Proc. Assoc. Advmt. Anim. Breed. Genet. 22:501-504

GENOME-WIDE MAPPING OF LOCI AFFECTING SEMEN VOLUME, SPERM CONCENTRATION AND TOTAL AND PROGRESSIVE MOTILITIES IN BOARS

T.S. Gaggini[1], R.C. Antunes[1], H. Barbosa[2], P. Charagu[2] and F.M. Rezende[1]

[1] College of Veterinary Medicine, Federal University of Uberlandia, Uberlandia, Brazil
[2] Hypor Inc., Regina, Canada

SUMMARY

This study was conducted to map genomic regions associated with semen quality traits of boars. Volume, sperm concentration and total and progressive motilities of 2,392 *in natura* ejaculates of 113 Duroc males were evaluated. Genotyping process was performed by Illumina PorcineSNP60 BeadChip (68,516 SNPs, 119 animals). After quality control (MAF <3% and call rate <90%), 118 boars, corresponding to 250 animals in pedigree, and 42,240 SNPs remained to be analysed. Genome wide-association analyses were realized by BLUPf90 using weighted single-step GBLUP method considering windows of 10 adjacent SNPs to estimate their effects. The animal model considered as fixed effect boar's litter size (except for ejaculate volume), mean age at measurement (except for total motility) and sperm concentration (only for motility evaluations) as covariates and the animal and residual random effects. It was mapped 22, 14, 10 and 11 genomic regions, distributed in 11, 9, 3 and 6 different chromosomes, explaining more than 1% of additive genetic variance of ejaculate volume, sperm concentration and total and progressive motilities, respectively. Genomic regions with a great influence on sperm quality traits' expression were identified and must be explored to understand their importance for the genetic control of these traits related to fertility.

INTRODUCTION

Artificial insemination has been extensively used in swine production and, over the years, it has promoted a considerable improvement in breeding results. However, to obtain and maintain a desirable reproduction performance it is necessary to use high quality semen originated from selected and approved boars.

Macroscopic evaluations, as ejaculate volume measurement, and microscopic exams, as sperm concentration and motility, are the most common analysis realized in boar studs (BS) routine. The objective of these evaluations is to process high quality doses of semen (Robaire and Chan 2010). Volume and sperm concentration are evaluated to determine the total number of cells in *in natura* semen and the number of possible doses to be produced per ejaculate once they are related to the number of cells per dose and the dilution rate of the doses (Flowers 1996; Waberski *et al*. 2011). Sperm motility indicates the percentage of mobile cells and, when using computer assisted sperm analysis (CASA), it can be differentiated in total motility and progressive motilities, according with the trajectory of the cells. In general, motility are positive correlated with *in vivo* fertility (Broekhuijse *et al*. 2012; Flowers *et al*. 2016) and are considered an important indicator of boar fertility (Kummer *et al*. 2013).

Despite the importance of boar semen quality traits, selection of boars to be used in BS almost does not take it into account and focuses mainly on growth and carcass characteristics (Flowers 2008). The possibility of associate sperm and genetic merit in boar selection could be interesting to reduce the number of boars required to service sows and maintain the improvement of growth and carcass quality (Oh *et al*. 2006). Although, sperm quality traits can only be evaluated on boars after puberty, the identification of genomic regions influencing their expression and their inclusion in breeding programs are an alternative to select for them in pre-pubertal stage. In that way, the aim of the study was to map chromosomal regions that potentially have association with volume,

sperm concentration and total and progressive motilities in Duroc boars.

