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Genomics to identify genetic variants for semen quality traits of fresh and frozen-thawed semen

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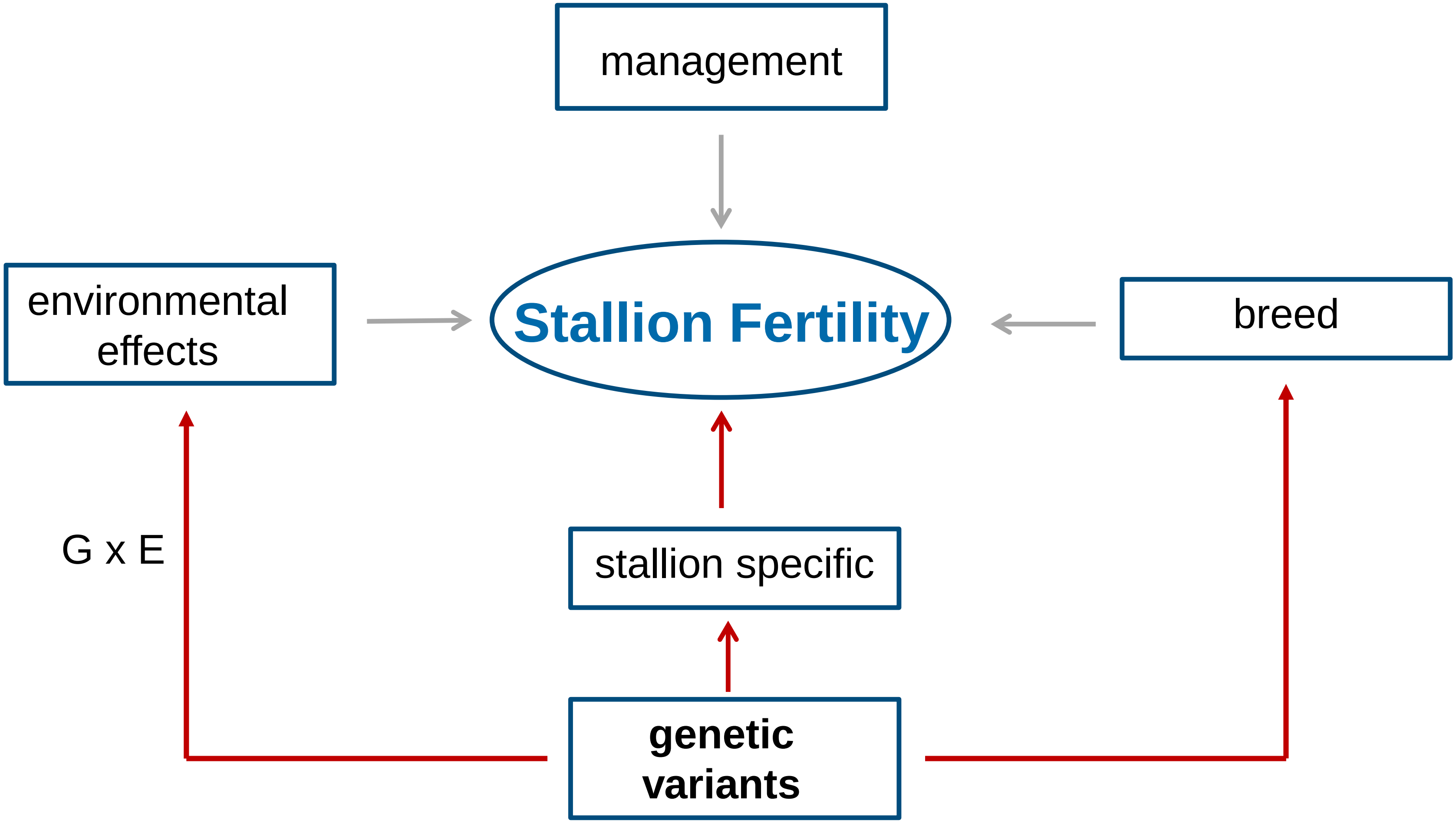
Stallion Fertility


management

environmental effects

Stallion Fertility

breed

G x E

stallion specific

genetic variants

Between-stallion variance of semen traits

- variation in semen quality among stallions of the same breed
  (Rousset et al., 1986; Parlevliet et al., 1994; Van Eldik et al., 2006; Labitzke et al., 2014)

