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# GENETICS AND GENOMICS OF BULL FERTILITY PHENOTYPES MEASURED AS PART OF THE BULL BREEDING SOUNDNESS EVALUATION

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## SUMMARY

Chromosomal regions that were associated ($P < 0.01$) with scrotal circumference (SC) and percentage of morphologically normal sperm (PNS) are reported according to genome-wide association studies in Tropical Composite cattle. Bulls were genotyped with Illumina SNP chips and association analyses were performed using animal models. Chromosome X had several SNP associated with SC, PNS or both traits (7,859 SNP). Polymorphisms associated with SC and PNS can contribute to new methods of estimating breeding values, which may enhance the selection of bulls with improved reproductive performance.

## INTRODUCTION

Sperm concentration and morphology are important semen parameters for bull fertility, both in field situations and for artificial insemination (AI). In multiple sire mating systems, sperm morphology was considered the best indicator for calf output (Holroyd et al., 2002). Sire bulls with enhanced fertility guarantee the efficiency of transmission of favourable alleles (for any trait of economical relevance. The consequences of fertile sires are improvement in fertility rates for the herd and increase economic return. In AI centres, bulls producing high volumes of semen with appropriate sperm concentration and without significant fluctuations in semen quality are preferred. These bulls can help avoid unexpected decreases in the number of straws produced for AI, reduce economic losses and disturbances in the distribution and marketing of semen (Hering et al., 2014). Sperm concentration and morphology are typically examined as part of the Bull Breeding Soundness Evaluations (BBSE).

Bull fertility is often an overlooked component of reproductive rate in genetic studies. Bull fertility influences not only fertilization but also the viability of the preimplantation embryos and the establishment of successful pregnancy (Saacke et al., 2000). Scrotal circumference (SC) and percent of morphologically normal sperm (PNS) are indicators of bull fertility that show high heritability in beef cattle (Corbet et al., 2013). Changes in favourable allele frequencies through selection can improve SC and PNS. A limitation in the use of SC and PNS as selection tools is that these reproductive traits cannot be measured before bulls reach 12–24 months of age (Lyons et al., 2014). Identifying SNP associated with these traits could be very useful for early recognition of young sires' fertility. In this study we investigate the heritability and genetic correlations between SC and PNS measured in BBSE are investigated. Genome-wide association analyses (GWAS) were performed for SC and PNS.

## MATERIAL AND METHODS

**Animals, Traits and Genotypes.** Blood for DNA extraction was obtained from 1,719 Tropical Composite (TC) bulls; bred by the Cooperative Research Centre for Beef Genetic Technologies. Details concerning the project design and traits measurements have been reported elsewhere (Burns et al., 2013; Corbet et al., 2013). In short, SC and PNS were measured at 24 months. BovineSNP50 chips (Matukumalli et al., 2009) were used to genotype all bulls and Bead Studio

software (Illumina Inc., San Diego, CA 2006) was used to call genotypes. SNP with call rates < 80% or minor allele frequency < 0.01 were discarded. High-density (HD) genotyping of selected TC cattle was performed and genotypes were imputed using BEAGLE (Browning and Browning, 2009). Quality control and imputation resulted in 729,069 SNP genotypes for 1,719 TC.

**Statistical Analyses.** Allele substitution effects were estimated for each SNP separately, using an animal model. Solutions were estimated with Qxpak5 (Perez-Enciso and Misztal, 2011), using a likelihood ratio test to compare the model containing each SNP versus the model without each SNP.

**RESULTS AND DISCUSSION**

Descriptive statistics, genetic parameters and number of SNP associated with SC and PNS are reported (Table 1). Both traits had high heritability and so they can be used as selection tools to increase fertility. These results corroborate those reported by Corbet et al. (2013). Moreover, PNS and specially, SC are easily measured and the operating cost-benefit is agreeable since they are part of BBSE. These traits have been associated with the genetic improvement of fertility in both male and female cattle in scientific and technical studies (Silva et al., 2013).

**Table 1. Descriptive statistics and genome-wide association results for percentage of normal sperm (PNS) and scrotal circumference (SC).**

| Traits* | N | Mean ± SD | $h^2$ | *r* | $P<10^6$ | SNP in both traits ($P<10^6$) |
|---|---|---|---|---|---|---|
| PNS | 1,648 | 0.72 ± 0.19 | 0.40 | 0.11 | 8,927 | 7,859 |
| SC | 1,719 | 31.42 ± 2.80 | 0.56 | | 14,635 | |

*Traits: Percentage of morphologically Normal Sperm (PNS) and Scrotal Circumference (SC). SD: standard deviation; $h^2$ Heritability; *r*: genetic correlation; P: P-value from Genome-Wide Association Study.

