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Association of polymorphisms in the *IGF-I*, *GHR* and *STAT5A* genes with serum IGF-I concentration and reproductive performance of Holstein dairy cows

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ABSTRACT

This study was conducted to evaluate associations of polymorphisms in the genes for the growth hormone receptor (*GHR*), insulin-like growth factor I (*IGF-I*) and signal transducer and activator of transcription 5A (*STAT5A*) with serum concentrations of IGF-I, reproductive performance and milk production of postpartum Holstein dairy cows. Days from calving to first ovulation (DTO) and calving to conception interval (CCI) were evaluated in 95 Holstein cows. Serum concentrations of IGF-I and β-hydroxybutyrate (BHBA) were quantified in samples collected in sequential blood collections. Genotyping of the *IGF-I* and *STAT5A* genes was performed. The *IGF-I* polymorphism distribution was 35.9% CC, 46.1% CT and 18% TT. The IGF-I concentrations in circulation were greater in cows of the TT compared with both the CT and CC groups ($P < 0.05$). Genotype had a linear association ($P < 0.05$) with DTO and CCI, which were less for cows of the TT group. There was no association of *STAT5A BstEII* on serum IGF-I or reproductive variables ($P > 0.05$). When combining the *GHR AluI* T allele, obtained in a previous study, and the *IGF-I SnaBI* T allele from the current study, for the same cows, there were additive associations of both with serum IGF-I, BHBA, number of services per conception, DTO and CCI ($P < 0.05$). Thus, the *IGF-1 SnaBI* TT appears to be associated with fewer DTO and lesser CCI of lactating dairy cows and had an additive association with the *GHR AluI* T allele on indicators for improvement of fertility.

1. Introduction

Growth hormone (GH; i.e., somatotropin) is synthesized and secreted by the anterior pituitary and stimulates the production of insulin-like growth factor I (IGF-I) by the liver, which mediates most of GH actions in target tissues (Jones and Clemmons, 1995). The GH-IGF axis includes GH, GH receptor (GHR), GH binding proteins (GHBP), IGF-I, IGF-II, IGF receptors and six IGF binding proteins (IGFBPs) (Martinelli et al., 2008; Blum et al., 2018). There is expression of *GH* and *IGF-I* receptor genes in several tissues of cattle with differential regulation occurring in liver and reproductive tissues (Rhoads et al., 2008). There is activation by GH of the intracellular Janus kinase (JAK) and signal transducer and activator of transcription (STAT) families, in particular STAT5, which promotes *IGF-I*

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gene expression (Argetsinger et al., 1993). This pathway is essential for cell growth, differentiation and development in various tissues, and especially in the modulation of gonadotropin actions during follicular growth in the ovary (Armstrong and Webb, 1997).

In the postpartum high-producing dairy cow, negative energy balance (NEB) is associated with loss of body condition, a delay in the return of cyclic ovarian functions resulting in ovulation and reduced conception rate after the first insemination (Butler, 2000, 2003). In early lactation, a NEB is associated with increased mobilization of adipose tissue as non-esterified fatty acids (NEFA) that alters liver metabolism and increases plasma β-hydroxybutyrate (BHBA) concentrations (Reist et al., 2003). Furthermore, liver expression of the *GHR* and *IGF-I* genes is reduced in dairy cows after calving due to the marked NEB (Radcliff et al., 2003), accounting for relatively lesser concentrations, as compared with other stages of reproduction, of serum IGF-I in the early postpartum period (Kobayashi et al., 1999). There are actions of IGF-I in the ovary to enhance the actions of the gonadotropins, increasing granulosa and theca cell proliferation and differentiation (Armstrong and Webb, 1997) and estradiol production (Spicer et al., 1993). Reduced postpartum serum IGF-I, therefore, is associated with delayed first postpartum ovulation (Butler et al., 2006; Kawashima et al., 2007a) that, is subsequently associated with a longer calving to conception interval (Walsh et al., 2007; Galvao et al., 2010; Vieira-Neto et al., 2014).

