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Polymorphisms within the *IGF1* and *IGF1R* genes associated with superovulation-related traits in Holstein dairy cows managed in a semiarid environment

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Abstract Embryo transfer (ET) is an artificial reproductive technology used for the genetic improvement of cattle. High variation has been observed in superovulation (SPO) and embryo flush recovery, which appear to be influenced by donor cow genetics. Then, the objective was to identify single nucleotide polymorphisms (SNPs) from the genes *IGF1* and *IGF1R* associated with reproductive traits related to SPO response in dairy cows raised in a semiarid region. Sixty-four Holstein cows were subjected to SPO, artificial insemination, and nonsurgical embryo collection. Individual blood samples were collected and used to genotype 13 SNPs from the genes *IGF1* and *IGF1R*. Additional blood samples were collected to measure anti-Mullerian hormone (AMH) concentrations. Ovarian and embryo traits related to SPO response were evaluated. A mixed-effects model was used to identify associations between SNPs and SPO-related traits. A regression model was implemented to calculate allele substitution effects. From 13 SNPs, the SNP rs109763947 in the gene *IGF1* and the SNPs rs110343126 and rs208140993 in the gene *IGF1R* were predictors for six traits evaluated in superovulated cows. The most favorable genotypes for these SNPs were CC, AA and GG, respectively. A linear trend was detected, suggesting an additive effect of the genes. Moreover, all traits evaluated in the current study improved as the number of favorable SNP genotypes increased, confirming a positive contribution of the SNP genes. In conclusion, three SNPs in the genes *IGF1* and *IGF1R* were marker predictors for reproductive traits related to SPO response and embryo production in Holstein cows managed in a semiarid region.

Keywords: genes, IGF1, holstein, SNP, superovulation

1. Introduction

Embryo transfer is a reproductive technology that involves the response to an SPO protocol and embryo recovery in donor cows, as well as the transfer of high-quality embryos to recipient cows (Hasler 2014). In addition to being considered a very valuable tool to study reproductive biology, ET has allowed the development of several biotechnologies, such as cloning, bipartition, sexing and gene editing (Hansen 2020).

Several factors influence the success of an ET program, with one of the most important being the highly variable response in cattle to SPO processes and embryo production (Rico et al 2012; Jaton et al 2016). Assisted reproductive techniques such as ovum pick-up (OPU) combined with *in vitro* fertilization (IVF) appear to overcome the limitations of ET programs, offering an alternative approach to produce a large number of high-quality embryos. However, an improvement in culture conditions is still required to ensure the expected efficiency of OPU-IVF technology in cattle (Balboula et al 2022).

Climatic factors such as ambient temperature and relative humidity have been associated with the reproductive response in superovulated cows. A reduction in SPO response, fertilization rate and embryo quality has been observed in cows raised in warm regions affected by heat stress (Hansen et al 2001; Chinchilla-Vargas et al 2018). It appears to be a consequence of physiological proliferation of oxygen-reactant chemical compounds caused by the warm climate (Roth 2015; Nabenishi et al 2017).

Physiological markers have been reported to be associated with reproductive responses in superovulated cattle. Serum concentrations of AMH higher than 400 ng/mL increased the number of follicles (FOL) and corpus luteum (CL), as well as the number of collected (NCE) and transferable embryos (NTE; Batista et al 2016). Similarly, a high antral follicle count (AFC) was positively associated with the number of healthy follicles, oocytes and

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transferable embryos in superovulated cows (Mossa et al 2017).

AMH and AFC have moderate heritability, and they are highly correlated with reproductive traits, which makes them potential markers useful to increase the genetic merit for cow fertility (Grala et al 2021).

Recently, molecular technologies have allowed the identification of genomic regions and SNPs that explain the variation associated with the SPO response and embryo production in cows subjected to an ET program (Parker Gaddis et al 2017). The combination of these technologies has provided evidence that a genetic component is associated with traits related to SPO and embryo yield. This suggests that genetic selection to improve both superovulatory and embryo production abilities in cows is possible (Thomasen et al 2016).