The genetic correlation between SC and PNS was low (Table 1) and so direct selection could be more efficient than indirect selection. However, the genetic correlation was positive indicating that the long-term increase of SC promoted by direct selection will result in an increment in PNS and consequent improvement of semen quality in young bulls. In addition, the number of SNP that were associated with the genetic variation for both traits was high: 7,859; all mapped to chromosome X. Genetic correlations are determined by either pleotropic effects or gene linkage, which depends on selection intensity, polymorphism effects, allele frequency and strength of linkage (Sheridan and Barker, 1974); factors that might vary across generations or herds. It is relevant to highlight that fixation of alleles with pleotropic effects and in linkage may reduce the genetic correlation between traits across generations (Sheridan and Barker, 1974). This might explain in part the low genetic correlation estimated between SC and PNS; selection for fertility might have been applied in this herd over generations.

The X chromosome harboured SNP that were significant for both traits, spread across millions of base pairs. Our results are evidence for polygenic regulation of these reproductive traits (Figure1).

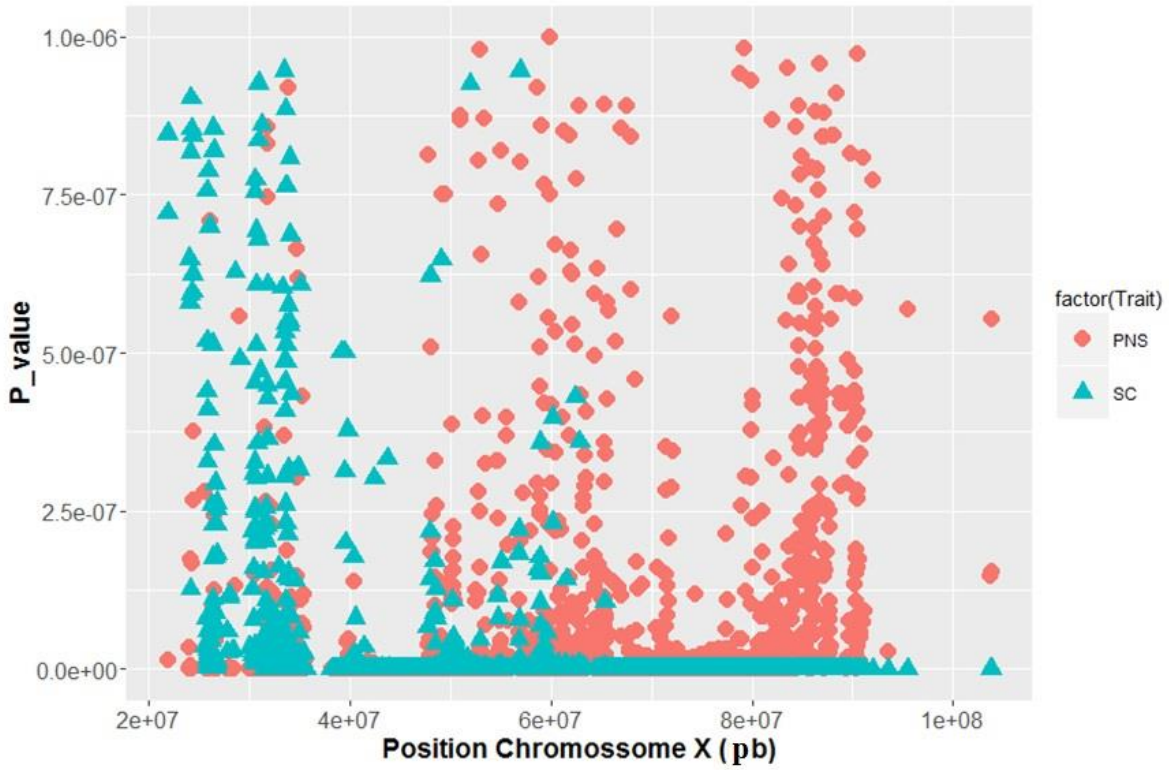


**Figure 1. Polymorphisms in the X chromosome associated with percentage of morphologically normal sperm (PNS) and scrotal circumference (SC) in Tropical Composite cattle.**

Associations that point to a QTL close to 32 Mb and another close to 110 Mb of chromosome X provide further evidence for results that were first reported in Holstein bulls (Blaschek et al., 2011). Candidate genes underpinning these QTL on chromosome X were proposed in Brahman bulls (Fortes et al., 2012). For example, the androgen receptor gene (*AR*) at 88 Mb of the X chromosome is a positional candidate gene, which is relevant because of its physiological role (Quigley 1998). The androgen receptor is paramount for testosterone signalling and, therefore, transcriptional regulation of genes that are critical for development and maintenance of male reproductive function.

## CONCLUSION

Reported SNP associated with SC and PNS may contribute to the selection of bulls with improved reproductive performance. Direct selection for each trait would be a more efficient route than indirect selection to improve bull fertility and semen quality.

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