Proc. Assoc. Advmt. Anim. Breed. Genet. 26: 399-402

EVIDENCE OF CHROMOSOME X ASSOCIATION WITH KNOBBED ACROSOMES

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SUMMARY

Sperm morphological defects are an important component trait of bull fertility. This paper reports a genome-wide association study targeting Knobbed Acrosomes (KA), a relatively common sperm morphological defect in tropical bulls. Phenotypes of bulls in a multi-breed population were available for analysis, and 25% had the KA defect in 1% or more of its sperm cells. Of genotyped bulls, 2,183 were classified as cases (KA ≥ 1%) and 3,657 were controls (no KA defects observed). Associated SNPs were aggregated to define QTL boundaries. Genes mapped to QTL were subject to functional annotation and enrichment analysis, aiming at identifying plausible candidate genes for KA. The estimated heritability for KA presence was 0.16. Significant associations defined a QTL on the X chromosome, containing eight candidate genes: *AMOT*, *ATRX*, *CYSLTR1*, *GPR174*, *HTR2C*, *LDHA*, *LPAR4*, and *P2RY10*. Selection against KA sperm defect may be possible and could improve fertilisation rates in tropical herds.

INTRODUCTION

The standardised Bull Breeding Soundness Evaluation (BBSE) measures traits relevant to male fertility (Entwistle *et al*. 2003). Sperm morphological defects are evaluated as part of BBSE. The proportion of sperm with morphological defects identified in a sample is an important criterion in BBSE. Bulls may be deemed unfit for mating if high percentages of sperm morphological defects are identified. One such defect is knobbed acrosomes (KA): the acrosome of the sperm is thickened or ridged, thus affecting the sperm's ability to bind to the zona pelucida, which is detrimental to fertilisation. Furthermore, in bulls that present with KA sperm, non-KA sperm's ability to form zygotes and bind to the zona pelucida is also affected (Thundathil *et. al*. 2000). Therefore, it is possible that the impact of KA presence is currently underestimated. In this study, we aimed to investigate the genetics underpinning the presence of KA defects in tropical bulls.

MATERIALS AND METHODS

Animals and phenotypes. BBSE records from 6,063 bulls comprising six different breeds were included in this study. Two breeds were research herds from the Cooperative Research Centre for Beef Genetic Technologies (Beef CRC) consisting of Brahman and Tropical Composite bulls. The remaining four breeds obtained from industry herds were Santa Gertrudis, Droughtmaster, Ultra black, and Belmont Red. This population of bulls and their BBSE phenotypes were first described in a previous paper (Porto Neto *et al.* 2023). Sperm morphology was observed before as a combined trait (i.e. percentage of normal sperm), but the occurrence of KA was not investigated previously.

The distribution of the percentage of KA-affected sperm (%KA) within this data was heavily skewed (i.e., not normally distributed). Therefore, we explored a case versus control GWAS design, by classifying bulls as affected (case) or unaffected (control). Cases were bulls with 1% or higher presence of sperm with KA defects. Controls were bulls with 0% KA (no KA sperm found on BBSE evaluation).

Genotypes and GWAS. Most animals were genotyped at ~ 50K. A reference panel that utilised Beef CRC and industry animals genotyped at higher density (~700K) were used to impute animals to higher density. The animals used in the reference population were representative of the bulls used in this study, as reported before (Porto-Neto *et al.* 2023). The reference population was phased using Eagle 2 (v2.4.1) and then used to impute the 50K genotypes using Minimac3 for autosomes and Minimac4 for Chromosome X. All SNP with imputation $r^2 > 0.8$, a call rate > 0.85 and a minor allele frequency > 0.05 were retained for further analysis. This resulted in a total of 661,037 SNPs.

The association between SNP and trait was calculated using EMMAX methods (Kang 2010). This method uses the following model iteratively for each SNP *j*:

$$y = X\beta + z_j g_j + Za + e$$

where y is a vector describing phenotypes for all individuals, X is the matrix for fixed effects, including continuous covariates (body weight and scrotal circumference) and categorical effects (contemporary groups defined by year and breed), β is the vector of coefficients for the fixed effects, z_j is the vector of genotypes SNP *j*, g_j is the fixed additive effect of SNP *j*, Z is the matrix of genotypes for all SNP, a is the random additive breeding value of each animal and assumed to follow a normal distribution with zero mean and variance $G\sigma_a^2$ where G is the genomic relationship matrix (GRM) computed using Method 1 of VanRaden (2008), and e represents the random residual effect assumed to follow a normal distribution with zero mean and variance $I\sigma_e^2$. The threshold of significance for SNP association with the presence of KA defect was set to $P \leq 10^{-8}$. We also considered SNPs with $P \leq 10^{-6}$ to define QTL boundaries. Methods for aggregating SNPs and defining QTL boundaries were described previously (Van den Berg *et al*. 2016; Fortes *et al*. 2020).

RESULTS AND DISCUSSION

Of all the bulls with BBSE records, 25% had KA ≥ 1%. However, less than 1% of bulls would have failed the BBSE test due to KA defects. In the industry, when KA is higher than 30%, the bull would be considered not fit for mating and would have failed the BBSE test according to clinical veterinarian practices. Thus, less than 1% of bulls in this multi-breed population are considered to have a clinical fertility problem due to the presence of KA alone. However, the implications of KA occurrence in Australian beef bulls will be considered more relevant or less severe, depending on whether this sperm defect is regarded as compensable. If KA is a compensable sperm defect, as suggested by industry standards (Fordyce *et al.* 2006), the <1% of the population with KA occurrence may seem of little consequence. However, sperm with KA also affect the fertilisation capacity of non-KA sperm in the same animal (Barth 1986; Thundathil *et al.* 2000). Thus, the consequences of KA occurrence are currently unknown. In the present dataset, 25% of bulls had KA occurrence in their ejaculate sperm. In other words, one-quarter of studied bulls may have lower fertility due to KA occurrence.

