships between polymorphisms in genes relating to the above functions and the first postpartum ovulation as a marker of the recovery of ovarian function in the cow. In immune function related-factors, the occurrence of first postpartum ovulation within 3 weeks in the C/C genotypes of tumor necrosis factor α (TNFα) exon (55.4%) and the A/G genotypes of TNFα promoter (55.4%) was significantly higher than that in T/T genotypes of TNFα exon (14.3%) and A/A genotypes of TNFα promoter (14.3%). Moreover, anovulatory cows with the T/T genotype of TNFα exon and the A/A genotype of TNFα promoter tended to have a prolonged days open compared with those of the other genotypes of TNFα polymorphisms. In metabolic function-related factors, ovulatory and anovulatory cows had a different distribution for alleles of the growth hormone receptor, but there were no significant differences in genotype and allele frequency of insulin-like growth factor-I polymorphism. No significant relationships were found between ovarian function after parturition and polymorphisms for reproduction-related genes. In conclusion, polymorphisms of TNFα gene both in exon and promoter regions have a strong association with the early first ovulation within 3 weeks after parturition in the high-producing dairy cow. Taken together, polymorphisms of TNFα gene could be strongly related to early first ovulation after parturition, thus being an effective tool of selection for improving reproductive performance in the high-producing dairy cow.

Key words: Cow, Ovulation, Polymorphism, Tumor necrosis factor α (TNFα)

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During the past 5 decades, milk production per cow has been dramatically increasing due to improvement of management, nutrition and genetic selection [1, 2]. In contrast, the decrease in fertility and conception rates of the modern high-producing dairy cow is the major cause of economic loss for dairy producers [2, 3]. In general, functions of leukocytes such as neutrophils and lymphocytes are suppressed after parturition in cows, and immunosuppression is related to the establishment of an intramammary infection resulting in mastitis [4, 5]. One of the associating factors for low reproductive performance and low immune function is negative energy balance that is due to a lower rate of feed intake compared with the energy necessary for milk production and is characterized by the loss of body weight and mobilization of body fat after parturition [6].

The recovery of ovarian activity plays a critical role in subsequent fertility after parturition in the cow [7]. In most dairy cows, medium-sized follicles appear by 5 days after calving, and large follicles appear by 10 days postpartum [8, 9]. Approximately half of all cows ovulate within 3 weeks postpartum, but in the other half, the dominant follicle of the first follicular wave regresses, and the first ovulation is delayed [10]. Metabolic hormones are related to the occurrence of first ovulation because ovulatory cows show higher levels of insulin-like growth factor-I (IGF-I) and lower levels of growth hormone (GH) than anovulatory cows during the peripartum period [11]. Moreover, we and others have shown that the occurrence of the early first ovulation within 3 weeks postpartum is positively associated with the recovery of normal ovarian function, first service and conception rate compared with anovulation in the high-producing dairy cow [12]. Therefore, it is suggested that ovulation within 3 weeks postpartum is a crucial phenomenon for subsequent recovery of ovarian function and conception and thus could be an initial index of improving reproductive performance [12].

Recently, the major interest of genomic studies in livestock species is the identification of molecular detectable markers promising quantitative traits. Polymorphisms, particularly single nucleotide polymorphisms (SNPs), within the genes may have potential to

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impair the ability of certain individual functions. There are many investigations of relationships between polymorphisms and important production traits such as milk production, milk composition and growth parameters in dairy cattle [13–15]. Recently, MacKey *et al.* (2007) reported that reproductive performance is negatively associated with both genetic merit for milk yield and actual level of milk production [16]. Moreover, Garmo *et al.* (2009) indicates that selection for milk yield negatively affects commencement of luteal activity (the recovery of ovarian function after parturition) [17]. They suggest that the increase in days to commencement of luteal activity caused by selection for high yields can be reduced if selection for milk yield is combined with fertility in the breeding program [17]. Therefore, we hypothesized that identification of candidate factors for the occurrence of the early first ovulation after parturition would prevent further reduction and foster improved reproductive status of the dairy herd.