Therefore, the objective was to identify single nucleotide polymorphisms (SNPs) from the genes *IGF1* and *IGF1R* associated with reproductive traits related to the SPO response and embryo production in Holstein cows raised in a semiarid region.

2. Materials and Methods

2.1. Animals

Sixty-four Holstein cows were included in this study, which received similar management and were housed in shaded barns with free access to water and shade. The floor and shade spaces were provided as per the needs of Holstein cows (Cartes et al 2021). Nutritional management followed the guidelines established by the NRC (2001). The diet was formulated to accomplish the requirements for dairy cows averaging 650 kg of body weight and 15 kg/d of milk yield (3.5% and 3.2% milk fat and protein composition, respectively).

2.2. Experimental location and environment

The study was conducted in a Holstein dairy farm located in the Yaqui Valley, Sonora. The geographical coordinates are 27°20'40" north latitude and 109°54'46" west longitude, with an average altitude of 20 m above sea level. The prevailing weather conditions were a climate that varied from dry to semihumid, with rainfall in summer (July September), average annual precipitation of 520.1 mm, and average annual temperatures of 33.6°C (maximum) and 17.4°C (minimum).

Ambient temperature (AT, °C) and relative humidity (RH, %) were collected from a nearby climatic station located in block 910 Valle del Yaqui. These data were used to calculate the temperature-humidity index (THI) following the procedures described by Hahn (1999): THI = (0.8 x AT) + [(RH/100) x (AT-14.4)] + 46.4.

2.3. SNP discovery and genotyping

Forty-four candidate genes within the PRL and GH/IGF1 metabolic pathways were selected based on their functional relationship with fertility, thermotolerance and milk production. Two of these genes, insulin-like growth factor 1 (*IGF1*) and IGF1 receptor (*IGF1R*), were resequenced using Sanger technologies to discover 2 and 11 SNPs, respectively. These intragenic SNPs were used in further genotyping analysis.

Blood samples (3 ml) were individually collected via venipuncture of the coccygeal vein. Five drops of whole blood were spotted on Fast Technology for Analysis of nucleic acids cards (FTA®). Cards were sent to Neogen GeneSeek (Lincoln, NE) to extract the DNA, which was genotyped using mass spectrometry in the Sequenom MassARRAY® platform (IPLEX GOLD, Sequenom, San Diego CA, USA) system. As a quality control, samples with a genotyping call rate < 80% were discarded from analysis.

2.4. Reproductive management

Cows received an intravaginal progesterone releasing device (CIDR 1.38 g vaginal delivery system for cattle, Zoetis, Dublin, Ireland), plus an intramuscular (IM) injection containing 2.76 mg estradiol benzoate (β-estradiol 3-benzoate, Sigma–Aldrich, St. Louis, MO, USA) and 50 mg of progesterone (Progesterone, Vetoquinol, Canada) given to synchronize the follicular wave emergence (day 0). The same day, a blood sample was collected from each cow by puncture of the coccygeal vein. Samples were centrifuged at 3,500 rpm for 30 min to collect serum, which was used to quantify AMH concentrations using the Minitube of America AMH-bovine specific immunoassay (AMH Fertility Assay™).

The superovulatory treatment started on day 4 and lasted until day 7. For 4 consecutive days, cows received decreasing IM doses of follicle-stimulating hormone (FSH; Folltropin-V, Bioniche Life Sciences, Belleville ON, Canada), which was supplied at 12-h intervals (60, 40, 20 and 10 mg). On day 6, cows received a double application (am/pm) of Prostaglandin F2α analog (Estrumate 50 mg IM; Schering-Plough, Union, NJ, USA). CIDR was removed on day 7 followed by a gonadotropin-releasing hormone injection (GnRH, Fertagyl 0.4 mg IM, Intervet/Merck Animal Health, Madison NJ, USA) on day 8. Finally, the recto-cervical technique of artificial insemination was performed at 12 and 24 h after GnRH injection using conventional frozen semen (15×10^6 sperm/straw) from Holstein sires with high genetic merit for milk yield and fertility traits. Microscopic analysis for semen quality was performed to confirm sperm motility > 60% at 0 h and < 10% of sperm abnormalities (Souza et al 2015).