The *Alu*I and *Sna*BI polymorphisms in genes encoding for the *GHR* and *IGF-I* proteins, respectively, are related to the hepatic abundance of *IGF-I* mRNA and plasma IGF-I concentration in Holstein steers (Maj et al., 2008). The *GHR-Alu*I and *IGF-I Sna*BI polymorphisms had no effect on values for milk production variables, although *IGF-I* genotype affected the calving to first insemination interval in dairy cows (Nicolini et al., 2013). There has been confirmation of the association of the *GHR Alu*I polymorphism with greater serum concentrations of IGF-I and a reduced calving to conception interval in Holstein dairy cows (Schneider et al., 2013). In addition to these reports on the GH-IGF axis, results of some other studies indicate there is an effect of polymorphisms on the GH intracellular mediator, *STAT5A,* in affecting fertilization and embryonic survival rates in cattle (Khatib et al., 2008, 2009), further emphasizing the important functions of the GH-IGF axis in reproduction. Because the serum concentration of IGF-I is a heritable trait ($h^2 = \sim 0.4$) and has been successfully used for genetic selection in cattle (Huang et al., 2011), it was of interest to further characterize the effects of polymorphisms in genes of the GH-IGF axis that may have important effects on dairy cattle fertility. Although in previous studies there has been evaluation of the effect of these polymorphisms individually on calving to ovulation and calving to conception interval (Nicolini et al., 2013; Schneider et al., 2013; Hax et al., 2017; Leyva-Corona et al., 2018) in none of these studies has there been an evaluation of the combined effects, or evaluation of more parameters such as serum concentrations of IGF-I and β-hydroxybutyrate (BHBA) concentrations in the same group of cows.

Based on all these previous finding, the aim of the present study was to investigate the effects of polymorphisms in the *GHR*, *IGF-I* and *STAT5A* genes and the combination of these effects on serum concentrations of IGF-I, reproductive performance and milk production of postpartum Holstein dairy cows.

2. Materials and methods

2.1. Animals and reproductive management

For this study, 95 Holstein dairy cows were evaluated from 21 days before calving to 210 days in milk (DIM). These are the same group of cows used and described in a previous study that were genotyped for the GHR *Alu*I polymorphism (Schneider et al., 2013). Cows were provided with *ad libitum* access to a total mixed ration by feeding twice daily. The pre- and post-partum diet, respectively, was composed of 10% and 16.6% of crude protein, 67.2% and 72.8% of total digestible nutrients, 45.4% and 32.6% of neutral detergent fiber and 1.56 and 1.72 Mcal/kg of net energy for lactation. At 55 DIM, the OvSynch-TAI program was initiated in all cows. The protocol was repeated in cows diagnosed as not pregnant by ultrasonic examination 30 and 60 days after AI. Cows having visual symptoms of estrus before ultrasonic examination were re-inseminated. The number of inseminations per conception, pregnancy rate at first postpartum insemination and calving to conception interval (CCI) were evaluated from the records until 210 days in milk (DIM). Body condition score was evaluated by a single technician at 60 DIM.

2.2. Sample collection and analyses

For determination of the genotype and values for biochemical/hormonal variables, blood collection from the coccygeal vein was performed at 0, 7, 21 and 60 days in milk (DIM). The samples were collected in vacutainer tubes containing EDTA for DNA extraction and without EDTA for serum hormonal and biochemical analyses. Blood was centrifuged and the serum separated and frozen at −20 °C for analysis of IGF-I and BHBA. Serum BHBA was quantified using an autoanalyzer (Boehringer Mannheim Hitachi 104, Diagnostic Laboratory Systems, Indianapolis, IN, USA). Serum concentrations of IGF-I were quantified using an RIA utilizing the method described by Butler et al. (2006) with an inter- and intra-assay CV of 17.8% and 13.3%, respectively.

Milk samples were collected twice a week from 7 until 60 DIM and progesterone concentrations in un-extracted milk samples were assayed using previously published procedures (Arnstadt et al., 1982; Waldmann, 1999). Progesterone concentrations greater than 1 ng/mL in two consecutive samples were considered indicative of an ovulation occurrence and the days from calving to first ovulation (DTO) were calculated.

2.3. Genotyping

The DNA extraction from whole blood was performed using the Wizard Genomic DNA Purification Kit (Promega Corporation) and