Based on the above evidence, we investigated the relationships between the first ovulation within 3 weeks postpartum and polymorphisms in immune-, metabolic- and reproductive-related genes to identify those associated with early first ovulation after parturition in the high-producing dairy cow. In the present study, we examined the SNPs for tumor necrosis factor α (TNFα) [18, 19], CD18 [20] and interleukin-8 (IL8) [21] as immune function-related genes, GH receptor (GHR) [22], IGF-I [23] and signal transducer and activator 5A (STAT5A) [15] as metabolic function-related genes and fibroblast growth factor 2 (FGF2) [14], follicle stimulating hormone receptor (FSHR) [24], luteinizing hormone receptor (LHR) [24], estrogen receptor (ER) [25] and progesterone receptor (PR) [26] as reproductive function-related genes.

Materials and Methods

All experiments were conducted at the Field Center of Animal Science and Agriculture, Obihiro University, and all experimental procedures complied with the Guidelines for the Care and Use of Agricultural Animals of Obihiro University.

Experimental design

Ninety-two Holstein cows at the Obihiro University farm and 78 Holstein cows in a commercial dairy herd were used between 2004 and 2009. Blood samples were obtained once or twice a week between parturition and 3 weeks postpartum using sterile 10 ml tubes containing 200 μl stabilizer solution (0.3MEDTA, 1% acetylsalicylic acid, pH 7.4) for progesterone analysis and a heparinized 5 ml tube (VP=H050K, Terumo, Tokyo, Japan) for SNPs analysis. Blood tubes were centrifuged at 2000 *g* for 20 min at 4 C, and the plasma samples were kept at –30 C until analysis. Data for days of first artificial insemination (AI) and days open of these 92 cows were collected at the Obihiro University farm.

Progesterone determination

The plasma progesterone concentration was determined by direct enzyme immunoassays (EIA) [27]. The progesterone was extracted using diethyl ether as described previously [27]. The recovery rate of progesterone was 88%. The intra- and interassay coefficients of variation were 6.2 and 9.3%, respectively. The standard curve ranged from 0.05 to 50 ng/ml, and the ED50 of the assay was 2.4 ng/ml.

Definition of ovulation and anovulation

When the plasma progesterone concentration first exceeded 1 ng/ml, luteal activity was assumed to have been initiated [12, 28]. Cows having resumed luteal activity by 3 weeks postpartum were defined as having ovulated (ovulatory), whereas those not having resumed luteal activity by 3 weeks postpartum were defined as anovulatory.

Determination of gene polymorphisms

Genomic DNA was extracted from isolated blood (including white blood cells) using a Wizard Genomic DNA Purification kit (Promega. Madison, WI, USA). The polymerase chain reaction (PCR) primers used in this study are shown in Table 1. All PCR reactions were performed as described previously [27]. To examine the genotypes for each factor, the PCR products were digested with several specific restriction enzymes (37 C for 3–16 h) which are shown in Table 1. Restriction fragments were next separated by electrophoresis in 2 or 4% agarose (Wako Pure Chemical Industries, Osaka, Japan) in 1x TAE buffer (Promega) with 0.5 μg/ml ethidium bromide and visualized under UV light. For analysis of IL8 polymorphism, a tetra-primer amplification refractory mutation system-PCR was used along with the primers published by Leyva-Baca *et al.* (2007) [21]. Analysis of TNFα promoter polymorphism utilized the primers reported by Kahl *et al.* (2009) [19], PCR amplicons were sequenced in both 5' and 3' orientation using an Applied Biosystems 3730xl DNA Analyzer (Applied Biosystems, Foster City CA, USA), and SNP was identified by visual inspection of the electropherograms. In the present study, we could not analyze 9 samples for TNFα exon, 6 samples for TNFα promoter, 8 samples for CD18, 32 samples for IL8, 2 samples for GHR, 5 samples for STAT5A, 3 samples for IGF-1, 5 samples for FGF2, 8 samples for FSHR, 1 samples for ER and 29 samples for PR, although we did re-extract DNA and re-analyze the genotypes; therefore, these samples were removed.

Collection of leukocytes

We selected 44 cows from the Obihiro University farm with previously determined polymorphisms, and white blood cells were isolated from heparinized blood. Briefly, 10 ml of blood was centrifuged at 2000 *g* for 20 min at 4 C, blood plasma was removed, 40 ml of PBS was added and the mixture was then centrifuged at 1000 *g* for 10 min at 4 C. After washing the cell pellet (centrifuged at 500 *g* for 10 min at 4 C), the samples were placed into a 1.5-ml microcentrifuge tube containing 0.4 ml TRIzol reagent, homogenized and stored at –80 C until analysis.

