



# Genome-wide association study for semen quality traits in German Warmblood stallions



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## ABSTRACT

We performed a genome-wide association study for semen quality traits in 139 German Warmblood stallions. Stallions were genotyped using the Illumina equine SNP50 Beadchip. Traits analysed were de-regressed estimated breeding values (EBVs) for gel-free volume, sperm concentration, total number of sperm, progressive motility and the total number of progressively motile sperm. The GWAS revealed 29 SNPs on 12 different chromosomes as genome-wide significantly associated with semen quality traits. For ten genomic regions we could retrieve candidate genes influencing stallion fertility. Among the candidate genes, we could find the genes encoding cysteine-rich secretory proteins (*CRISP1*, *CRISP2* and *CRISP3*). This was the first GWAS in horses performed for semen quality traits.

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## 1. Introduction

As stallions are primarily selected for breeding based on their athletic performance and other phenotypic traits fertility and therewith an adequate semen quality is usually at most of secondary importance. Nevertheless, an efficient production of high quality semen is very desirable in stallions due to the widespread use of artificial insemination (AI) in horse breeding. Besides management and environmental factors, particularly, genetic effects lead to significant variation in semen quality among stallions. In recent years, approximately 60 candidate genes with functional importance for male fertility have been reported (Roser, 2008; Giesecke et al., 2010; Sieme and Distl, 2012). Candidate genes for stallion fertility including *ACE*, *CRISP3*,

*FKBP6*, *FSHB*, *INHBA*, *PLCz1*, *PRLR*, *SPATA1* and *SP17* have been screened for polymorphisms and tested for association with stallion fertility using estimated breeding values for the paternal component of the pregnancy rate per estrus cycle (EBV-PAT) as target trait (Giesecke et al., 2010; Sieme and Distl, 2012; Schrimpf et al., 2014, 2015). However, genes affecting semen traits in stallions are poorly clarified. A genome-wide association study (GWAS) conducted by Raudsepp et al. (2012) implicated testis-sperm specific *FKBP6* as a susceptibility locus for impaired acrosome reaction (IAR) of sperm causing subfertility in stallions. Schrimpf et al. (2015) searched *FKBP6* for mutations using whole-genome sequence data of seven stallions to perform an association study in Hanoverian stallions with EBV-PAT as target trait. Genotyping of five exonic mutations within *FKBP6* revealed a significant association of the single nucleotide polymorphism (SNP) g.11040379C > A with EBV-PAT in 216 Hanoverian stallions. They concluded that

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**Table 1**

Single nucleotide polymorphisms (SNPs) associated with gel-free semen volume in 139 German Warmblood stallions. Given are the SNP-ID, its position and motif, minor allele (MA), minor allele frequency (MAF), permuted P-values, estimates for the SNP effect with its standard error (SE) and trait variance explained (%).

SNP ID	SNP-Position and motif	ECA	Location	MA	MAF	P-value <sub>perm</sub>	SNP-effect ± SE	Variance explained
BIEC2-930017	g.93992337A>G	5	<i>MIER1</i>	A	0.39	0.002	-18.9 ± 3.0	24.7
BIEC2-723816	g.1673754C>T	28	Intergenic	T	0.39	0.039	17.0 ± 3.1	19.4

**Table 2**

Single nucleotide polymorphisms (SNPs) associated with sperm concentration in 139 German Warmblood stallions. Given are the SNP-ID, its position and motif, minor allele (MA), minor allele frequency (MAF), permuted P-values, estimates for the SNP effect with its standard error (SE) and trait variance explained (%).

SNP ID	SNP-Position and motif	ECA	Location	MA	MAF	P-value <sub>perm</sub>	SNP-effect ± SE	Variance explained
BIEC2-39318	g.94832928T>C	1	Intergenic	T	0.16	0.041	27.6 ± 5.4	17.7
BIEC2-928481	g.90129827A>G	5	<i>NEGR1</i>	G	0.24	0.007	-23.9 ± 4.3	20.1
BIEC2-535169	g.47313526A>G	20	Intergenic	G	0.34	0.046	-21.0 ± 4.1	17.8
BIEC2-561771	g.34550411T>G	21	Intergenic	T	0.28	0.004	-22.1 ± 3.9	20.7
BIEC2-561772	g.34550625A>G	21	Intergenic	A	0.28	0.006	-22.0 ± 4.0	20.5
BIEC2-561784	g.34563579A>G	21	Intergenic	A	0.28	0.006	-22.0 ± 4.0	20.5
BIEC2-692110	g.27827952T>C	26	Intergenic	C	0.10	0.054	30.9 ± 6.1	17.4

**Table 3**

Single nucleotide polymorphisms (SNPs) associated with progressive motility in 139 German Warmblood stallions. Given are the SNP-ID, its position and motif, minor allele (MA), minor allele frequency (MAF), permuted P-values, estimates for the SNP effect with its standard error (SE) and trait variance explained (%).