quantified by measuring the absorbance at 260 nm in a spectrophotometer. Single nucleotide polymorphisms in the genes of interest were identified using PCR-restriction fragment length procedures (PCR-RFLP). For determination of the *IGF-I* alleles by PCR, a 249-bp fragment was amplified using the primers: forward, 5′-ATTACAAAGCTGCCTGCCCC-3′; reverse, 5′-ACCTTACCCGTATGAAAGGAATATACGT-3′ (Ruprechter et al., 2011). For the *STAT5A* alleles the primers: forward, 5′-GAGAAGTTGGCGGAGATTATC-3′; reverse, 5′-CCGTGTGTCCTCATCACCTG-3' were used (Khatib et al., 2008). The annealing of the primers was performed at 64 and 58 °C for *IGF-I* and *STAT5A*, respectively. The amplified fragments were digested in reactions containing 5 μL of PCR product and 5 U of restriction enzyme *Sna*BI for *IGF-I* and 3 U of restriction enzyme *BstE*II for *STAT5A,* respectively. The digestion was performed in the thermocycler at 37 °C for 2 to 3 h. After digestion of the amplified products, the DNA fragments were separated on a 2% agarose gel. A standard molecular weight marker of 100-bp was used in each gel to record the size (base pairs) of the digested fragments. The DNA fragments were labeled with SYBR Safe (Life technologies) and visualized on the agarose gel under ultraviolet light. Gels were photographed for data summarization and statistical analysis.

Individual genotypes were determined by analysis of the fragment size of the digestion products. The different IGF-I genotypes formed the following products: *Sna*BI (TT): 223 and 26 bp; *Sna*BI (CT): 249, 223 and 26 bp; *Sna*BI (CC): 249 bp (undigested). For the different STAT5A genotypes, there were the following fragments: *BstE*II (GG): 676 bp; *BstE*II (CG): 820 and 676 bp; *BstE*II (CC): 820 bp.

2.4. Statistical analysis

Data from this experiment were analyzed using the SAS statistical program (SAS Institute Inc., Cary, USA). The data from the current study were combined with GHR genotyping results for the same group of cows from a previous study (Schneider et al., 2013). Lactation number was used as a covariate and the least square means and standard errors are presented. Genotypes were classified as 0, 1 and 2 for either *IGF-I* (T) or *STAT5A* (G), with two indicating the homozygous presence of an individual allele (*IGF-I* T and *STAT5A* G, respectively). Additionally, there was combining and testing for the presence of the individual alleles for the *GHR* and *IGF-I* polymorphisms in each cow to assess its associations with serum IGF-I and fertility parameters. Thus, the combined presence of the alleles in the two genes was indicated by 0, 1, 2 and 3 + 4, indicating the presence of none or an increasing abundance specifically of T alleles for GHR and IGF-I. The means for milk production, IGF-I, BHBA, DTO, CCI, and AI/conception were analyzed using the GLM method and a *post-hoc* Tukey test to evaluate the linear, quadratic and cubic associations with the presence of the alleles. The DTO and CCI were also evaluated using Kaplan-Meier survival analysis procedures utilizing the Log-rank (Mantel-Cox) test on GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA, USA). The *P*-values that were less or equal to 0.05 were considered significant.

3. Results

For the *IGF-I Sna*BI polymorphism, 35.9% of the cows had the CC genotype, 46.1% had the CT genotype and 18% the TT genotype (Table 1). The mean serum IGF-I concentrations were greater in cows of the TT group compared with cows of both the CT and CC groups ($P < 0.05$, Table 1). Conversely, the mean serum BHBA concentration was less in cows of the TT group compared with cows of both the CT and CC group ($P < 0.05$; Table 1). There were no significant associations of *IGF-I* genotypes with milk production and BCS.

The mean DTO and CCI were less for cows with the TT genotype for the *IGF-I Sna*BI polymorphism compared with cows with both the CT and CC group and there was a linear association with the alleles ($P < 0.05$; Table 1). The number of AI/conception was also less for cows of the TT group ($P < 0.05$). The pregancy rate as a result of the initial postpartum insemination was not different

Table 1
Serum concentrations of IGF-I and BHBA, milk production and values for reproductive variables during lactation among cows with the *IGF-I Sna*BI genotypes.

Variable	Genotype *IGF-I Sna*BI			*P* value[a]	
	CC	CT	TT	Linear	TT compared with CT and CC
Cows, %(number)	35.9 (32/89)	46.1 (41/89)	18 (16/89)	–	–
IGF-I, ng/mL	61.6 ± 4.6	70.9 ± 4.3	76 ± 7.4	0.09	0.05
BHBA[b], mg/dL	7.2 ± 0.4	6.7 ± 0.4	5.5 ± 0.7	0.03	0.05
Milk, kg/day	33.8 ± 1.0	34.0 ± 0.9	32.8 ± 1.5	0.59	0.67
BCS[c]	3.3 ± 0.1	3.3 ± 0.1	3.5 ± 0.1	0.14	0.31
DTO[d]	30.9 ± 2.6	31 ± 2.5	20.2 ± 4.8	0.05	0.15
CCI[e]	108.8 ± 7.4	97.4 ± 6.7	78.4 ± 13.2	0.05	0.05
AI/Conception, n	2.8 ± 0.3	2.7 ± 0.3	1.4 ± 0.5	0.01	0.05
Pregnancy rate at 1 st AI, % (*n*)	22.6 (7/31)	27.5(11/40)	46.1 (6/13)	> 0.05	

[1a] $P < 0.05$ was considered to be significant.
[b] β-Hydroxybutyrate.
[c] Body condition score at 60 DIM.
[d] Days to first post-partum ovulation.
[e] Calving to conception interval (days).