2.5. Ultrasonography evaluation

Cows were subjected to an ultrasonography examination on day -14 to confirm uterine involution, as well as the reestablishment of ovarian activity (i.e., follicles > 3 mm and CL > 1.2 cm). Transrectal ultrasonography was performed again on day 0 to determine the number of antral follicles > 3 mm in diameter, which was recorded as the antral follicular count (AFC). This reproductive evaluation was repeated on days 8 and 15 to determine the

number of ovulatory follicles (< 0.8 mm) and corpus luteum (CL), respectively.

2.6. Embryo collection and evaluation

Nonsurgical uterine flushing was performed twice on day 15 for embryo recovery. Before starting this procedure, cows received 5 mL of lidocaine spinal anesthesia (lidocaine hydrochloride injectable 2%; Phoenix Pharmaceutical, St. Joseph, MO). A catheter with a metallic guide was then inserted into the uterine body through the vaginal-cervical channel (Silicon catheter CH16/CH18, 2-way Foley, 30-mL balloon). The catheter was connected to a Y-junction tube to allow the flow of medium for embryo recovery (Dulbecco's phosphate buffered saline supplemented with 1% fetal calf serum, Nutricell, Brazil). Medium with embryos was recovered from the uterus and collected inside an embryo filter (Mini-Flush Embryo System) to determine the number of collected embryos (NCE).

Embryos were placed into a Petri dish and washed several times using holding medium (BoviPro Holding Medium, with BSA). A stereomicroscope was used to search the collected embryos, which were evaluated for quality following the Guidelines of the International Embryo Transfer Society (IETS, 2010). Embryos were classified as good (grade 1), fair (grade 2), poor (grade 3) and degenerated (grade 4). Only embryos with quality 1 and 2 were considered to determine the number of transferable embryos (NTE) due to the direct association between embryo quality and higher pregnancy rates in recipient cows (Rocha et al 2016).

2.7. Statistical analysis

Mean values for SPO and embryo traits were calculated using the MEANS procedure. The assumption of normality and equality of variances were tested using the UNIVARIATE procedure (Littell et al 2002). Allele and genotypic frequencies for each individual SNP and deviation from Hardy-Weinberg equilibrium were estimated through the ALLELE procedure (Saxton et al 2004). All statistical analyses were performed in SAS software (V9.3, SAS Inst. Inc., Cary, NC).

Only SNPs that met the criteria of minor allele frequency greater than 10% (MAF > 0.10) and no deviation from Hardy-Weinberg equilibrium ($X^2 > 0.05$) were included in a further validation analysis, which was an associative study between genotype and phenotype using the MIXED procedure for continuous variables. This analysis was performed with a mixed-effects model, which included the fixed effects of the SNP genotype and dam age, the covariate of days in milk, the random effect of the sire (i.e., using the Z statistic to test whether Ho: $\delta w^2 = 0$), and the residual effect (mean= zero, variance= δe^2).

When the genotype term resulted as a significant ($P < 0.05$) source of variation, the PDIFF option was used to generate preplanned pairwise comparisons of least squares means. These mean separation tests were executed within LSMEANS in the MIXED procedure including Bonferroni adjustment (Weir 2001). The allele substitution effect (i.e., the effect of substituting 1 allele in the population with another allele) was calculated by regressing the phenotype on the number of copies of one´s polymorphism allele as a covariate (Falconer and Mackay 1996). If the genotype term was significant ($P < 0.05$), additive and nonadditive effects were calculated following the procedures described by Sherman et al. (2008). To confirm or negate such effects, linear and quadratic effects were also estimated.

3. Results

3.1. Mean values for climatic and reproductive traits

The climatic data described in Figure 1 confirmed that cows included in the current study were managed under harsh semiarid conditions. Descriptive statistics for reproductive traits related to SPO are presented in Table 1. These values suggested wide variation for traits associated with SPO response.

