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Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1687002> since 2019-02-05T17:13:54Z

Published version:

DOI:10.1111/rda.12747

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(Article begins on next page)

This is the author's final version of the contribution published as:

Puglisi R, Gaspa G., Balduzzi D., Severgnini A., Vanni R., Macciotta Npp, Galli A.,
Genomewide analysis of bull sperm quality and fertility traits
Reproduction In Domestic Animals, 51:840:843, 2016,
doi: 10.1111/rda.12747

The publisher's version is available at:

<https://onlinelibrary.wiley.com/doi/full/10.1111/rda.12747>

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1 **Genome-wide analysis of bull sperm quality and fertility traits**

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12

13 Running title: GWAS of bull fertility traits

14

15 **Keywords:** high-density panel, genome-wide association study, chromatin status,

16

17 **Abstract**

18 Because the priority of AI industry is to identify sub fertile bulls, a predictive model that allowed for
19 the prediction of 91% bulls of low fertility was implemented based on seminological (motility)
20 parameters and DNA status assessed both as DNA fragmentation index (DFI) and by TUNEL assay
21 using sperm of 105 Holstein Friesian bulls (4 batches per bull) selected based on *in vivo* estimated
22 relative conception rates (ERCR). Thereafter, sperm quality and male fertility traits of bulls were
23 explored by GWAS using a high density (777K) Illumina chip.
24 After data editing, 85 bulls and 591,988 SNPs were retained for GWAS. Out of 12 SNPs with false
25 discovery rate < 0.2 , four SNPs located on BTA28 and BTA18 were significantly associated (LD
26 adjusted Bonferroni < 0.05) with the non-compensatory sperm parameters DFI and TUNEL. Other
27 SNPs of interest for potential association with TUNEL were found on BTA3, in the same
28 chromosome where associations with non-compensatory *in vivo* bull fertility were already reported.
29 Further suggestive SNPs for sperm membrane integrity were located on BTA28, the chromosome
30 where QTL studies previously reported associations with sperm quality traits. Suggestive SNPs for
31 ERCR were found on BTA18 in the vicinity of a site already associated with *in vivo* bull fertility.
32 Additional SNPs associated with ERCR and sperm kinetic parameters were also identified. In contrast
33 to other, but very few GWAS on fertility traits in bovine spermatozoa, which reported significant
34 SNPs located on BTX, we have not identified SNPs of interest in this sexual chromosome.

35

36 **Introduction**

37

38 Numerous authors investigating the genetic basis of fertility suggest that genome-wide association
39 studies (GWAS) are more effective in detecting causal variants associated to complex fertility traits
40 when compared to traditional quantitative trait loci (QTL) mapping (Zhang et al. 2012). However,
41 only few GWAS have focused on bull fertility (Fortes et al. 2013). Although the accurate estimate of
42 fertility allows to identify the critical number of viable sperm required to obtain adequate pregnancy

43 rates, there are some uncompensable characteristics, such as the state of nuclear chromatin, which
44 cannot be overcome by simply increasing the sperm number (Evenson and Wixon 2006). Because
45 the priority of AI industry is to identify hypofertile bulls, which require more sperm in the dose to
46 reach maximum fertility, a predictive model for the low level of fertility as estimated *in vivo* was
47 developed in the present work based on standard seminological and DNA status assessments.
48 Furthermore, sperm quality and male fertility traits were explored by GWAS using a high density
49 Illumina chip.

50

51 **Materials and methods**

52

53 **Estimate of *in vivo* bull fertility**

54 Four batches of commercial frozen sperm (years 2002-2014; 13 AI centres) of 105 Holstein Friesian
55 bulls were selected according to their fertility, based on 56-day non-return to oestrus adjusted for
56 environmental effects, calculated as the random effect of service sire (estimated relative conception
57 rates, ERCR; 90% reliability) using the model described in Puglisi et al. (2012). Fifteen bulls were of
58 low fertility (ERCR < -2.46; mean = -3.8 ± 0.8) and 90 bulls were of middle-high fertility (ERCR >
59 -2.46; mean = $+0.4 \pm 1.7$), based on the threshold fixed at 3 standard deviations below the mean
60 ERCR calculated on a dataset of 4989 bulls (mean ERCR = 0.0005 ± 0.82).