RNA extraction and cDNA production

Total RNA was extracted from leukocytes following the protocol of Chomczynski and Sacchi using TRIzol reagent [29] as in our previous study [30]. The extracted total RNA was stored in RNA storage solution (Ambion, Austin, TX, USA) at –80 C until being used for cDNA production. The synthesized cDNA was stored at –30 C.

Table 1. Primers used in real-time PCR

Gene		Sequence of nucleotide (5'-3') [a]	Restriction enzyme	Reference No.
TNFα exon	FWD	GGGTGACTTGCTCTAACACTCATC	RsaI	[18]
	REV	AGGCCTCACTTCCCTACATCCCTA		
TNFα promoter	FWD	CCTGCTGTGCTGGAGTTCGTG		[19]
	REV	CTCATTCAACCAGCGGAAAAC		
CD18	FWD	GAGGAAATCGGCTGGCGCAATG	Fnu4HI	[20]
	REV	GTCATTGGGGGTGAGATG		
IL8	FWD inner	CTGTGTGGGTCTGGTGTCGA		[21]
	REV inner	GACATCCTGTATTTTATCTGACACCC		
	FWD outer	TTCCATTGCTTCTAAGAATTCCTCA		
	REV outer	AAACCAAGGCACAGTTCAACAG		
GHR	FWD	TGCGTGCACAGCAGCTCAACC	Fnu4HI	[22]
	REV	AGCAACCCCACTGCTGGGCAT		
IGF-I	FWD	ATTACAAAGCTGCCTGCCCC	SnaBI	[23]
	REV	ACCTTACCCGTATGAAAGGAATATACGT		
STAT5A	FWD	GAGAAGTTGGCGGAGATTATC	BstEII	[15]
	REV	CCGTGTGTCCTCATCACCTG		
FGF2	FWD	CATAGTTCTGTAGACTAGAAG	Csp6I	[14]
	REV	CCTCTAAAGAAGGATTAAGTCAAAATGGGGCTGGTA		
FSHR	FWD	CTGCCTCCCTCAAGGTGCCCCTC	AluI	[24]
	REV	TCAAGAACCGATTTACAGAATCCCCC		
LHR	FWD	CAAACTGACAGTCCCCCGCTTT	HhaI	[24]
	REV	GGAGGCTCGTACTGACCTTACCG		
ER	FWD	TTTGGTTAACGAGGTGGAG	BgII	[25]
	REV	TGTGACACAGGTGGTTTTTC		
PR	FWD	GTGAATTTGCTCCAAGATTC	DraIII	[26]
	REV	GCCCGACCTTCCCATAAC		

[a]FWD, forward; REV, reverse.

Real-time reverse transcription-polymerase chain reaction (real-time RT-PCR)

Quantification of mRNA expressions for TNFα and β-actin was performed using synthesized cDNA via real-time PCR with a LightCycler (Roche Diagnostics, Mannheim, Germany) using a commercial kit (QuantiTect™ SYBR Green PCR, QIAGEN GmbH, Hilden, Germany). The amplification program consisted of 15 min activation at 95 C followed by 40 cycles of PCR steps (15 sec denaturation at 94 C, 30 sec annealing at 58 C and a 20 sec extension at 72 C). For quantification of the target genes, a series of standards was constructed by amplifying a fragment of DNA (150–250 bp) that contains the target sequence for real-time PCR. The primers used for real-time PCR were as follows: 5-taacaagccg-gtagcccacg-3, forward, and 5-gcaagggctcttgatggcaga-3, reverse, for TNFα and 5-ccaaggccaaccgtgagaaaat-3, forward, and 5-ccacattc-cgtgaggatcttca-3, reverse, for β-actin. The values were normalized using β-actin as the internal standard.

Statistical analysis

Data about the number of ovulatory or anovulatory cows were analyzed by chi-square analysis and Fisher's exact tests. Data of mRNA expression, days of first AI and days open are presented as means ± SEM, and were analyzed by ANOVA followed by Bonferroni's multiple comparison test. Probabilities less than 5% ($P<0.05$) were considered to be significant.