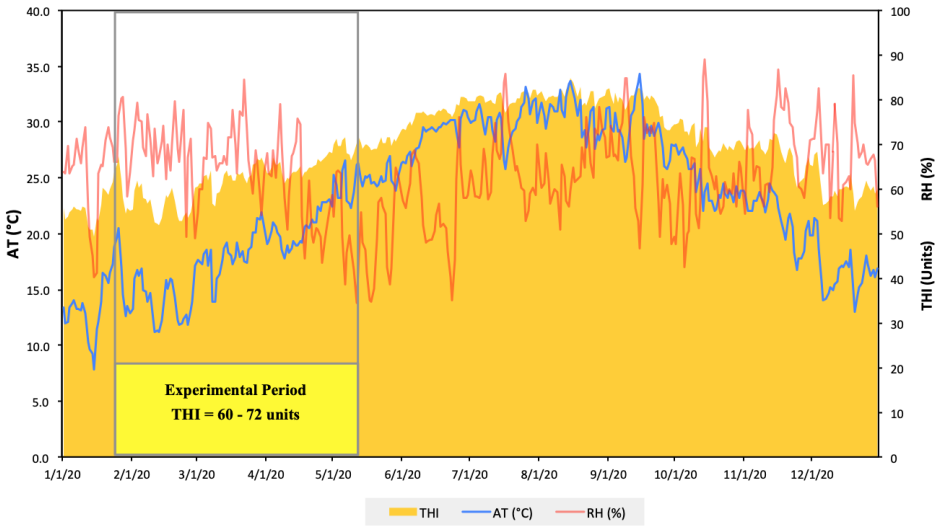

Figure 1 Average daily values for ambient temperature (AT, ºC), relative humidity (RH, %) and temperature-humidity index (THI) during the year 2020 in the Yaqui Valley, Sonora, Mexico. The experimental period lasted from January 20th to May 10th.

Table 1 Mean values ± SE for superovulation related reproductive traits in Holstein donor cows from the Yaqui Valley, Sonora, Mexico.

Variable	Mean ± SE
Anti-Mullerian hormone (AMH; ng/mL))	1245.0 ± 112.7
Antral follicle count (AFC; number of FOL)	32.6 ± 2.5
Follicles (FOL; number of FOL)	18.9 ± 1.1
Corpus luteum (CL; number of CLs)	13.6 ± 0.9
Number of collected embryos (NCE)	9.9 ± 0.8
Number of transferable embryos (NTE)	7.6 ± 0.6

3.2. SNP marker effects

The two SNPs from the *IGF1* gene and six SNPs from the *IGF1R* gene met the criteria for minor allele frequency greater than 0.10 (MAF > 0.10) and no deviation from Hardy-Weinberg equilibrium (χ^2 > 0.05). Therefore, only these SNPs were considered appropriate to be included in genotype-to-phenotype association analyses.

From these eight SNPs, the association study identified three SNPs as predictor markers (P < 0.05) for reproductive traits in superovulated Holstein cows. These markers were the SNP rs109763947 in the *IGF1* gene and the SNPs rs110343126 and rs208140993 in the *IGF1R* gene. The most favorable genotypes for these SNPs were CC, AA and GG because they had the most beneficial values for the traits evaluated in the current study (Table 2).

Although the three markers had a significant effect on SPO-related traits, allele substitution effects revealed that the SNP rs208140993 in the gene *IGF1R* had the highest influence on reproductive traits. In addition, a linear trend was detected for the three SNP markers, suggesting an additive effect with the *IGF1* and *IGF1R* genes (Table 3).

3.3. Effects of favorable SNP genotypes

Average values for all reproductive traits improved (P < 0.05) in superovulated cows that carried at least 1 favorable SNP genotype compared to those cows carrying nonfavorable genotypes (Table 4). Ovarian and embryo traits evaluated in the current study improved as the number of favorable SNP genotypes increased, which confirmed the favorable contribution of the three SNP markers in the *IGF1* and *IGF1R* genes (Figure 2).

Table 2 Least square means ± SE according to SNP genotypes for superovulation-related traits in Holstein cows from the Yaqui Valley, Sonora, Mexico.