Table 2
Serum concentrations of IGF-I and BHBA, milk production and values for reproductive variables during lactation among cows with the *STAT5A BstE*II genotypes.

Variable	Genotype *STAT5A BstEII*			*P* value[a]	
	CC	CG	GG	Linear	CC compared with CG and GG
Cows, % (number)	28.9 (24/83)	39.8 (33/83)	31.3 (26/83)	–	–
IGF-I, ng/mL	86.2 ± 5.7	76.5 ± 5.1	81.9 ± 5.5	0.59	0.31
BHBA[b], mg/dL	7.0 ± 0.5	6.7 ± 0.5	6.6 ± 0.5	0.56	0.57
Milk, kg/day	34.5 ± 1.2	33.5 ± 1.0	33.2 ± 1.1	0.41	0.41
BCS[c]	3.4 ± 0.1	3.3 ± 0.1	3.2 ± 0.1	0.08	0.11
DTO[d]	31.1 ± 3.2	29.9 ± 2.9	28.7 ± 3.3	0.59	0.63
CCI[e]	101.3 ± 9.3	102.0 ± 7.3	93.8 ± 9.3	0.57	0.76
AI/Conception, n	2.5 ± 0.4	2.6 ± 0.3	2.6 ± 0.3	0.88	0.88
Pregnancy rate at 1st AI, % (n)	30.4 (7/23)	27.3 (9/33)	34.8 (8/23)	> 0.05	

[a] $P < 0.05$ was considered to be significant.
[b] β-Hydroxybutyrate.
[c] Body condition score at 60 DIM.
[d] Days to first post-partum ovulation.
[e] Calving to conception interval (days).

among these allele groups.

For the *STAT5A Bst*EII genotypes, 28.9% of the cows had the CC genotype, 39.8% the CG genotype and 31.3% the GG genotype (Table 2). There were no differences among *STAT5A* genotypes for serum IGF-I concentrations, milk production, BCS, postpartum DTO and CCI or when there was assessment of other variables (Table 2).

There was combining of the alleles for *IGF-I Sna*BI with the alleles for the *GHR Alu*I polymorphisms from a previous study (Schneider et al., 2013). From the cows of the TT group with the *GHR* polymorphisms, 10% also were TT and 70% were CT for the *IGF-I* polymorphism. From the cows of the AT group with the *GHR* polymorphisms, 28% were TT and 39% were CT for the *IGF-I* polymorphism. Because the T allele for both *GHR* and *IGF-I* polymorphisms was associated with the greatest serum IGF-I concentrations, there was evaluation of the additive association of the presence of T alleles in these genes. Cows having three or four T alleles for both *GHR* and *IGF-I* polymorphisms combined had greater serum IGF-I concentrations, lesser serum BHBA concentrations, a lesser CCI and less services per conception ($P < 0.05$) as compared with cows with no, one or two T alleles (Table 3). Results from the survival analysis for DTO indicate there was a tendency for cows having two, three or four favorable T alleles to have ovulations earlier in the postpartum period ($P = 0.06$; Fig. 1A) and to have a shorter CCI ($P = 0.04$; Fig. 1B). There was a quadratic association with BCS, where cows classified as 4 had the greatest BCS (Table 3).

4. Discussion

The polymorphism in the *IGF-I* gene evaluated in the current study was associated with serum IGF-I concentration and postpartum fertility in dairy cows, without associations with milk production. These results were consistent with results from a previous study using the same cows genotyped for a *GHR* polymorphism (Schneider et al., 2013). In this previous study the TT genotype for the *GHR*

Table 3
Serum concentrations of IGF-I and BHBA, milk production and values for reproductive variables during lactation for cows with combined genotypes (*GHR* and *IGF-I*); 0, 1, 2 and 3/4 indicate the presence of none or an increasing combination of T alleles in both polymorphisms.

