Association of *STAT5A* and *FGF2* gene mutations with conception rate in dairy cattle

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INTRODUCTION

During the last decade there has been growing scientific interest in the use of genomic information as an additional tool in conventional dairy cows' breeding schemes. It is now well established that dairy cows' fertility has declined during the last decades and this may not be remedied only by improved management. Given that there is a substantial genetic background in this decline, emphasis should be placed in efforts to improve dairy cows' fertility through genetic selection. Although there are substantial genetic effects that contribute to this infertility, little progress has been made on the identification of major genes affecting reproduction traits. There is an urgent need to identify the genetic factors responsible for the decline in fertility in cattle.
The objective of this study was to investigate the association of two specific polymorphisms, the SNP12195 and SNP11646 polymorphisms of the Signal Transducer and Activator of Transcription 5A (*STAT5A*) and Fibroblast Growth Factor 2 (*FGF2*) genes respectively with fertility in cattle. These genes have been previously reported to be associated with *in vitro* fertilization and embryonic survival rate in cattle.

MATERIALS AND METHODS

A total of 120 1st lactation Holstein cows, raised on the same farm and under the same conditions, were divided into 2 performance groups (n=60 per group), the 1st consisting of animals that conceived and the 2nd that did not conceive with the 1st artificial insemination (A.I.).
DNA was extracted from whole blood samples using the NucleoSpin Blood kit (Macherey-Nagel, Germany) according to manufacturer's instructions. The integrity of the DNA samples was examined by electrophoresis through a 1.5% agarose gel. Genotyping was performed using RFLP-PCR.
For genotyping of SNP12195 (G/C) of the *STAT5A* gene a 820 bp fragment was amplified by PCR using the primer pair: 5′-GAGAAGTTGGCGGAGATTATC-3′ and 5′-CCGTGTGTCCTCATCA CCTG-3′.
For genotyping of SNP11646 (A/G) of the *FGF2* gene a 207 bp fragment was amplified by PCR using the primer pair: 5′-CATAGTTCTGTAGACTAGAAG-3′ and 5′-CCTCTAAAGAAGGATTAAG TCAAAATGGGGCTGGTA-3′.
PCR amplification was performed using approximately 300ng of genomic DNA as template, 200 nM primers each, 1 mM dNTPs and 1 unit Taq DNA Polymerase Recombinant (Invitrogen) in 25 μl total volume reaction. PCR conditions were 94°C for 3 min, 35 cycles of 94°C for 30 sec, 53°C (*STAT5A*) or 50°C (*FGF2*) for 30 sec, 72°C for 30 sec and a final extension period at 72°C for 10 min.
For the *STAT5A* polymorphism PCR products were digested using *Bst*EII restriction enzyme (Fermentas) and resolved by electrophoresis on 1% agarose gels, visualised with ethidium bromide and imaged under UV illumination. Using this RFLP-PCR method the C and G alleles of SNP12195 were indicated by bands of 820 and 676 bp, respectively.
For the *FGF2* polymorphism PCR products were digested using *Csp6*I restriction enzyme (Fermentas) and resolved by electrophoresis on 1.5% agarose gels, visualised with ethidium bromide and imaged under UV illumination. Using this RFLP-PCR method the A and G alleles of SNP11646 were indicated by bands of 207 and 171 bp, respectively.
Statistical analysis was performed using the statistical program SPSS 18.0. A Chi-square test was used to investigate differences in genotypic and allelic frequencies between the 2 groups.

RESULTS

As illustrated in figures 1 and 2 the PCR conditions used in this study amplified successfully a 820 bp and a 207 bp DNA fragment of the *STAT5A* and *FGF2* genes respectively.
In addition, as illustrated in figures 3 and 4, genotyping was performed using RFLP-PCR.