MATERIALS AND METHODS

Repeated observations of volume, concentration and total and progressive motilities of *in natura* ejaculates of 113 Duroc boars housed at the same boar stud were collected from February 2015 until May 2016. Automated semen collection system (Collectis®, IMV) was used and each ejaculate was collected in a pre-warmed (36ºC) plastic container. Gel fraction of each ejaculate was filtered and discarded. After that, the ejaculate was weighted and, to a better estimation of its real volume, it was assumed that one gram corresponds to one millilitre of semen. For microscopic evaluations, samples of ejaculates were prepared (90 µL of raw semen plus 810 µL of pre-warmed extender) and submitted to CASA system (Sperm Vision® Minitüb), which determined sperm concentration and total and progressive motilities. Sperm concentration was determined through counting the cells in eight fields and establishing an average of them. Total motility corresponded to the percentage of mobile cells, independent of their trajectory, and progressive motility corresponded to progressive forward motility of the cells (>4.5 µm of distance sperm travels in straight line). It was evaluated 2,392 ejaculates and the number of ejaculates per boar was 21.17 ± 12.63. Mean values of boar's age at measurement, volume, concentration and total and progressive motilities were considered in the analysis.

Boars were genotyped with Illumina PorcineSNP60 BeadChip (Ramos *et al*. 2009), according to the manufacturer protocols (119 animals for 68,516 SNPs). The quality control of markers was made excluding those with unknown genomic position, placed in sexual chromosomes, with MAF (minor allele frequency) lower than 3% and markers and animals that presented call rate lower than 90%. After quality control, 118 animals and 42,240 SNPs remained to be analysed. Genome wide-association analysis were realized by BLUPf90 (Misztal *et al*. 2002) using weighted single-step GBLUP method (WssGBLUP, Zhang *et al*. 2014), considering windows of 10 adjacent SNPs to estimate their effects by postGSf90 (Aguilar *et al*. 2010; Wang *et al*. 2012). A total of three iterations of BLUPf90 and postGSf90 were used for the WssGBLUP. Each run of postGSf90 updated weights for SNP, whereas each run of BLUPf90 used the updated weights to constructed G matrices (Zhang *et al*. 2016). The iterations increase the weights of SNPs with large effects and decrease those with small effects.

The animal model considered as fixed effect boar's litter size (except for ejaculate volume), besides, as covariates, mean age at measurement (except for total motility) and sperm concentration (only for motility evaluations) and, as random effects, animal and residual effects. Analyses were performed using a pedigree composed by 250 animals.

The results of GWAS were reported as the percentage of the additive genetic variance explained by the windows of 10 adjacent SNPs presented in Manhattan plots drawn by R software.

RESULTS AND DISCUSSION

In Table 1 descriptive statistics for ejaculate volume, sperm concentration, total and progressive motilities were presented.

Table 1. Descriptive statistics for mean values of ejaculate volume, sperm concentration, total and progressive motilities of Duroc boars used for GWAS analysis

Semen traits	N	Mean	SD	Range
Volume (mL)	110	160.19	45.42	75.11 – 286.60
Concentration (x10^6/mL)	110	0.57	0.18	0.20 – 1.11
Total motility (%)	113	86.53	7.47	50.45 – 95.24
Progressive motility (%)	110	76.52	9.99	38.21 – 91.32

N – Number of animals evaluated; SD – Standard deviation

In Figures 1, 2, 3 and 4 were represented the genomic regions and the percentage of genetic variance explained by windows of 10 adjacent SNPs in each chromosome for ejaculate volume, sperm concentration, total and progressive motilities, respectively.

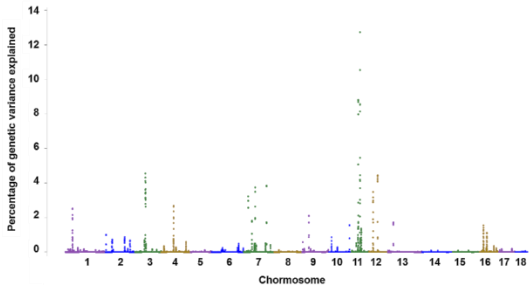

Figure 1. Manhattan plot of genomic regions associated with ejaculate volume.