Results

Genotype distribution in relation to immune function depending on the first postpartum ovulation in two herds

Table 2A shows the genotype frequencies observed for immune related factors such as TNFα exon, TNFα promoter, CD18 and IL8 in the two herds. Table 3 shows the genotype distribution in relation to immune function depending on the first postpartum ovulation in the two herds. In the present study, the numbers of cows with ovulation and anovulation within 3 weeks postpartum were 78 (45.8%) and 92 (54.1%), respectively. The occurrence of first postpartum ovulation within 3 weeks in the C/C genotype animals of TNFα exon (55.4%) was by 40% higher than that in T/T genotype (14.3%). With polymorphism of TNFα promoter, the occurrence of first postpartum ovulation in the A/G genotype animals (55.4%) was higher than that in A/A genotype (14.3%). In

Table 2. Distribution of genotypes in the two herds

A) Immune function related factors

	TNFα exon			TNFα promoter			CD18			IL8		
	T/T	T/C	C/C	A/A	A/G	G/G	C/C	C/T	T/T	A/A	A/T	T/T
Univ. farm	12	33	38	18	38	30	56	21	8	10	38	20
Comm. farm	2	40	36	10	36	32	47	19	11	15	34	21

B) Metabolic function related factors

	GHR			IGF-1			STAT5		
	T/T	C/T	C/C	A/A	A/B	B/B	G/G	G/C	C/C
Univ. farm	12	41	38	20	57	15	20	36	32
Comm. farm	2	20	55	24	35	16	19	36	22

C) Reproductive function related factors

	FGF2			FSHR		LHR		ER		PR	
	A/A	A/G	G/G	G/G	G/C	C/C	C/T	G/G	G/A	G/G	G/C
Univ. farm	17	53	19	79	9	92	0	64	27	27	53
Comm. farm	5	40	31	74	0	48	30	70	8	11	50

Table 3. Genotype distribution in relation to immune function depending on the first postpartum ovulation in the two herds

Factors		Ovulatory	Anovulatory	Ovulatory ratio (%)	P-value
TNFα exon (n=161)					
Genotype	T/T	2	12	14.3	0.018
	T/C	34	39	46.6	
	C/C	41	33	55.4	
Allele	T	38	63		0.013
	C	116	105		
TNFα promoter (n=164)					
Genotype	A/A	4	24	14.3	< 0.001
	A/G	41	33	55.4	
	G/G	31	31	50.0	
Allele	A	49	81		0.011
	G	103	95		
CD18 (n=162)					
Genotype	C/C	51	52	49.5	0.052
	C/T	21	19	52.5	
	T/T	4	15	21.1	
Allele	C	123	123		0.048
	T	29	49		
IL8 (n=138)					
Genotype	A/A	13	12	52.0	0.783
	A/T	33	39	45.8	
	T/T	21	20	51.2	
Allele	A	76	63		0.307
	T	75	79		

regard to polymorphism of CD18, the occurrence of first postpartum ovulation in the T/T genotype animals tended to decrease compared with the allele frequency of CD18 having a different distribution in ovulatory and anovulatory cows. There were no significant differences in genotype and allele frequency of IL8.

Genotype distribution in relation to metabolic function depending on the first postpartum ovulation in the two herds

Table 2B shows the genotype frequencies observed for metabolic-related factors such as GHR, IGF-1 and STAT5A in the two herds. Table 4 shows genotype distribution in relation to metabolic function depending on the first postpartum ovulation in the two

Table 4. Genotype distribution in relation to metabolism function depending on the first postpartum ovulation in the two herds

Factors		Ovulatory	Anovulatory	Ovulatory ratio (%)	P-value
GHR (n=168)					
Genotype	T/T	8	6	57.1	0.059
	C/T	34	27	55.7	
	C/C	35	58	37.6	
Allele	T	50	39		0.022
	C	104	143		
STAT5A (n=165)					
Genotype	G/G	22	17	56.4	0.165
	G/C	35	37	48.6	
	C/C	20	34	37.0	
Allele	G	79	71		0.046
	C	75	105		
IGF-1 (n=167)					
Genotype	A/A	18	19	48.6	0.856
	A/B	44	45	49.4	
	B/B	14	18	43.8	
Allele	A	80	83		0.719
	B	72	81		

herds. The genotype of GHR (P=0.059) tended to have a different distribution, and the allele frequency of GHR (P=0.022) had a different distribution in ovulatory and anovulatory cows. In regared to polymorphism of STAT5A, alleles had a different distribution in ovulatory and anovulatory cows. There were no significant differences in genotype and allele frequency of IGF-I polymorphism.