61

62 **Sperm analysis**

63 Sperm quality parameters of the 105 bulls were assessed as follows: membrane integrity (MI) was
64 evaluated by the NucleoCounter SP100 (ChemoMetec A/S, Allerød, Denmark); motility (total, TM;
65 progressive, PM; average path velocity, VAP) was evaluated by CASA System-HTM IVOS v.12
66 (Hamilton Thorne); DNA status, assessed both as DNA fragmentation index (DFI) implemented in
67 the sperm chromatin structure assay (SCSA[®]) and by the TUNEL assay, was determined using the

68 flow cytometer Guava EasyCyte Plus® (IMV Technologies, l'Aigle, France) as described (Evenson
69 and Wixon 2006).

70

71 **Genomic analysis**

72 Sperm genomic DNA of bulls was genotyped with the Illumina BovineHD chip (777K) (Illumina,
73 San Diego, CA). Both SNPs and bulls with call rate < 95% and < 97.5%, respectively, were
74 discharged. SNPs were removed if the Minor Allele Frequency (MAF) was lower than 0.02, or if they
75 statistically deviated from the Hardy Weinberg equilibrium ($p < 0.0001$).

76

77 **Statistical analysis**

78 Statistical analysis was implemented by R procedures (R Core Team, 2012).

79 At first, seminological data were evaluated by general linear mixed model (GLMM) using bull and
80 batch as random effects and semen production centres as fixed effect. Thereafter, in order to
81 implement the model for the identification of the bulls of low fertility, the variable LowFERT was
82 defined as follows: LowFERT = 1 for ERCR < -2.46 and LowFERT = 0 for ERCR > -2.46.

83 A first logistic model was implemented with the continuous seminal variables and the discrete
84 variable BATCH, as follows:

$$85 \text{ LowFERT} = \beta_0 + \beta_1 TM + \beta_2 PM + \beta_3 VAP + \beta_4 MI + \beta_5 DFI + \beta_6 TUNEL + BATCH_i + e$$

86 A second model was, then, implemented including only the effects identified in the first model as
87 significant, as follows:

$$88 \text{ LowFERT} = \beta_0 + \beta_1 PM + \beta_2 MI + \beta_3 DFI + \beta_4 TUNEL + e$$

89 Results were validated by bootstrapping with nonparametric resampling (1000 trials) using package
90 “boot”.

91

92 For GWAS, sperm parameters were pre-corrected for the effect of production batch. The GWAS was
93 carried out with the Grammar genomic control (GC) approach, that account for genetic substructure

in the population (Aulchenko et al. 2007), implemented in the GenABEL R package (*polygenic* and *grammar* functions).

At first, data were analysed with the following linear mixed model:

$$y_{jk} = AI_cent_j + a_k + e_{jk}$$

where y_{jk} is the sperm parameter for the k-th bull; AI_cent is the fixed effect of the j-th AI centre; a_k is the random polygenic additive effect of the k-th bull $\sim N(0, G\sigma_a^2)$; e_{jk} is the random residual $\sim N(0, I\sigma_e^2)$, where G and I are the genetic (co)variance and identity matrices, respectively. The genetic (co)variance between animals was structured using the genomic relationship matrix. Residual of the model were, then, analysed with a linear model that included the fixed effect of the SNP genotype. Given that Bonferroni is the simplest and more conservative correction for multiple testing assuming independence of performed test, and that its application largely ignores the correlation between markers due to linkage disequilibrium, the genome wide significance was assessed by LD adjusted Bonferroni (Sun et al. 2014; Wu et al. 2014). To discover SNPs potentially associated to seminal parameters the threshold was fixed at 8.06×10^{-7} ($0.05/N$), where N is the number of haplotype blocks estimated with `-blocks` flag in `plink` ($N = 62,062$). False discovery rate (FDR) and q-values were also calculated: SNPs with $FDR < 0.20$ are discussed.

Results and discussion

Results of seminological and DNA status assessments are presented in Table 1. Statistical analysis shown high variability among bulls for all the parameters, and a moderate variability among batches for TM, PM and MI (Table 2). Differently, for DFI and TUNEL a negligible variability was reported among sperm batches, thus confirming these parameters as intrinsic-not compensable characteristics of individual bulls (Evenson 1999). The effect of the semen production centre was not significant. The statistical model implemented for the identification of hypofertile bulls allowed for the prediction of 91% ($n = 14$) bulls of low fertility and data was further validated by bootstrapping (89-91%; 95% CI).