SNP	Gene	Trait	Least square means by SNP genotype ± SE			P-value
			CC	CT	TT	
rs109763947	*IGF1*	AMH	1806.6 ± 214.2 ^{a}	1292.4 ± 106.2 ^{b}	947.3 ± 91.5 ^{c}	0.005
		AFC	36.5 ± 2.8 ^{a}	32.7 ± 2.3 ^{b}	28.2 ± 2.0 ^{c}	0.011
		FOL	24.2 ± 1.6 ^{a}	18.5 ± 1.5 ^{b}	16.4 ± 1.2 ^{b}	0.029
		CL	18.7 ± 1.1 ^{a}	14.2 ± 1.0 ^{b}	11.4 ± 0.8 ^{b}	0.012
		NCE	14.6 ± 1.2 ^{a}	9.5 ± 1.0 ^{b}	7.7 ± 0.9 ^{b}	0.008
		NTE	11.9 ± 1.3 ^{a}	7.1 ± 0.8 ^{b}	5.6 ± 0.7 ^{b}	0.022
			AA	AG	GG	
rs110343126	*IGF1R*	AMH	2009.7 ± 196.1 ^{a}	1264.2 ± 115.3 ^{b}	990.1 ± 96.7 ^{c}	0.002
		AFC	37.2 ± 2.8 ^{a}	30.5 ± 2.5 ^{b}	27.9 ± 2.4 ^{b}	0.015
		FOL	24.9 ± 2.1 ^{a}	19.5 ± 1.7 ^{b}	17.1 ± 1.6 ^{b}	0.007
		CL	18.3 ± 1.5 ^{a}	14.9 ± 1.4 ^{ab}	12.5 ± 0.9 ^{b}	0.019
		NCE	14.7 ± 1.1 ^{a}	9.9 ± 1.0 ^{ab}	8.3 ± 0.7 ^{b}	0.006
		NTE	10.9 ± 0.8 ^{a}	6.8 ± 0.5 ^{ab}	4.7 ± 0.3 ^{b}	0.023
			AA	AG	GG	
rs208140993	*IGF1R*	AMH	820.9 ± 77.3 ^{a}	1112.0 ± 102.6 ^{b}	1968.3 ± 187.2 ^{c}	0.001
		AFC	28.5 ± 2.6 ^{a}	32.9 ± 3.1 ^{b}	39.6 ± 3.3 ^{c}	0.017
		FOL	16.3 ± 1.5 ^{a}	19.6 ± 1.6 ^{a}	24.9 ± 2.2 ^{b}	0.002
		CL	10.9 ± 1.0 ^{a}	13.1 ± 1.2 ^{a}	18.5 ± 1.7 ^{b}	0.002
		NCE	7.1 ± 0.5 ^{a}	8.7 ± 0.6 ^{a}	14.7 ± 1.1 ^{b}	0.001
		NTE	4.6 ± 0.3 ^{a}	6.1 ± 0.5 ^{ab}	11.8 ± 0.6 ^{b}	0.025

SNP= *SNP Reference of the NCBI*; Gene= *Gene symbol (IGF1*= Insulin-like growth factor 1; *IGF1R*= Insulin-like growth factor 1 receptor); Trait= superovulation-related traits (AFC = antral follicle count; FOL = number of follicles > 3 mm; CL = number of CLs > 1.2 cm; NCE = number of collected embryos; NTE = number of transferable embryos); ^{a,b,c}Indicate statistical difference among genotype least square means in the mixed model (PROC MIXED in SAS); *P-value*= Statistical value for genotypes comparisons.

4. Discussion

Embryo transfer (ET) is an artificial reproductive technology able to improve economically important traits faster than traditional breeding and management programs. However, this is only observed if genetically superior animals are involved in an ET program. Marker association studies have been used as a strategy to identify candidate genes functionally linked with fertility traits. In the current study, we provided evidence that three SNPs from the *IGF1*

and *IGF1R* genes were molecular markers useful to predict traits associated with response to SPO and embryo production in Holstein cows managed in a semiarid environment, where cows experience heat stress. These SNPs are proposed to be included in marker-assisted programs, which are aimed at improving the genetic selection of donor dams for an embryo transfer program.

Table 3 Allele substitution effects and fixed additive and dominant effects of the favorable SNP allele for superovulation related traits in Holstein cows from the Yaqui Valley, Sonora, Mexico.