Parameter	*GHR/IGF-I* T alleles combined				*P* value[a]		
	0	1	2	3/4	Linear	Quadratic	Cubic
Cows, % (number)	16.8(15/89)	34.8 (31/89)	24.7 (22/89)	23.6 (21/89)	–	–	–
IGF-I, ng/mL	63.3 ± 6.3	70.3 ± 4.5	54.5 ± 5.7	83.3 ± 6.0	0.10	0.05	0.01
BHBA[b], mg/dL	8.7 ± 0.8	6.8 ± 0.5	8.3 ± 0.7	5.5 ± 0.7	0.01	0.52	0.01
Milk, kg/day	33.7 ± 1.4	34.0 ± 1.0	33.5 ± 1.2	33.4 ± 1.4	0.80	0.84	0.83
BCS[c]	3.3 ± 0.1	3.3 ± 0.1	3.0 ± 0.1	3.5 ± 0.1	0.44	0.01	0.02
DTO[d]	31.2 ± 3.5	28 ± 2.6	38.4 ± 3.2	20.4 ± 3.6	0.17	0.02	0.01
CCI[e]	121.6 ± 11.5	104.5 ± 7.1	92.7 ± 9.3	76.7 ± 10.2	< 0.01	0.95	0.81
AI/Conception, n	3.4 ± 0.4	2.9 ± 0.3	2.3 ± 0.4	1.5 ± 0.4	< 0.01	0.81	0.95
Pregnancy rate at 1st AI, % (n)	20.0 (3/15)	22.6(7/31)	26.3 (5/19)	47.4 (9/19)	> 0.05		

[1a] $P < 0.05$ was considered to be significant.
[b] β-Hydroxybutyrate.
[c] Body condition score at 60 DIM.
[d] Days to first post-partum ovulation.
[e] Calving to conception interval (days).

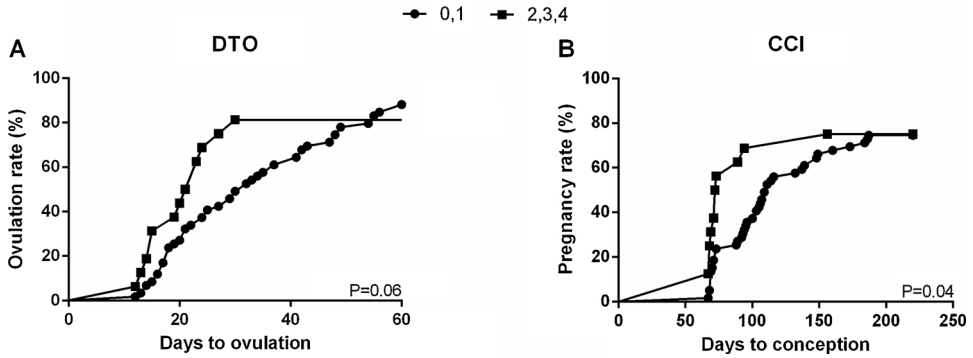

Fig. 1. Survival analysis of days from parturition to first ovulation (DTO, Panel A) and calving to conception interval (CCI, Panel B) for cows having no or one compared with two, three, or four favorable T alleles for *GHR* and *IGFI* gene polymorphisms combined.

polymorphism was associated to increased IGF-I concentration, fewer DTO and a shorter CCI (Schneider et al., 2013). Interestingly, when combining genotypes of cows that specifically had T alleles for both polymorphisms, there was an additive association. Cows with a more T alleles for *GHR* and *IGF-I* genes combined had greater serum IGF-I concentrations and a lesser calving to conception interval. There, however, was not any association of the *STAT5A* genotypes with the evaluated parameters and, therefore, these were excluded from the combined analysis.

When there was assessment of the *IGF-I* SNP alone or in combination with the *GHR* SNP, there was an increase in serum IGF-I concentration for cows with the T allele compared with cows with the C allele. Most of the synthesis of IGF-I is in the liver in response to GH (Jones and Clemmons, 1995). The GHR is present in many tissues and the liver is the site of its greatest abundance of this receptor (Rhoads et al., 2008). Expression of *GHR* and *IGF-I* genes in the liver is acutely responsive to nutritional status (Bornfeldt et al., 1989) and during NEB the liver becomes refractory to GH stimulation and circulating IGF-I concentrations are markedly reduced (Vicini et al., 1991). Importantly, high producing dairy cows have greater serum GH concentrations during early lactation, but lesser IGF-I and insulin concentrations (Gong et al., 2002). The *IGF-I* polymorphism evaluated in the current study occurs in the promoter region of the *IGF-I* gene (Ge et al., 2001), which was suggested to affect the transcription of the gene, and thereby, is reflected in serum IGF-I concentrations. Similarly, the *GHR* polymorphism was associated with regulation of liver IGF-I mRNA transcript abundance and circulating concentrations of IGF-I in Holstein bulls and heifers (Maj et al., 2008). Results from the current study further indicate polymorphisms in both *GHR* and *IGF-I* genes are associated both independently and additively with serum IGF-I concentrations in postpartum dairy cows.

