Theriogenology 88 (2017) 67–72

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

# Association between polymorphisms in somatotropic axis genes and fertility of Holstein dairy cows

Lucas Teixeira Hax [a], Augusto Schneider [b], Carolina Bespalhok Jacometo [a], Patrícia Mattei [a], Thaís Casarin da Silva [a], Géssica Farina [a], Marcio Nunes Corrêa [a,*]

[a] Research Center, Education and Extension in Livestock (NUPEEC), College of Veterinary, Federal University of Pelotas, Pelotas, RS, Brazil

[b] Department of Nutrition, College of Nutrition, Federal University of Pelotas, Pelotas, RS, Brazil

ARTICLE INFO

*Article history:*
Received 22 October 2015
Received in revised form 29 March 2016
Accepted 31 March 2016

*Keywords:*
*GHR*
*IGF-I*
*STAT5A*
SNP
Reproduction

ABSTRACT

The aim of this study was to determine the effect of growth hormone receptor (GHR) *Alu*I, insulin-like growth factor type 1 (IGF-I) *Sna*BI, and signal transducer and activator 5A (STAT5A) *Bst*EII polymorphisms in the reproductive performance of Holstein dairy cows and the frequency of this genotypes in cows managed in different systems. This work studied 381 and 506 Holstein cows from semiextensive and intensive systems, respectively. The frequency of genotypes *GHR AluI* (+/−), *IGF-I SnaBI* (−/−) and (+/−), and *STAT5A BstEII* (−/−) was higher in animals from semiextensive system, whereas the frequency of the genotypes *GHR AluI* (+/+) and *IGF-I SnaBI* (+/+) was higher in animals from intensive system ($P < 0.05$). In the intensive system, cows from the *STAT5A BstEII* (−/−) genotype had a longer calving-first heat interval ($P = 0.03$). In conclusion, there was no association between the genotypes of *GHR AluI* and *IGF-I SnaBI* and fertility of Holstein cows raised in semiextensive or intensive regimes, although the genotype frequencies of the evaluated polymorphisms were different between the studied systems. The *STAT5A BstEII* polymorphism was associated with calving-first heat interval in Holstein cows raised in the intensive system, indicating that this gene could be a molecular marker for genetic selection to improve reproductive performance.



## 1. Introduction

The function of the GH/insulin-like growth factor-I (IGF-I) axis was affected by the intensive selection of dairy cattle for milk production [1,2]. As a consequence, with the increased milk production, fertility was dramatically reduced in Holstein dairy cows in the last 60 years [3,4]. In agreement with this, some studies have suggested that single nucleotide polymorphisms (SNPs), single nucleotide mutations in a gene potentially affecting its function [5], in genes of the somatotropic axis can severely affect reproductive performance [6], and its use as candidate genes in selection programs was suggested [7].

The somatotropic axis is composed by the genes encoding the GH, IGF-I, their receptors (growth hormone receptor [GHR] and insulin-like growth factor receptor [IGFR]) and several intracellular signaling proteins, among them, the signal transducer and activator 5A (STAT5A). The somatotropic axis has a key role in the regulation of the metabolism and physiology of mammals [8]. Polymorphisms in these genes have been linked to some characteristics of economic interest, such as the synthesis and composition of milk [7], carcass traits and meat production [3], and reproductive performance [6,9]. The *GHR/AluI* polymorphism, an A→T point mutation located at position -1182, in the promoter region of *GHR* gene, was

* Corresponding author. Tel.: +55 53 3275 7136; fax: +55 53 3275 7561.
*E-mail address:* marcio.nunescorrea@gmail.com (M.N. Corrêa).

0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2016.03.044

identified by Aggrey et al. [10]. The *IGF-I/Sna*BI polymorphism is a T→C transition located at 512 bp in the first exon identified by Ge et al. [11]. Khatib et al. [12] identified the *STAT5A/Bst*EII polymorphism to be located in the exon 8 of the *STAT5A* gene. Serum IGF-I is synthesized by the liver in response to GH acting through its receptor GHR [13,14]. The transcription factor STAT5A is one of the prime mediator of the actions of GH, especially important in regulating IGF-I mRNA synthesis [15]. In the ovary, IGF-I stimulates the proliferation of granulose and theca cells of the follicle [16] and inhibits follicular atresia [17]. In addition, IGF-I stimulates the response of follicular cells to the hypothalamic gonadotropins [16]. Dairy cows with delayed return to postpartum ovarian activity have lower serum concentration of IGF-I when compared with cows that ovulate earlier [18]. Serum concentration of IGF-I also increases linearly until the moment of first postpartum ovulation and has a negative correlation with the duration of postpartum anestrous in beef cows [19,20]. Therefore, higher serum IGF-I is essential for an earlier return to postpartum cyclicity and more estrous cycles before the first insemination is associated with a higher pregnancy rate [21].