SNP ID	SNP-Position and motif	ECA	Location	MA	MAF	P-value <sub>perm</sub>	SNP-effect ± SE	Variance explained
BIEC2-23463	g.54934045A>G	1	<i>CTNNA3</i>	G	0.36	0.049	20.3 ± 4.3	15.6
BIEC2-23479	g.55034558C>T	1	<i>CTNNA3</i>	T	0.33	0.026	-18.9 ± 4.9	20.1
BIEC2-777972	g.39054277T>C	3	Intergenic	T	0.50	0.038	18.8 ± 4.5	12.8
BIEC2-777975	g.39060021T>C	3	Intergenic	T	0.50	0.038	18.8 ± 4.5	12.8
BIEC2-809120	g.108016029G>A	3	<i>CIQTNF7</i>	A	0.08	0.020	-22.0 ± 4.0	20.5
BIEC2-809121	g.108016418A>C	3	<i>CIQTNF7</i>	C	0.09	0.020	34.5 ± 7.6	14.2

**Table 4**

Single nucleotide polymorphisms (SNPs) associated with total number of sperm in 139 German Warmblood stallions. Given are the SNP-ID, its position and motif, minor allele (MA), minor allele frequency (MAF), permuted P-values, estimates for the SNP effect with its standard error (SE) and trait variance explained (%).

SNP ID	SNP-Position and motif	ECA	Location	MA	MAF	P-value <sub>perm</sub>	SNP-effect ± SE	Variance explained
BIEC2-804322	g.95981423C>A	3	Intergenic	C	0.29	0.032	26.5 ± 5.0	19.1
BIEC2-1103296	g.68034922A>G	9	Intergenic	G	0.26	0.022	-25.6 ± 4.8	19.1
BIEC2-445903	g.53770904A>G	19	<i>NXPE3</i>	G	0.26	0.013	28.5 ± 5.1	20.0
BIEC2-579931	g.8781707T>G	22	Intergenic	G	0.35	0.041	-24.5 ± 4.7	18.5

**Table 5**

Single nucleotide polymorphisms (SNPs) associated with total number of progressively motile sperm in 139 German Warmblood stallions. Given are the SNP-ID, its position and motif, minor allele (MA), minor allele frequency (MAF), permuted P-values, estimates for the SNP effect with its standard error (SE) and trait variance explained (%).

SNP ID	SNP-Position and motif	ECA	Location	MA	MAF	P-value <sub>perm</sub>	SNP-effect ± SE	Variance explained
BIEC2-22928	g.53958189T>C	1	Intergenic	T	0.19	0.011	-27.6 ± 5.1	20.5
BIEC2-23557	g.55909321C>T	1	<i>CTNNA3</i>	C	0.35	0.022	25.1 ± 4.7	19.0
BIEC2-1053493	g.56115677A>G	8	Intergenic	A	0.39	0.022	22.5 ± 4.2	18.7
BIEC2-1104137	g.70859944G>A	9	Intergenic	A	0.35	0.025	-23.1 ± 4.4	18.4
BIEC2-1104140	g.70863096A>G	9	Intergenic	G	0.37	0.021	-23.5 ± 4.4	18.8
BIEC2-1104189	g.70889211A>C	9	Intergenic	C	0.36	0.025	-23.1 ± 4.4	18.4
BIEC2-1104198	g.70893252G>T	9	Intergenic	T	0.35	0.025	-23.1 ± 4.4	18.4
BIEC2-1104242	g.70960794G>A	9	Intergenic	A	0.35	0.014	-23.6 ± 4.4	19.3
BIEC2-311811	g.54632135A>C	15	Intergenic	A	0.36	0.015	22.8 ± 4.2	19.3
BIEC2-579931	g.8781707G>T	22	Intergenic	G	0.35	0.015	-24.8 ± 4.6	19.3

this *FKBP6*-associated SNP leads to higher conception rates in stallions homozygous for the mutant allele. Previous GWAS using Illumina BovineSNP50 Beadchip genotypes on AI-bulls identified SNPs and candidate genes associated with semen quality traits in Holstein-Friesian bulls (Hering

et al., 2014a,b,c; Suchocki and Szyda, 2015). Corresponding studies for semen quality traits in stallions are missing. Therefore, the objective of our study was to perform a GWAS for semen quality traits in German Warmblood stallions and to identify associated SNPs and candidate genes.

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