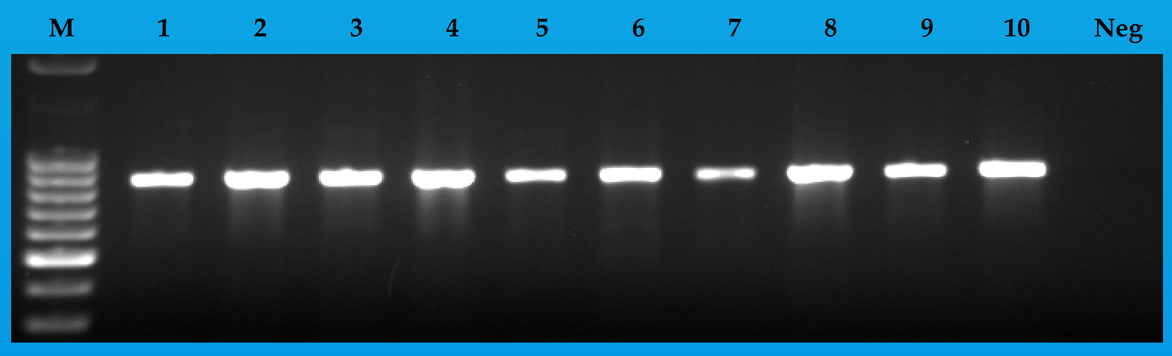

Figure 1. PCR amplification of *STAT5A* gene. Lanes 1-10: PCR amplification in 10 Holstein cows, Neg: negative control, M: 100bp DNA marker.

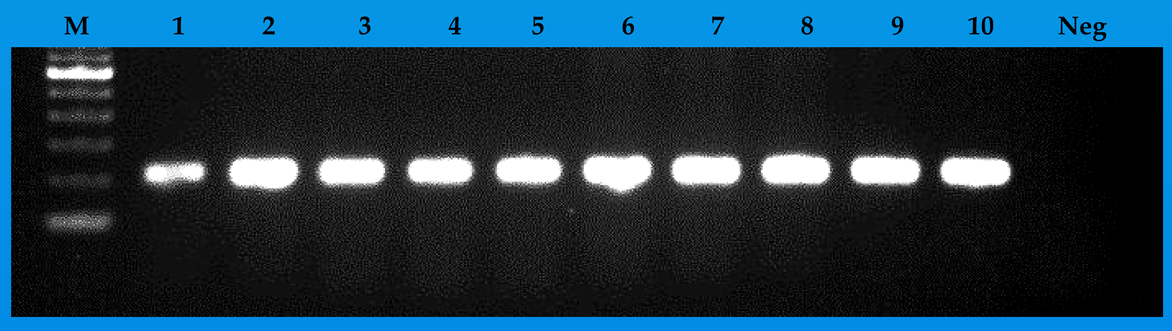

Figure 2. PCR amplification of *FGF2* gene. Lanes 1-10: PCR amplification in 10 Holstein cows, Neg: negative control, M: 100bp DNA marker.

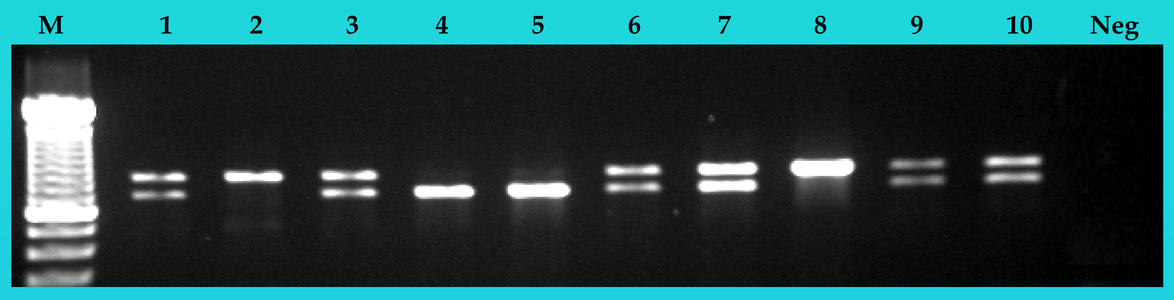

Figure 3. RFLP-PCR analysis for the SNP12195 of the *STAT5A* gene. PCR products were restricted with *Bst*EII. Lanes 1-10: RFLP-PCR analysis in 10 Holstein cows, Neg: negative control, M: 100bp DNA marker.