On the basis of the information previously mentioned, it may be inferred that the SNPs in genes encoding the somatotropic axis can influence the reproductive performance of cattle. Maj et al. [7] observed an association between the *GHR Alu*I and *IGF-I Sna*BI polymorphisms with plasma concentrations of IGF-I in Holstein steers. Schneider et al. [6] reported an association of the *GHR Alu*I polymorphism with increased serum IGF-I concentration and a reduced calving conception interval (CCI) in Holstein dairy cows. Khatib et al. [12] reported an association between SNPs in the *STAT5A* gene and fertility of dairy cows. However, most of these studies were conducted with high-producing dairy cows managed in free-stall systems. For these reasons, more information is necessary about the effects of these SNPs in dairy cows raised in semiextensive and free-stall systems in the southern hemisphere, with different climate, feed, and management conditions.

The general hypothesis of this study was that SNPs in the *GHR*, *IGF-I*, and *STAT5A* genes are associated with the reproductive performance of dairy cows. Then, we aim to determine the effect of the *GHR Alu*I, *IGF-I Sna*BI, and *STAT5A Bst*EII SNPs in the calving-first heat interval (CHI), CCI and number of AI per pregnancy of Holstein dairy cows, and the genotype frequency in semiextensive or intensive systems.

## 2. Material and methods

The procedures adopted in this study were approved by the Ethics Committee of the Federal University of Pelotas.

### 2.1. Semiextensive management system

This experiment was conducted in a commercial farm in southern Brazil evaluating 381 Holstein cows. The CCI and number of AI per pregnancy were obtained from the farm database (ALPRO Herd Management System, DeLaval), including only cows that became pregnant within 250 days in milk (DIM). The animals were managed in a semiextensive farming system and the diet was based on cultivated pasture, ryegrass (*Lolium multiflorum*) during fall/winter, and sorghum (*Sorghum bivolor*) during spring/summer. In addition, the cows received mineral, concentrated, and additional forage supplement containing 53% of concentrate, 13.3% of haylage, and 33.7% of ryegrass for adjusting the nutritional requirements according to milk production level and number of lactations [22]. The cows were milked and fed twice daily.

Cows were presynchronized at 47 ± 5 DIM with 25-mg intramuscular (i.m.) of $PGF2_{\alpha}$ (Lutalyse, Pfizer Animal Health, Brazil). At 60 ± 3 DIM, cows underwent gynecological examination and the fixed-time artificial insemination (FTAI) protocol was started with the injection of 100-μg i.m. of a GnRH analog (Cystorelin, Merial, USA) and the insertion of a progesterone-releasing intravaginal device (controlled internal drug release [CIDR]; Pfizer Animal Health, Brazil). On Day 7 of the protocol, the intravaginal device was removed and 25-mg i.m. of an analog of $PGF2_{\alpha}$ (Lutalyse, Pfizer Animal Health, Brazil) was injected. On Day 8 of the protocol, cows were injected with 1-mg i.m. of estradiol cypionate (Pfizer Animal Health, Brazil). The FTAI was performed 72 hours after CIDR removal by a single technician using semen of different commercial bulls according to the farm selection criteria. Cows observed in heat before and after FTAI were inseminated 12 hours after heat detection. Pregnancy diagnosis was performed with an ultrasound 30 and 60 days after AI, and cows diagnosed as nonpregnant were immediately subjected to the same FTAI protocol again.

### 2.2. Intensive management system

This experiment was conducted in a commercial farm in southeastern Brazil using 506 Holstein cows. The CHI, CCI, and number of AI/pregnancy were obtained from the farm database and only cows with CCI lower or equal than 200 DIM were used in this study. Cows were managed in a free-stall system, receiving total mixed ration, with *ad libitum* access to water and were milked three times a day. Nutritional requirements were adjusted according to milk production level and number of lactations [22]. The diet ingredients were: corn silage (33.3%); haylage (7.3%); core cotton (7.2%); citrus pulp (12.3%); dry corn (17%); soybean meal (15.3%); water (0.2%); protected fat (0.9%); vitamin and mineral mix (6.5%).

After 60 DIM, cows had estrus monitored through commercial pedometers and were inseminated 12 hours after estrous detection. The pregnancy diagnosis was performed by ultrasound 28 and 60 days after AI. Cows not inseminated or not pregnant at the pregnancy diagnosis underwent a fixed-time embryo transfer protocol. For that, cows received 100 μg i.m. of GnRH (Fertagyl; Schering-Plough Co., São Paulo, Brazil) and a progesterone-releasing intravaginal device (CIDR containing 1.9 g of progesterone, Pfizer Animal Health, Sao Paulo, Brazil). Seven days after the beginning of the protocol, cows received an injection of 25 μg i.m. of $PGF2_{\alpha}$ (Lutalyse; Pfizer Animal Health, Sao Paulo, Brazil) and the CIDR was removed. On Day 8, cows received 1 mg i.m. of estradiol cypionate (Pfizer Animal Health, São Paulo, Brazil). Fixed-time embryo transfer was performed on Day 15, after