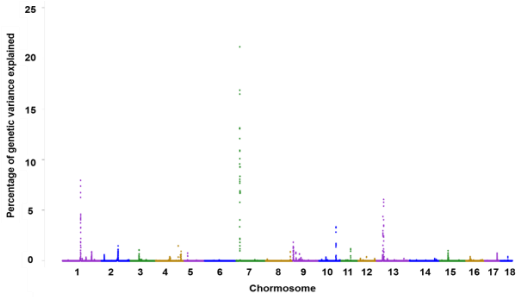

Figure 2. Manhattan plot of genomic regions associated with sperm concentration.

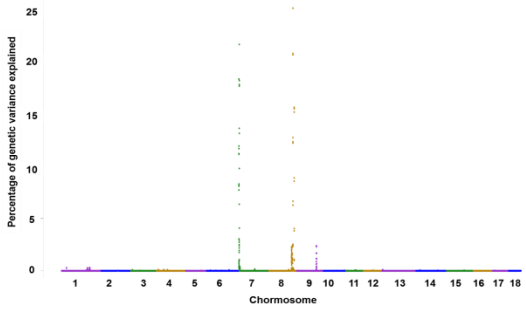

Figure 3. Manhattan plot of genomic regions associated with total motility.

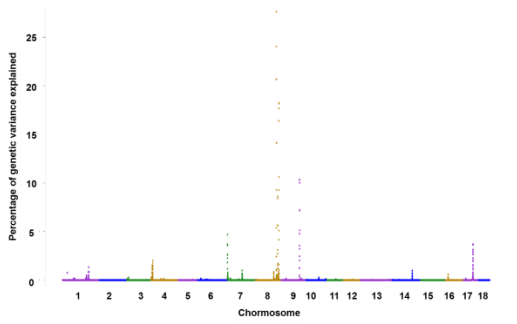

Figure 4. Manhattan plot of genomic regions associated with progressive motility.

Genomic regions that explained more than 1% of additive genetic variance of ejaculate volume were distributed in chromosomes 1, 2, 3, 4, 7, 9, 10, 11, 12, 13 and 16. Of these, chromosome 11 has two important regions explaining 8.81% (stating position 26634721 – final position 27221215) and 14.38% (starting position 49822501 – final position 50200669) of its additive genetic variance. Similarly to our results, Xing *et al.* (2009) also reported that chromosome 3 has significative quantitative trait loci (QTL) for semen volume.

Chromosomes 1, 2, 3, 4, 7, 9, 10, 11 and 13 presented genomic regions that explained more than 1% of genetic variance of sperm concentration. Windows of 10 adjacent SNPs located between 147860835-149305559 pb of chromosome 1, 8326917-8526138 pb and 8833246-8980813 pb of chromosome 7 and 22293935-22666436 pb of chromosome 13 account for 7.94%, 26.03%, 9.28% and 6.05% of its additive genetic variance, respectively. Other studies also reported significant genomic regions in chromosome 7 affecting sperm concentration, total sperm per ejaculate (Zhao *et al.* 2016) and testicular weight (Ren *et al.* 2009).

For total motility, chromosomes 7, 8 and 9 presented windows that explained more than 1% of additive genetic variation. Windows placed between 58555-740616 pb and 1637113-1806683 pb of chromosome 7 and 138036642-138190631 pb and 140609271-141043305 pb of chromosome 8 explained more than 15% of additive genetic variance (18.32%, 21.62%, 25.10% and 15.63%, in this order).

Regions that explain more than 1% of genetic variation for progressive motility were in chromosomes 1, 4, 7, 8, 9 and 17. Higher percentages of additive genetic variance (27.63%, 18.24% and 10.33%) were observed in two windows located on chromosome 8 (133531944-133969475 pb and 140609271-141043305 pb) and one on chromosome 9 (125924965-126346678 pb). There is no reference about progressive motility in literature, but Xing *et al*. (2009) and Diniz *et al*. (2014) have reported significant genomic regions in chromosome 1 affecting total motility, which can also influence sperm motility and their trajectory.

This study identified important genomic regions associated with sperm quality traits of Duroc boars. A total of 57 SNPs windows that explained more than 1% of genetic variance were identified for ejaculate volume, sperm concentration, total and progressive motilities.