SNP ID (FA)	Trait	Allele substitution effects			Fixed effect estimates		
		P-value[a]	Estimate[b]	SE	*P*-value[c]	Additive effect[d]	Dominant effect[e]
rs109763947 (C)	AMH	0.001	403.9	36.5	0.001	429.6	84.5
	AFC	0.009	3.8	0.4	0.013	4.2	0.4
	FOL	0.015	3.3	0.2	0.011	3.9	1.8
	CL	0.003	3.1	0.2	0.002	3.7	0.9
	NCE	0.008	3.2	0.3	0.007	3.5	1.7
	NTE	0.012	2.9	0.1	0.016	3.2	1.6
rs110343126 (A)	AMH	0.001	481.9	40.2	0.001	509.8	235.7
	AFC	0.026	4.5	0.3	0.021	4.7	2.1
	FOL	0.014	3.5	0.3	0.019	3.9	1.5
	CL	0.006	3.7	0.3	0.002	2.9	0.5
	NCE	0.004	3.3	0.2	0.007	3.2	1.6
	NTE	0.012	2.9	0.1	0.016	3.1	1.0
rs208140993 (G)	AMH	<0.001	590.8	43.1	<0.001	573.7	282.6
	AFC	<0.001	5.1	0.4	<0.001	5.6	1.2
	FOL	<0.001	4.2	0.4	<0.001	4.3	1.0
	CL	<0.001	3.7	0.3	0.001	3.8	1.6
	NCE	<0.001	4.1	0.4	0.002	3.9	2.2
	NTE	<0.001	4.3	0.3	0.015	3.8	2.1

SNP ID (FA)= SNP Reference of the NCBI (favorable allele for the SNP); Trait= superovulation (AMH, AFC, FOL and CL) and embryo production traits (NCE and NTE).

[a]*P*–values were obtained from allele substitution analysis in SAS which included the term genotype as covariate.

[b]Estimates of the effect expressed in units of the traits.

[c]*P*–values for fixed effects were obtained from substitution of favorable allele analysis that included the genotype term as fixed effect.

[d]Additive effect was estimated as the difference between the 2 homozygous means divided by 2.

[e]Dominant effect was calculated as the deviation of the heterozygous from the mean of the 2 homozygous.

Table 4 Average values ± SE according to the presence or absence of favorable SNP genotypes in Holstein cows from the Yaqui Valley, Sonora, Mexico.

Variable	Presence of favorable genotypes (1, 2 or 3)	Absence of favorable genotypes
Anti-Mullerian hormone (AMH; ng/mL)	1532.4 ± 142.1 [a]	967.4 ± 94.5 [b]
Antral follicle count (AFC; follicles)	35.2 ± 2.87 [a]	27.2 ± 2.34 [b]
Number of follicles (FOL)	22.3 ± 1.87 [a]	15.7 ± 1.36 [b]
Number of corpora lutea (CL)	16.7 ± 1.37 [a]	11.9 ± 1.02 [b]
Number of collected embryos (NCE)	12.7 ± 0.91 [a]	8.1 ± 0.79 [b]
Number of transferable embryos (NTE)	9.5 ± 0.72 [a]	4.9 ± 0.54 [b]

[a, b] Different letters between columns indicate statistical difference ($P < 0.05$).

Reproductive technologies combined with selective breeding programs enhance genetic improvement programs (Loi et al 2016). Advances in molecular technologies have allowed the development of panels of SNP markers to genotype economically relevant traits in a cost-effective manner (DeAtley et al 2011; Leyva-Corona et al 2018). Then, identification of SNP markers and candidate genes associated with SPO response appear to be a useful strategy to ensure an efficient ET program in dairy cattle (Jaton et al 2016), especially if the donor dams experience heat stress. Chromosomal regions associated with NCE and NTE were detected in superovulated Holstein cows, suggesting a genetic component underlying the SPO response (Parker Gaddis et al 2017).

In the current study, we analyzed 11 SNPs from the *IGF1* and *IGF1R* genes that belong to the GH/IGF1 metabolic pathways. The IGF1 peptide acts by binding with IGF1R, a process mediated by several IGF1 binding proteins (i.e., IGFBPs) that are also regulated by proteolytic proteinases (i.e., PAPPAs). These genetically induced proteins regulate the bioavailability and function of IGF1 (Boldt et al 2006).