- inter-stallion variance 55–65% of the total variance in semen traits in Hanoverians (Gottschalk et al., 2015)

- > 1000 candidate genes with functional importance for stallion fertility
  (Sieme and Distl, 2012, Schrimpf et al., 2016)

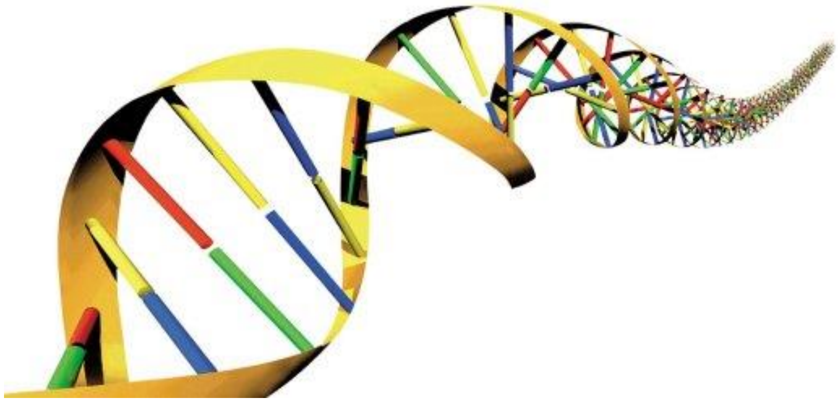
Objectives

Estimation of genetic parameters for semen quality traits of German Warmblood employed in Artifical Insemination (AI)

- **heritabilities of semen traits**

Search for genetic variants contributing to semen quality traits

- **genome-wide association study for semen traits**



Heritability of semen traits

Material and Methods:

- 271 fertile German Warmblood stallions routinely employed in AI (Lower Saxon National Stud Celle and the North Rhine-Westphalian National Stud Warendorf)

- seasonal conception rates ≥ 70%

  (> 9 mares bred from a stallion)

Heritability of semen traits

Material and Methods:

- 63,972 fresh semen reports
- 3,681 frozen-thawed semen samples
- semen samples collected once daily on six consecutive days every week in breeding seasons of 1998-2014

- **Traits** analysed:

VOL	gel-free volume (ml)
CONC	sperm concentration (x10^6/mL)
TNS	total number of sperm (x10^9)
PMS	progressive motility (%)
TPMS	total number of progressively motile sperm (x10^9)
MPT	post-thawing motility (%)
DFI	DNA fragmentation index via flow cytometer
NVI	sperm membrane integrity (non-viable sperm, %)

Heritability of semen traits

Methods:

- estimation of heritabilities and correlations among semen traits
- estimated breeding values (EBVs) and their reliabilities

- multivariate linear animal model using all 8 traits with restricted maximum likelihood (REML)

$$Y_{ijklmnoqr} = \mu + Year_i + Month_j + b_1(Age)_k + b_2(Age)^2_l + b_3(logAge)_m$$

$$+ Stud_n + Stbook_o + permanent\text{-}env\text{-}stallion_p + animal_q + e_{ijklmnopqr}$$