Those regions must be explored to understand their importance for the genetic control of these these traits related to fertility. In the future, the markers identified in this research may be useful to improve the selection of boars to be used in boar studs.

REFERENCES

Aguilar, I., Misztal, I., Johnson, D. L., Legarra, A., Tsuruta, S., and Lawlor, T. J. (2010) *J. Dairy Sci*. **93**:743.

Broekhouijse, M.L.W.J., Sostaric, E., Feitsma, H. and Gadella, B.M. (2012) *Theriogenology* **76**:1473.

Diniz, D.B., Lopes, M.S., Broekhuijse, M.L.W.J., Lopes, P.S., Harlizius, B., Guimarães, S.E.F., Duijuvensteijn, N., Knol, E.F. and Silva, F.F. (2014) *Anim. Reprod. Sci*. **151**:201.

Flowers, W.L., Deller, F. and Stewart, K.R. (2016) *Anim. Reprod. Sci*. **174**:123.

Flowers, W.L. (1996) *Proceedings of American Association od Swine Practitioners* **1**:469.

Flowers, W.L. (2008) *Theriogenology* **70**:1297.

Kummer, A.B.H.P.; Gaggini, T.S.; Bernardi, M.L.; Mcmanus, C.; Gonçales, E.M.; Wentz, I. and Bortolozzo, F.P. (2013) *Reprod. Domest. Anim.* **48**:747.

Misztal I., Tsuruta S., Strabebl T., Auvray B., Drueta, T. and Lee, D.H. (2002) *Proceedings of 7th World Congress on Genetics Applied to Livestock Production* **28**:21.

Oh, S.H., See, M.T., Long, T.E. and Galvin, J.M. (2006) *Asian-Aust. J. Anim. Sci*. **19**:160.

Ramos, A.M., Crooijmans, R.P., Affara, N.A., Amaral, A.J., Achibald, A.L., Beever, J.E., Bendixen, C., Churcher, C., Clark, R., Dehais, P., Hansen, M.S., Hedegaard, J., Hu, Z.L., Kerstens, H.H., Law, A.S., Megens, H.J., Milan, D., Nonneman, D.J., Rohrer, G.A. Rothschild, M.F., Smith, T.P., Schnabel, R.D., Van Tassel, C.P., Taylor, J.F., Wiedmann, R.T., Schook, L.B. and Groenen, M.A. (2009) *PLoS One* **4**:6524.

Ren, D.R., Ren, J., Xing, Y.Y., Guo, Y.M., Wu, Y.B., Yang, G.C., Mao, H.R. and Huang, L.S. (2009) *J. Anim. Sci*. **87**:17.

Robaire, B. and Chan. P. (2010) 'The handbook of andrology' Schaumburg: The American Society of Andrology, Illinois. **2**.

Xing, Y., Ren, J., Ren, D., Guo, Y., Wu, Y., Yang, G., Mao, H., Brenig, B. and Huang, L. (2009) *Anim. Reprod. Sci*. **114**:210.

Zhang, X., Lourenco, D.A.L., Aguilar, I., Legarra, A. and Misztal, I. (2016) *Front. Genet*. **7**:151.

Zhang, X., Lourenco, D.A.L., Misztal, I., Aguilar, I. and Legarra, A. (2014) *Proceedings World Congress on Genetics Applied to Livestock Production* **10**.

Zhao, X., Zhao, K., Ren, J., Zhang, F., Jiang, C., Hong, Y., Jiang, K., Yang, Q., Wang, C., Ding, N., Huang, L., Zhang, Z., Xing, Y. (2016) *Anim. Sci. J*. **87**:646.

Waberski, D., Henning, H. and Pretrunkina, A.M. (2011) *Reprod. Domest. Anim.* **46**:45.

Wang, H., Misztal, I., Aguilar, I., Legarra, A. and Muir, W. M. (2012) *Genet. Res*. **94**:73.