Our study revealed one SNP within the *IGF1* gene (rs109763947) and two SNPs in the *IGF1R* gene (rs110343126 and rs208140993) that were associated with six SPO-related traits. Such traits were the serum level of AMH, ovarian traits such as AFC, number of FOLs and number of CLs, and embryo-related traits such as NCE and NTE.

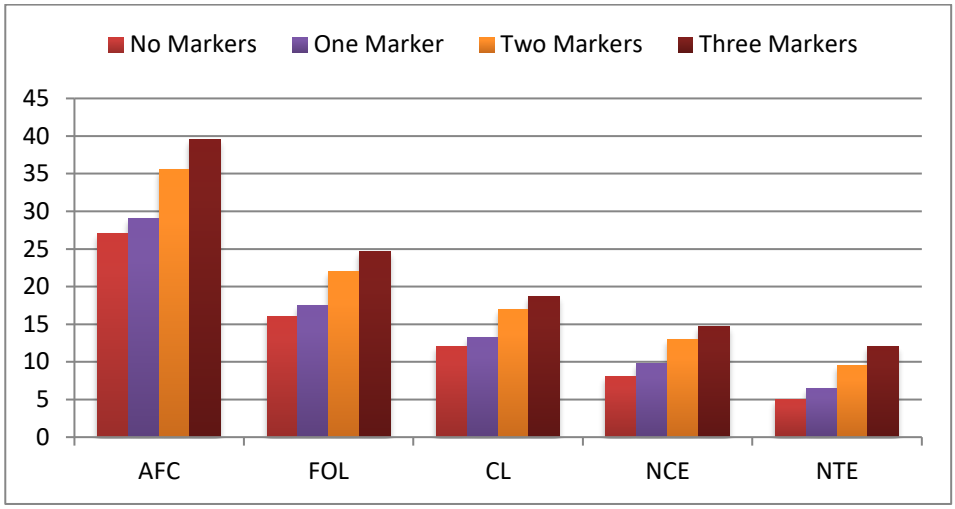

Figure 2 Average values for superovulation-related traits (AFC = antral follicle count; FOL = number of follicles > 3 mm; CL = number of CLs > 1.2 cm; NCE = number of collected embryos; NTE = number of transferable embryos) according to the number of favorable SNP genotypes (i.e., 0, 1, 2 or 3).

Our study revealed one SNP within the *IGF1* gene (rs109763947) and two SNPs in the *IGF1R* gene (rs110343126 and rs208140993) that were associated with six SPO-related traits. Such traits were the serum level of AMH, ovarian traits such as AFC, number of FOLs and number of CLs, and embryo-related traits such as NCE and NTE.

The serum concentration of AMH has been reported as an endocrine marker associated with the number of FOLs, number of CLs, NCE and NTE (Souza et al 2015; Nabenishi et al 2017; Torres-Simental et al 2021). Similarly, AFC has been proposed as a marker for the number of FOLs, NCEs and NTEs in superovulated cows (Ireland et al 2008; Center et al 2018; Mossa and Ireland 2019). Grigoletto et al. (2020) identified several candidate genes associated with AMH and AFC and suggested that both variables were heritable and suitable for use as indicators to genetically improve SPO response and pregnancy rates in ET programs in cattle. Grala et al. (2021) identified genomic regions associated with AFC and anogenital distance (AGD) in dairy cows and proposed both traits as candidate markers to improve fertility. Nawaz et al. (2018) reported genomic regions and gene members of the TGF-β family associated with AMH and AFC in superovulated Holstein heifers. These genes are involved in fertility-related processes such as follicular development, steroidogenesis and ovulation.

Both AMH and AFC require only a single measure, making them more popular in the field of assisted reproductive technologies (Umer et al 2019). In addition, a relevant overlap was observed between the genes associated with AMH and AFC and the genes affecting SPO-related traits in cattle. In this regard, the prostaglandin-endoperoxide synthase 1 gene (*PTSGS1*), which is associated with AMH, was also positively correlated with ovulation, NCE and NTE (Gobikrushanth et al 2018; Jaton et al 2016). Similarly, SNPs and genes associated with AFC were involved in superovulatory-related processes, such as regulation of primordial germ cells and cellular maintenance (Alward et al 2023).