Heritability of semen traits

Trait	Vol	Conc	PMS	TNS	TPMS	MPT	DFI	NVI
Vol	**0.28**	-0.69	-0.16	0.54	0.46	-0.30	-0.22	0.36
Conc	-0.37	**0.21**	0.11	0.18	0.24	0.52	0.31	-0.51
PMS	-0.09	0.02	**0.14**	-0.20	0.16	0.39	-0.79	-0.45
TNS	0.55	0.40	-0.06	**0.14**	0.94	0.03	0.21	-0.02
TPMS	0.47	0.37	0.34	0.90	**0.13**	0.16	-0.05	-0.19
MPT						**0.13**	-0.29	-0.01
DFI						-0.21	**0.45**	0.30
NVI						-0.33	0.18	**0.11**

h^2-SE: 0.01-0.04; r_g: above diagonal; r_e: below diagonal

Estimated breeding values of semen traits

EBVs on a scale of 100 ± 20 and their reliabilities (r^2)

Trait	min	max	range	r^2_{mean}
Vol	56	189	133	0.68
Conc	51	173	122	0.37
PMS	26	156	130	0.41
TNS	43	177	134	0.43
TPMS	35	165	130	0.41
MPT	51	156	105	0.28
DFI	49	181	132	0.43
NVI	52	155	103	0.28

Variance components of semen traits

Permanent environmental (PERM), additive genetic (ADD) and total **variance among stallions** (STALL)

Trait	PERM	ADD	STALL
Vol	0.10	0.28	0.38
Conc	0.38	0.21	0.59
PMS	0.19	0.14	0.33
TNS	0.18	0.14	0.32
TPMS	0.19	0.13	0.32
MPT	0.16	0.13	0.29
DFI	0.26	0.45	0.71
NVI	0.17	0.11	0.28

Genetic parameters of semen traits

- semen quality traits show moderate heritabilities
- heritability estimates indicate a large additive genetic variation in semen traits of stallions regularly used in AI

- EBVs:
  - publication of EBVs may inform breeders on semen quality of stallions
  - implementation of EBVs in a selection index for future stallions

- large impact of the stallion on semen quality traits in Warmblood horses

GWAS

Genome-wide association study for semen traits

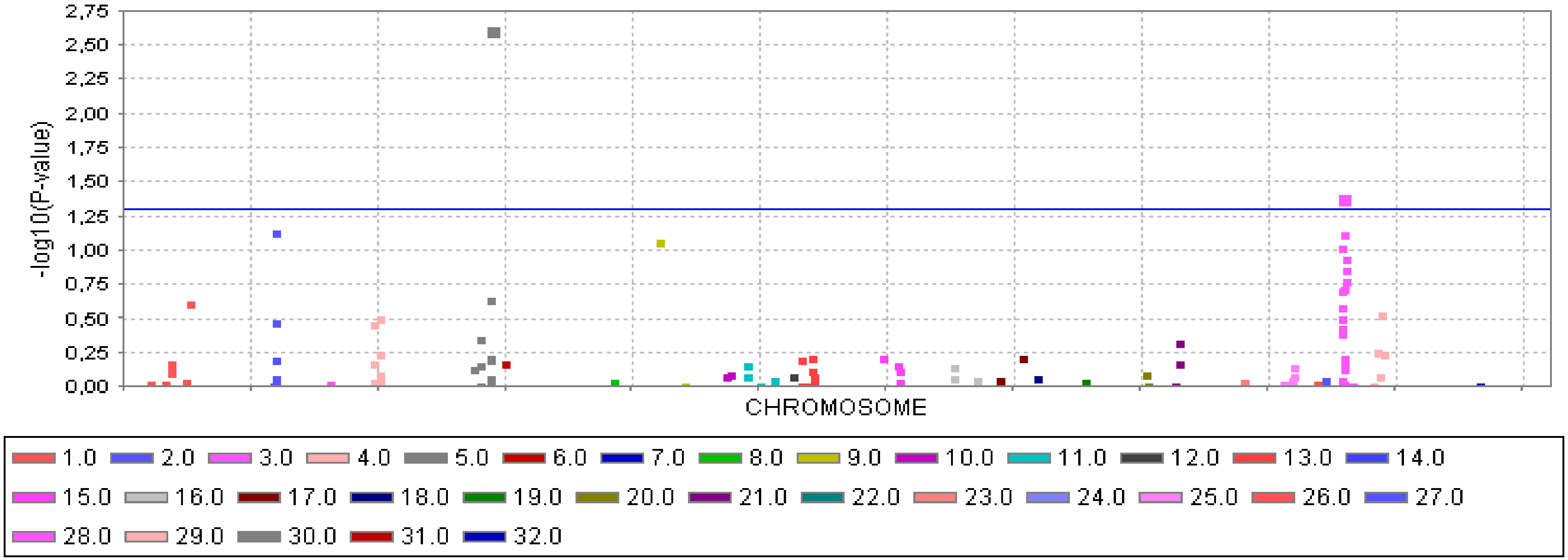
- identification of polymorphisms associated with complex traits