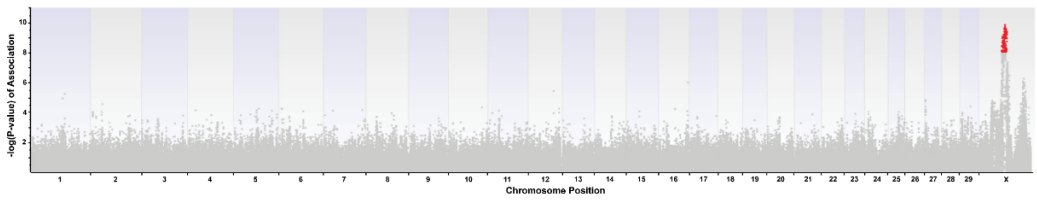

Figure 1. Manhattan plot showcasing genome-wide association results for the sperm morphological defect known as Knobbed Acrosomes. Note the significant associations identified in chromosome X. SNP marked in red are considered significantly associated (-log(*P*-value) > 8).

The heritability estimate for KA presence as a binary trait was 0.16. Among genotyped bulls, 2,183 were classified as cases (KA ≥ 1%) and 3,657 were controls. The association study points to two QTL regions in the X chromosome (Figure 1). Previous studies identified sex-linked recessive inheritance patterns for KA occurrence in Friesian bulls (Donald and Hancock 1953). Charolais bulls are disproportionally affected by KA presence and severely affected bulls have lower fertility (Barth 1986). Barth (1996) also observed pedigree evidence for a recessive inheritance of KA defects in Charolais bulls. Our results corroborate the view that KA inheritance might be sex-linked and point more specifically to QTL regions on the X chromosome.

Table 1. Quantitative trait loci boundaries and details

SIG	START (bp)	END (bp)	SIZE (bp)	PEAK POS	N SNP
10^{-8}	X:63.859.185	X:74.316.807	10.457.622	69.611.250	989
10^{-6}	X:61.130.332	X:77.980.200	16.849.868	69.611.250	1252

SIG = significance in *P*-value, bp = base pairs, POS= position, N SNP= number of SNPs.

The results point to large QTL regions on chromosome X (Table 1). Inside the defined QTL boundaries, we discovered eight candidate genes: *AMOT*, *ATRX*, *CYSLTR1*, *GPR174*, *HTR2C*, *LDHA*, *LPAR4*, and *P2RY10*. Of these genes, *ATRX* is a promising functional candidate. *ATRX* has been linked to Sertoli cell proliferation, and Sertoli-mediated spermatogenesis (Bagheri-Fam *et al.* 2011). Previous GWAS review and prioritisation analyses have linked *ATRX* with bull fertility traits (Fonseca *et al*. 2018). Knockout mice demonstrate that spermatogenesis defects may result from the impaired interaction between the faulty ATRX protein and the androgen receptor (AR), supporting *ATRX* as a key candidate gene (Bagheri-Fam *et al*. 2022).

The other candidate genes mentioned above cannot be excluded from future work. *CYSLTR1* expression can aid in cases of testicular inflammation (Awad *et al.* 2023*). GPR174* codes for a molecule involved with spermatogenesis due to its role in testosterone signalling (Tsutsui *et al.* 2011). A recent study linked *LPAR4* expression to testicular function and immunity (Dai *et al.* 2024). The conditional deletion *LDHA* in Sertoli cells disrupts spermiogenesis in mice by impairing lactate production, highlighting a mechanism critical for sperm development (Zhang *et al.* 2022). In short, there is literature evidence to support a role in spermatogenesis for 5 of the positional candidates found in this study: *ATRX, CYSLTR1, GPR174, LPAR4,* and *LDHA***.** They might act independently or in a coordinated manner to affect spermatogenesis. The peak SNP is an intergenic variant (rs110162429) mapped to a large copy number variant, and its closest annotated feature is an enhancer. Their hypothetical impact on KA occurrence needs further investigation.

When the acrosome of the sperm is thickened or ridged, the sperm's ability to bind to the zona pelucida of the oocyte is compromised (Thundathil *et al.* 2000). Not binding to the zona pelucida impairs fertilisation. Thus, this research contributes to understanding acrosome-mediated infertility, a problem reported across mammalian species, including cattle and humans (Thundathil *et al*. 2000; Aitken *et al*. 1990; Moretti *et al.* 2005). The candidate genes suggested herein need further research to validate their association and elucidate their roles in acrosome-mediated infertility.

CONCLUSION

The occurrence of KA sperm is a heritable and relatively common sperm defect in tropical bulls, with significant associations mapped to the X chromosome. Selective breeding against KA occurrence could enhance fertility rates, especially considering that 25% of bulls in this multi-breed population had KA-affected sperm. The notion that KA defects might be inherited in a recessive

Mendelian fashion further contributes to hopes of identifying risk (or causative) alleles that could aid in discarding bulls with the deleterious genotype.

ACKNOWLEDGMENTS

This work had support from CSIRO, the University of Queensland, and Meat & Livestock Australia. The authors thank the beef producers who contributed to this project (L.GEN.1818).

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