Leyva-Corona et al. (2018) reported that the SNPs rs109763947 and rs110343126 in the genes of *IGF1* and *IGF1R* were associated with services per conception in Holstein cows exposed to a heat-stressed environment. Such results suggested that these genes were involved in ovulation and embryo development processes needed to ensure pregnancy. Sigdel et al. (2021) reported *IGF1R* and *IGF2* as candidate genes associated with pregnancy maintenance in Holstein cows. This study detected the TGF-β pathway linked to fertility.

A genomic region controlling ovulation rate was detected on cattle chromosome 10. This region contains three candidate genes, *SMAD3*, *SMAD6* and *IQCH* (Kirkpatrick and Morris 2015). Interestingly, SMAD genes encode an intracellular protein acting as a key mediator of the TGF-β signaling pathway, which counteracts the *IGF1* gene (Zeng et al 2021). SMAD3 is highly involved in the metabolism of adipose tissue (Faylon et al 2015), a critical nutrient that signals for neural control of reproduction.

Allele substitution analysis confirmed the positive contribution of the favorable SNP alleles in the *IGF1* and *IGF1R* genes to improve the reproductive response in superovulated cows. Yang et al. (2013) reported two SNPs in the gene *IGF1R* associated with a high ovulation rate and enhanced transferable embryo yield in superovulated Holstein cows, suggesting variants in the *IGF1R* gene as potential markers for donor dam selection. A similar association study reported decreases of 0.14 and 0.09 services per conception for SNPs in the *IGF1* (rs109763947) and *IGF1R* (rs110343126) genes, respectively, in Holstein cows subjected to artificial insemination (Leyva-Corona et al 2018).

IGF1 acting through its receptor IGF1R has a synergistic effect with follicle-stimulating hormone (FSH) to regulate ovarian synthesis of progesterone, aromatase,

estradiol, LHR, and inhibin, which are crucial hormones in the ovulatory process (Baumgarten et al 2017). IGF1 plays a critical role in both the ovarian response and embryo production in cows subjected to SPO by participating in follicular growth, oocyte competence acquisition, oocyte secretion of hormones and growth factors, synthesis of gonadotropin receptors, and embryo development and viability (Velazquez et al 2009; Dai et al 2022).

A further analysis of the individual marker effects detected superior performance in cows carrying favorable genotypes from the three SNPs, while cows with no favorable genotypes experienced an attenuated SPO response. Moreover, average values for all SPO-related reproductive traits improved as the number of favorable genotypes increased.

Favorable genotypes from genes belonging to the GH/IGF1 endocrine pathways were used to construct a molecular breeding value for fertility in Holstein cows, making genetic selection to improve reproductive performance possible (Zamorano-Algandar et al 2021). Cochran et al. (2013) reported SNPs from the gene *STAT5*, which belongs to the GH/IGF1 endocrine axis, as associated with pregnancy rate in Holstein cows. Similarly, Yang et al. (2013) reported SNPs in the *FSHR* gene associated with superovulation traits.

5. Conclusions

Cattle included in embryo transfer programs have shown large variation in superovulatory response and embryo production. The identification of molecular markers associated with such variability is a useful strategy to improve the efficiency and sustainability of artificial reproductive technologies. Based on the results of this study, we proposed three SNPs in the *IGF1* and *IGF1R* genes as marker predictors for SPO-related traits such as AMH, AFC, FOL, CL, NEC and NET in superovulated Holstein cows. This study also provided additional evidence of a genetic basis associated with the superovulatory response and embryo production. However, further research exploring genomic regions associated with SPO traits is needed to identify more candidate genes, which will facilitate the selection of genetically superior donor cows.

Ethical considerations

Animal care and handling procedures performed in this study followed the guidelines of the Ethics Committee on the Use of Animals in Experiments (Institutional Animal Care and Use Committee of the Instituto Tecnologico de Sonora; Code 2018-0089).

Conflict of Interest

The authors declare no conflicts of interest.

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