➢ identify single nucleotide polymorphisms (SNPs) and candidate genes associated with semen quality traits

- Illumina equine SNP50 Beadchip including 54,602 SNPs
- genotyping rate per stallion >0.98
- de-regressed EBVs evaluated with mixed linear model
- P-values adjusted for multiple testing

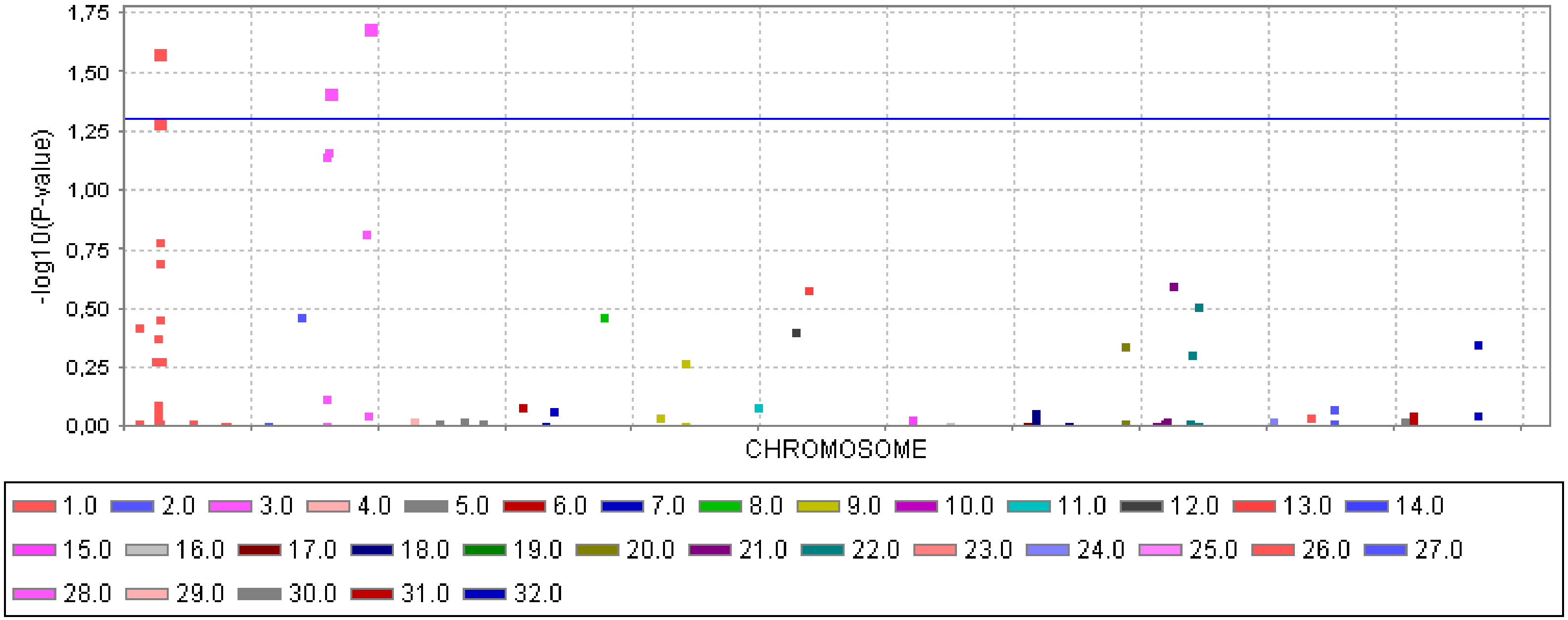
➢ application of a set of genetic markers for future genomic selection of breeding stallions