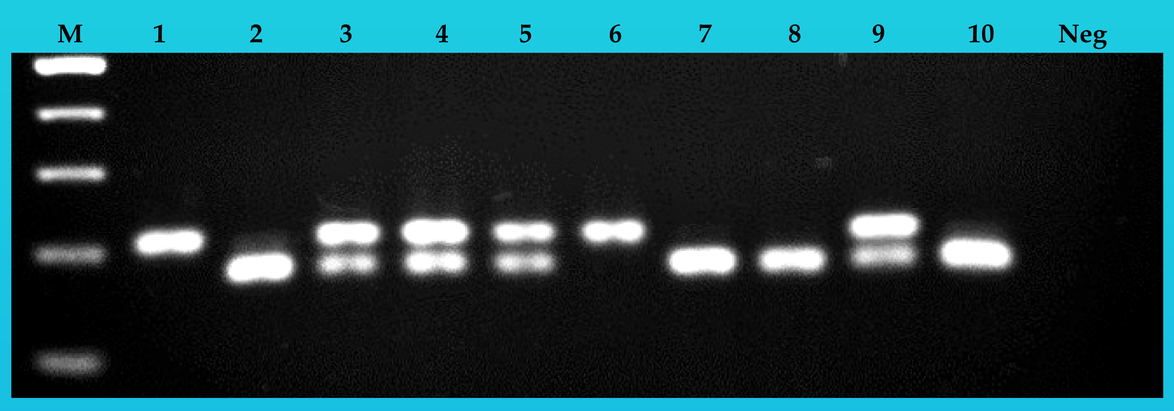

Figure 4. RFLP-PCR analysis for the SNP11646 of the *FGF2* gene. PCR products were restricted with *Csp6*I. Lanes 1-10: RFLP-PCR analysis in 10 Holstein cows, Neg: negative control, M: 100bp DNA marker.

Once genotypes were determined, allelic frequencies at each gene locus were calculated by gene counting. Deviations from Hardy-Weinberg equilibrium were examined for each locus using chi-squared tests.
As illustrated in Tables 1 and 2, in the 1st group, genotype CC of *STAT5A* had a frequency of 0.32, GC 0.45 and GG 0.23, while the frequency of allele C was 0.54 and of G 0.46. For *FGF2*, genotype AA was had a frequency of 0.15, AG 0.60 and GG 0.25, while the frequency of allele A was 0.45 and of G 0.55. In the 2nd group, CC had a frequency of 0.40, GC 0.42 and GG 0.18 (*STAT5A*), with frequencies of alleles C and G being 0.61 and 0.39, respectively. For *FGF2*, genotype AA had a frequency of 0.22, AG 0.50 and GG 0.28, while the frequency of allele A was 0.47 and of allele G 0.53.
Statistical analysis revealed that differences were not significant ($P>0.05$) implying no association between *STAT5A* and *FGF2* and fertility.

1st Group (Conceived with 1st A.I.)					
STAT5A	CC	GC	GG	C freq	G freq
	0.32	0.45	0.23	0.54	0.46
FGF2	AA	AG	GG	A freq	G freq
	0.15	0.60	0.25	0.45	0.55

Table 1. Genotypes and allele frequencies of cows which conceived with 1st A.I. for the SNP12195 and SNP11646 in the *STAT5A* and *FGF2* genes respectively.

2nd Group (Did not conceive with 1st A.I.)					
STAT5A	CC	GC	GG	C freq	G freq
	0.40	0.42	0.18	0.61	0.39
FGF2	AA	AG	GG	A freq	G freq
	0.22	0.50	0.28	0.47	0.53

Table 2. Genotypes and allele frequencies of cows which did not conceived with 1st A.I. for the SNP12195 and SNP11646 in the *STAT5A* and *FGF2* genes respectively.

CONCLUSIONS

In this study we investigated the association of the SNP12195 and SNP11646 polymorphisms of the Signal Transducer and Activator of Transcription 5A (*STAT5A*) and Fibroblast Growth Factor 2 (*FGF2*) genes respectively with fertility, in a total of 120 1st lactation Holstein cows.
Results presented in this study suggest that there is no significant association between *STAT5A* and *FGF2* gene polymorphisms with cow fertility, indicating that the polymorphisms of these 2 genes cannot be used in gene-assisted selection to improve conception rate in cattle.
However confirmation of these results on a larger number of animals is necessary, as well as on different cow breeds, before definitive conclusions can be made.