In dairy cattle, both the duration and severity of early postpartum NEB affect the interval to resumption of ovulations following parturition (Beam and Butler, 1999). The capacity of ovarian follicles to produce sufficient estradiol for induction of the pre-ovulatory LH surge and subsequently ovulation seems to depend on the availability of IGF-I and insulin in circulation, mainly at the time of onset of the postpartum NEB (Beam and Butler, 1999; Butler et al., 2006; Cheong et al., 2016). Cows that had ovulations from the first dominant follicle that developed postpartum had greater serum concentrations of IGF-I (Butler et al., 2006; Kawashima et al., 2007a, b). These previous findings are consistent with the findings in the current study, where SNPs in the *GHR* and *IGF-I* genes that were associated with greater serum IGF-I concentrations were also associated with a reduced calving to first ovulation interval. This is important because the resumption of ovulations postpartum and ovulation from the dominant follicle that develops during the first wave of follicular development postpartum that occurs during the first 3 weeks postpartum are related to greater pregnancy rates at the first postpartum AI and fewer days non-pregnant during lactation (Galvao et al., 2010; Vieira-Neto et al., 2014). In previous studies of the IGF-I polymorphism, dairy cows with the *IGF-I Sna*BI TT genotype had ovulations earlier postpartum (Nicolini et al., 2013) which is consistent with findings in the present study where the T allele was associated with a lesser postpartum interval to ovulation and conception. In a subsequent study, there was not an association between *IGF-I* alleles and the fertility of cows when comparing different nutrition and management systems (Hax et al., 2017).

In the current study, there were greater serum IGF-I concentrations that were associated with a shorter DTO and CCI for cows with the *IGF-I* TT genotype and overall shorter CCI for cows having the TT genotype for both *GHR* and *IGF-I* polymorphisms. Cows with at least three T alleles located in both genes had greater serum IGF-I concentrations, a shorter interval from calving to first ovulation, fewer number of inseminations per conception and a reduced CCI. Overall, results of the present study support the hypothesis that SNPs in the GH-IGF-I axis associated with serum IGF-I concentrations will have a positive effect on the fertility of postpartum dairy cows.

Relatively lesser circulating concentrations of IGF-I and relatively greater concentrations of BHBA and NEFA have all been associated with impaired reproductive performance in dairy cows (Abdelli et al., 2017). There was an inverse relationship for IGF-I and BHBA in the current research with cows having greater serum IGF-I concentrations having the least concentrations of BHBA (i.e., cows with the TT genotype for *IGF-I* and for the combined *GHR* and *IGF-I* TT genotype had the least concentrations of BHBA). Cows with relatively greater blood concentrations of BHBA had impaired hepatic GH-mediated JAK2-STAT5 pathway signaling and downregulated expression of the IGF-I gene and lesser IGF-1 secretion (Du et al., 2018). Additionally, results from other previous studies indicated there was a need to evaluate the association of the *STAT5A* polymorphism with serum IGF-I and fertility. In some

studies, results indicate there was an effect of *STAT5A* polymorphisms on the fertilization of oocytes and embryonic survival rates in cattle (Khatib et al., 2008, 2009). In another study, however, there was no effect of the STAT5A polymorphism on the fertility of Holstein cows (Leyva-Corona et al., 2018) which is consistent with results from the current study. Overall, the findings in the present study indicate there is no association of the *STAT5A* mutations with serum IGF-I concentrations in lactating cows.

In conclusion, the presence of the *IGF-I Sna*BI T allele in lactating dairy cows is associated with increased serum IGF-I concentrations, fewer days from calving to first ovulation and a shorter CCI. In addition, the simultaneous presence of T alleles with both the *GHR Alu*I and *IGF-I Sna*BI polymorphisms is associated with greater serum IGF-I concentrations (additive association) and a lesser CCI in postpartum dairy cows. Together, these results indicate selection to increase the prevalence of the *IGF-I Sna*BI T and *GHR Alu*I T alleles in the population may be a logical goal to increase reproductive efficiency in dairy cattle in the future.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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