Genotype distribution in relation to reproductive function depending on the first postpartum ovulation in the two herds

Table 2C shows the genotype frequencies observed for reproductive-related factors such as FGF2, FSHR, LHR, ER and PR in the two herds. Table 5 shows the genotype distribution in relation to reproductive function depending on the first postpartum ovulation in the two herds. However, there were no significant differences in genotype and allele frequency of polymorphisms for reproductive-related factors.

Association of TNFα exon and TNFα promoter polymorphisms with TNFα mRNA expression levels in white blood cells

The TNFα gene expression was measured in white blood cells carrying different TNFα exon and TNFα promoter genotypes using real-time PCR as shown in Fig. 1. Higher relative TNFα mRNA expression was found for animals with the TNFα exon C/C genotype (n=16) than for those with the T/T (n=10) and T/C (n=18) genotypes. In regard to polymorphism for TNFα promoter, animals with the A/G (n=19) and G/G (n=8) genotypes tended to have higher TNFα mRNA expression than those with the A/A genotype (n=11, P=0.06).

Association of TNFα exon and TNFα promoter polymorphisms with days open in dairy cows

The days open (duration from parturition to next conception) was calculated for the different TNFα exon (Fig. 2A) and TNFα promoter genotypes (Fig. 2B) separated by parity. There were no significant differences in days open after 1st parturition. For days open after 2nd parity, animals with the T/T genotype of TNFα exon and the A/A genotype of TNFα promoter tended to have a longer days open than in those with the C/C of TNFα exon and other genotypes of TNFα promoter. For days open after 3rd parity, animals with the T/T genotype of TNFα exon and the A/A genotype of TNFα promoter had significantly longer days open than those with other genotypes of TNFα exon and the G/G genotypes of TNFα promoter. However, the duration between parturition and days to first AI service did not differ among either polymorphisms of both regions of TNFα or parity (data not shown).

Discussion

The recovery of ovarian activity postpartum plays a critical role in subsequent fertility after parturition in high-producing dairy cows. To examine the possibility that this physiological trait relates to polymorphisms of relating genes, we investigated the statistical relationships between the occurrence of first postpartum ovulation and polymorphisms in genes relating to immune, metabolic and reproductive function. Consequently, we found a strong correlation between reproductive performance and polymorphisms of immune function-related genes, especially TNFα.

TNFα is an essential cytokine that plays a key role in the initiation of innate proinflammatory responses and protecton against infectious pathogens. Indeed, polymorphisms in the TNFα gene have been associated with increased susceptibility to autoimmune and infectious diseases in mice [31] and humans [32] and bovine leukemia virus-induced disease [33]. In the present study, the occurrence of first postpartum ovulation within 3 weeks in the C/C genotype animals of TNFα exon (55.4%) was higher than that in T/T genotypes (14.3%). Likewise, in regard to polymorphism of

Table 5. Genotype distribution in relation to reproductive function depending on the first postpartum ovulation in the two herds

Factors		Ovulatory	Anovulatory	Ovulatory ratio (%)	P-value
FGF2 (n=165)					
Genotype	A/A	10	12	45.5	0.783
	A/G	43	50	46.2	
	G/G	24	26	48.0	
Allele	A	63	74		0.723
	G	94	102		
FSHR (n=162)					
Genotype	G/G	69	84	45.1	0.490
	G/C	3	6	33.3	
Allele	G	141	174		0.496
	C	3	6		
LHR (n=170)					
Genotype	C/C	66	74	49.5	0.476
	C/T	12	18	52.5	
Allele	C	144	176		0.498
	T	12	18		
ER (n=169)					
Genotype	G/G	68	66	50.7	0.151
	G/A	13	22	37.1	
Allele	G	149	154		0.177
	A	13	22		
PR (n=141)					
Genotype	G/G	22	16	57.9	0.163
	G/C	46	57	44.7	
Allele	G	90	89		0.363
	C	46	57		