For GWAS, 85 bulls and 591,988 SNPs were retained, while 130,462 SNPs were discarded because did not reach the MAF threshold. Table 3 lists the top 12 significant SNPs with FDR < 20%, among which, four SNPs located on BTA28 and BTA18 were also significantly associated (Bonferroni-LD) with DFI and TUNEL. Further SNPs for DFI were found on BTA1-4-16-23-28. With respect to BTA28, in this chromosome QTL were previously detected for several semen quality traits (Valour et al. 2015). The complete list of suggestive SNPs for seminological traits and ERCR is presented as supplemental Table 4. Of interest, suggestive SNPs ($p\text{-value} < 1.61 \times 10^{-5} = 1/62,062$; Sun et al. 2014) for ERCR were found on BTA18 in the vicinity of the site where Peñagaricano et al. (2012) found association with *in vivo* bull fertility. Other SNPs, ranked by their values of nominal significance, were found on BTA3 for TUNEL, in the chromosome where associations with noncompensatory bull fertility were reported (Blaschek et al. 2011). Similarly, GWAS was successfully used for identifying candidate genes associated with several sperm traits in bulls (Fortes et al. 2013; Hering et al. 2014). In contrast to other studies reporting significant SNPs located on BTX (Suchocki and Szyda 2015), our work has not identified SNPs of interest on this sexual chromosome.

134

135 **Acknowledgements**

The authors wish to thank Dr. Giorgio Burchiellaro, the General Director of the “Associazione Nazionale Allevatori di Frisone Italiana (ANAFI), for providing ERCR data. This work was funded by the Italian Ministry of Agriculture (Grant INNOVAGEN).

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140 **References**

141

Aulchenko YS, Ripke S, Isaacs A, van Duijn CM, 2007: GenABEL: an R library for genome-wide association analysis. *Bioinformatics* **15**, 23(10), 1294-1296.

144

145 Blaschek M, Kaya A, Zwald N, Memili E, Kirkpatrick BW, 2011: A whole-genome association
 146 analysis of noncompensatory fertility in Holstein bulls. *J Dairy Sci* **94**, 4695-4699.
 147
 148 Evenson DP, 1999. Loss of livestock breeding efficiency due to uncompensable sperm nuclear defects.
 149 *Reprod Fertil Dev* **11**, 1-15.
 150
 151 Evenson DP, Wixon R, 2006: Clinical aspects of sperm DNA fragmentation detection and male
 152 infertility. *Theriogenology* **65**, 979-991.
 153
 154 Fortes MR, DeAtley KL, Lehnert SA, Burns BM, Reverter A, Hawken RJ, Boe-Hansen G, Moore
 155 SS, Thomas MG. 2013: Genomic regions associated with fertility traits in male and female cattle:
 156 Advances from microsatellites to high-density chips and beyond. *Anim Reprod Sci* **141**, 1-19.
 157
 158 Hering D, Olenski K, Kaminski S, 2014: Genome-wide association study for poor sperm motility in
 159 Holstein-Friesian bulls. *Anim Reprod Sci* **146**, 89-97.
 160
 161 Peñagaricano F, Weigel KA, Khatib H, 2012: Genome-wide association study identifies candidate
 162 markers for bull fertility in Holstein dairy cattle. *Anim Genet* **43**, 65-71.
 163
 164 Puglisi R, Pozzi A, Foglio L, Spanò M, Eleuteri P, Maria G. Grollino, Bongioni G., Galli A, 2012:
 165 The usefulness of combining traditional sperm assessments with in vitro heterospermic insemination
 166 to identify bulls of low fertility as estimated in vivo. *Anim Reprod Sci* **132**, 17-28.
 167
 168 R Core Team (2012). R: A language and environment for statistical computing. R Foundation for
 169 Statistical Computing, Vienna, Austria.
 170

171 Suchocki T, Szyda J, 2015: Genome-wide association study for semen production traits in Holstein-
172 Friesian bulls. *J Dairy Sci* **98**, 5774-5780.
173
174 Sun YF, Liu RR, Zhao GP, Zheng MQ, Sun Y, Yu XQ, Li P, Wen J, 2014: Genome-Wide linkage
175 analysis and association study identifies loci for polydactyly in chickens. *G3-Genes Genomes*
176 *Genetics* **4**, 1167-1172.
177
178 Valour D, Michot P, Eozenou C, Lefebvre R, Bonnet A, Capitan A, Uzbekova S, Sellem E, Ponsart
179 C, Schibler L, 2015: Dairy cattle reproduction is a tightly regulated genetic process: Highlights on
180 genes, pathways, and biological processes. *Animal Frontiers* **5**. doi:10.2527/af.2015-0006
181
182 Zhang H, Wang Z, Wang S, Li H, 2012: Progress of genome wide association study in domestic
183 animals. *J Anim Sci Biotech* **3**, 26. doi: 10.1186/2049-1891-3-26.
184
185 Wu Y, Fan H, Wang Y, Zhang L, Gao X, Chen Y, Li J, Ren H, Gao H, 2014: Genome-wide
186 association studies using haplotypes and individual SNPs in Simmental cattle. *PLOS ONE* **9**, e109330.
187

188 Table 1

189 Sperm quality parameters of 105 bulls.

Parameter	Low fertility		Medium-high fertility	
	Mean ± SD	Range	Mean ± SD	Range
Total Motility (%)	46.2 (14.8) ^a	11.0-72.0	53.2 (15.5) ^b	8.0-88.0
Progressive Motility (%)	34.9 (13.7) ^a	6.0-63.0	42.2 (13.8) ^b	6.0-70.0
Average Path Velocity (µm/sec)	90.5 (14.4)	59.0-120	92.5 (16.4)	11.0-138.0
Membrane Integrity (%)	50.0 (11.2)	25.0-71	52.0 (15.2)	0.0-89.0
DNA fragmentation Index (%)	9.8 (4.2) ^a	3.0-25.0	5.8 (5.5) ^b	1.0-96.0
TUNEL (%)	8.11 (4.4) ^a	2.0-22.0	5.6 (3.3) ^b	1.0-32

190 ^{a,b} Different superscripts within rows indicate statistical difference at the ANOVA test (p < 0.001)

191

192 Table 2

193 Random (bull and batch) and fixed (production centre) effects by general linear mixed model.

Parameter	Variance		
	Bull	Batch	Centre
Total Motility (%)	64.88 (27.49%)	20 (8.47%)	< 0.0001
Progressive Motility (%)	70.76 (37.69%)	15.46 (8.24%)	< 0.0001
Average Path Velocity (µm/sec)	96 (44.20%)	8 (3.50%)	< 0.0001
Membrane Integrity (%)	89.24 (46.05%)	19.4 (10.01%)	< 0.0001
DNA fragmentation Index (%)	11 (35.62%)	0.30 (1.00%)	0.0571
TUNEL (%)	9 (20.91%)	0.38 (0.89%)	< 0.0001

194

195 Table 3

196 Top significant SNPs from GWAS with false discovery rate < 0.2.

Trait	SNP	BTA	bp	p-Bonf-LD	q-value
DFI	BovineHD2800009025	28	33,677,489	0.001	0.006
DFI	BovineHD2800009027	28	33,682,118	0.008	0.020
TUNEL	BovineHD0800005232	18	16,773,834	0.016	0.149
DFI	BovineHD2800008609	28	32,601,290	0.026	0.050
DFI	BovineHD2800006900	28	26,638,772	0.100	0.135
DFI	BovineHD1600001050	16	3,700,646	0.191	0.139
DFI	ARS-BFGL-NGS-117941	16	4,095,536	0.254	0.150
DFI	BovineHD2600011042	26	40,124,425	0.327	0.150
DFI	BovineHD0400022506	4	81,577,828	0.383	0.150
DFI	BovineHD1600000982	16	3,486,636	0.428	0.150
DFI	BovineHD2300001056	23	4,581,363	0.483	0.157
DFI	BovineHD0100016322	1	57,604,927	0.712	0.192

197

198 DFI, DNA fragmentation index; p-Bonf-LD = nominal p-value/62,062.

199