TNFα promoter, the occurrence of first postpartum ovulation in the A/G genotypes animals (55.4%) was also higher than that in A/A genotypes (14.3%). Briefly, cows with the C/C genotypes of TNFα exon and the A/G genotypes of TNFα promoter are more likely to express early first ovulation after parturition. Brannstrom *et al.* (1995) reported that injection of TNFα concomitantly with LH increases the LH-induced ovulation rate in the rat ovary [34]. Moreover, intrafollicular injection of TNFα antiserum blocks ovulation together with the decreased apoptosis in granulosa cells in ewes, suggesting that TNFα is an essential component of the ovulatory mechanisms [35]. Interestingly, mRNA expression of TNFα in leukocytes was higher in cows of the ovulatory types (C/C of TNFα exon and A/G of TNFα promoter) compared with the anovulatory types (T/T of TNFα exon and A/A of TNFα promoter). Therefore, polymorphisms of TNFα gene may affect the transcription levels of TNFα. Moreover, anovulatory-type cows with the T/T genotype of TNFα exon and the A/A genotype of TNFα promoter had a tendency for prolonged days open compared with the other genotypes of TNFα polymorphisms after the 2nd and 3rd deliveries. These findings from the present study suggest that polymorphisms of TNFα are a factor strongly related to early first ovulation after parturition and that they may provide an effective tool of selection for improved reproductive performance in high-producing dairy cows. This hypothesis should be confirmed by large-scale field studies.

Integrins are essential for cell adhesion to the surfaces of other cells including leukocytes and are composed of a common chain such as CD18 and CD11. The dysfunction of CD18 caused by the D128G mutation (polymorphisms other than the present study) leads to the bovine leukocyte adhesion deficiency syndrome. Czarnik *et al.* (2004) reported that C/T mutation of CD18 gene is associated with milk protein content [20]. Although the occurrence of first postpartum ovulation in the T/T genotypes animals tended to decrease compared with other genotypes, the intensity of the relationships between CD18 and the early first ovulation is weak compared with those in TNFα polymorphisms. Interleukin-8 is a strong chemoattractant for neutrophils and has been associated with bovine mastitis [36]. Leyva-Baca *et al.* (2007) reported the associations of IL8 SNPs with milk fat yield [21], but in the present study, there were no significant differences in genotype and allele frequency of IL8 in regard to the occurrence of the first ovulation after parturition.

Growth hormone is a major regulator of growth and metabolism and thus affects growth rate, body composition, health and milk production. GH action is mediated by GHR, which is a transducer of GH signals within the cell for IGF-I secretion [37]. IGF-1 is also an important factor for development of the dominant follicle during the first follicular wave postpartum [6, 11]. In Polish Holstein-Friesian cattle, a genotype of GHR was related to IGF-I gene expression and plasma IGF-I concentration [22]. Therefore, we

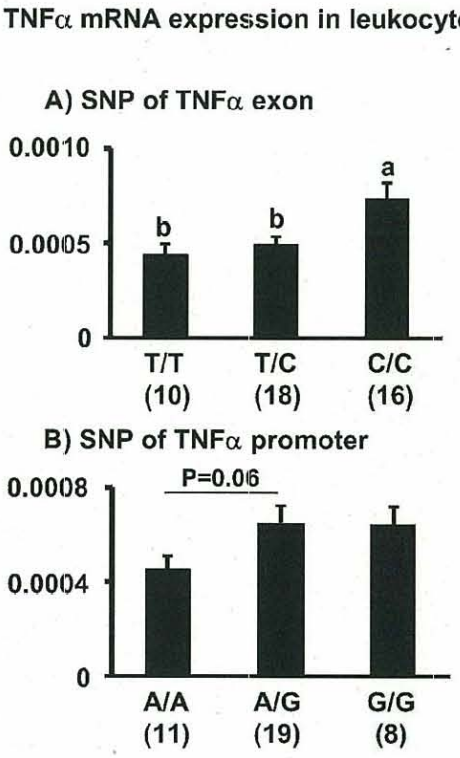

Fig. 1. Changes of mRNA expressions of TNFα in leukocytes depending on polymorphisms of TNFα. A: TNFα mRNA expression depending on SNP of TNFα exon. B: TNFα mRNA expression depending on SNP of TNFα promoter. In each figure, the numbers of cows with each types of SNP are shown under each type of SNP. Means ± SEM are presented. Different superscripts indicate statistically different values ($P<0.05$).

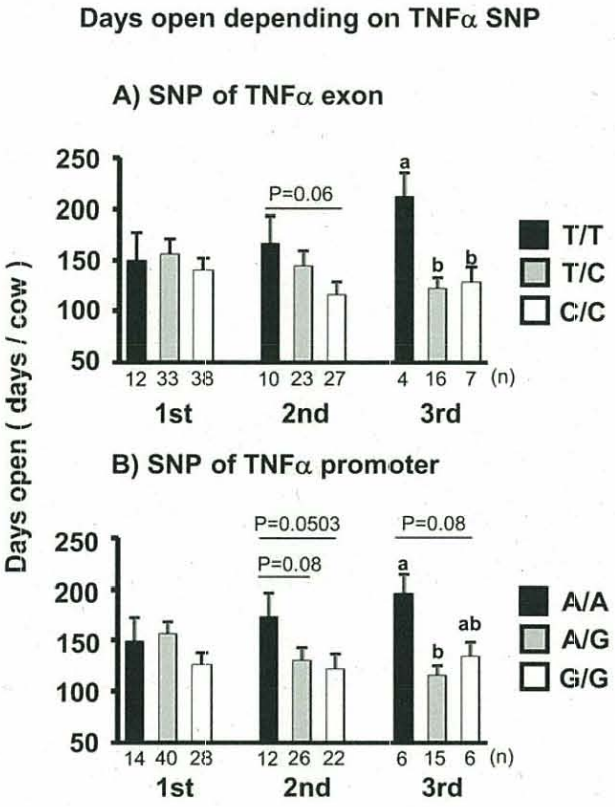

Fig. 2. Changes of days open depending on polymorphisms of TNFα. A: Days open after parturition depending on SNP of TNFα exon. B: Days open after parturition depending on SNP of TNFα promoter. Days open are analyzed for the 1st, 2nd and 3rd parity animals. In each figure, the numbers of cows with each type of SNP are shown under each type of SNP. Means ± SEM are presented. Different superscripts indicate statistically different values ($P<0.05$).

hypothesized that polymorphisms of the GH-GHR-IGF-I axis may strongly affect ovarian function during severe negative energy balance in the postpartum period. In the present study, the genotype of GHR ($P=0.059$) tended to have a different distribution, and the allele frequency of GHR ($P=0.022$) had a different distribution in ovulatory and anovulatory cows, but there were no significant differences in the genotype and allele frequency of IGF-1 polymorphism. On the other hand, STAT5A is known as a main mediator of GH action on target genes and to be important for body growth [37]. Khatib *et al.* reported that the G allele of STAT5A polymorphisms is associated with a significant decrease in milk protein and fat percentages and with low embryonic survival [15]. The polymorphism of the G allele of STAT5A is higher for the ovulatory cows in the present study. Interestingly, polymorphism of the bovine GH gene has no effect on the interval from calving to first ovulation in Holstein-Friesian cows [38]. These data suggest that GH signaling including GHR and STAT5A, but not polymorphisms of metabolic hormones such as GH and IGF-I, is important and related to the occurrence of the first ovulation after parturition, probably through due to modulation of the mRNA levels and blood concentration of IGF-I [23]. However, further investigations are needed to clarify the association between polymorphisms of the GH-GHR-STAT5A-IGF-I axis and reproductive performance in the high-producing dairy cow.

Finally, we investigated the relationships between polymorphisms of reproductive function-related genes and early first ovulation after parturition in dairy cows. Recently, Wang *et al.* (2008) reported that FGF2 polymorphisms are associated with fat percentage and yield, somatic cell score and productive life [14]. Also, FSH plays a major role in the involvement of follicle recruitment via FSHR, and Yang *et al.* (2010) demonstrated that FSHR genotypes affect ovarian responsiveness to superovulation in Holstein cows [39]. Additionally, Driver *et al.* (2009) indicated that the GG genotype of PR polymorphism was found to be associated with both fertilization and embryo survival rates [26]. However, we did not find any clear relationship between ovarian function and these SNPs for reproduction-related genes after parturition.

In conclusion, polymorphisms of TNFα gene both in exon and promoter regions have a strong association with early first ovulation within 3 weeks after parturition in high-producing dairy cows.

Additionally, these polymorphisms of TNFα genes appear to affect transcription levels, and the anovulatory type of TNFα SNP is related to prolongation of the interval between parturition and conception. Taken together, polymorphisms of TNFα gene are related to early first ovulation after parturition in high-producing dairy cows.

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