GWAS – gel-free volume



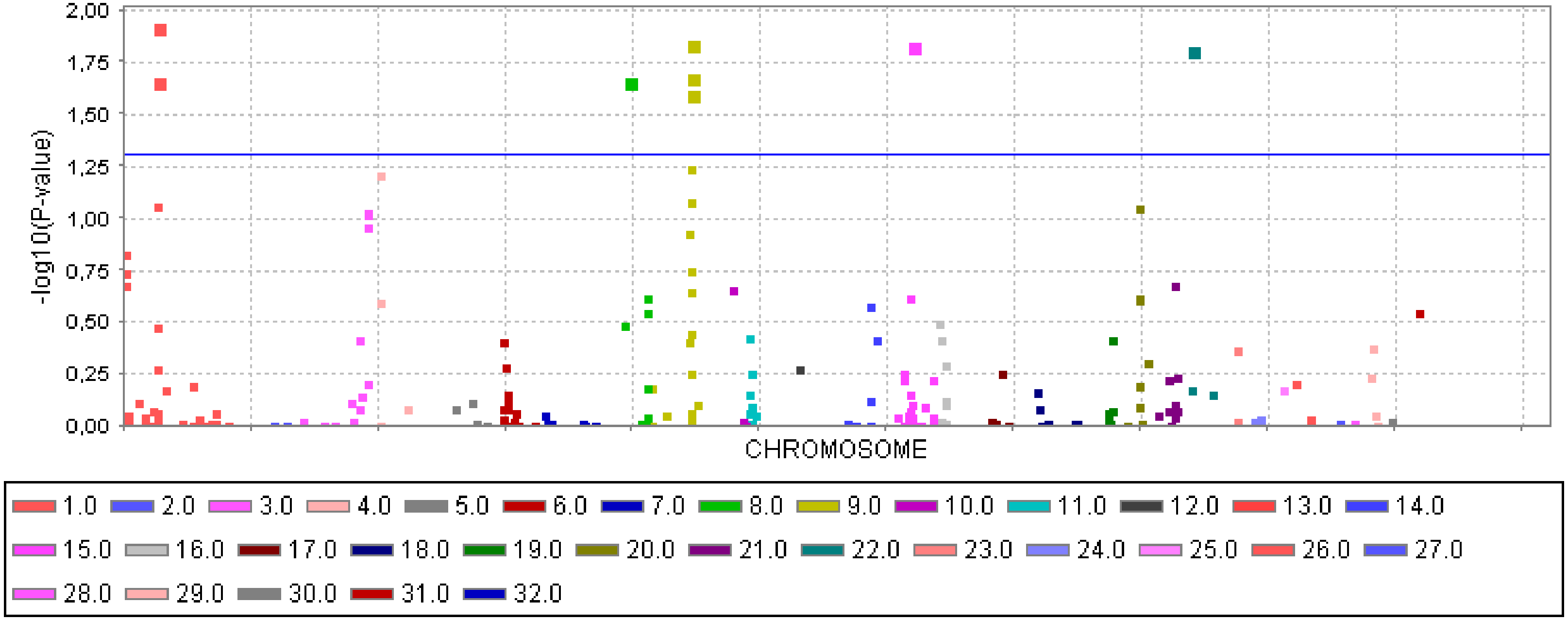
ECA	Position (Mb)	P-value	Effect	Variance (%)
5	93	0.002	-19	25
28	1.6	0.04	17	19

GWAS – progressive motility



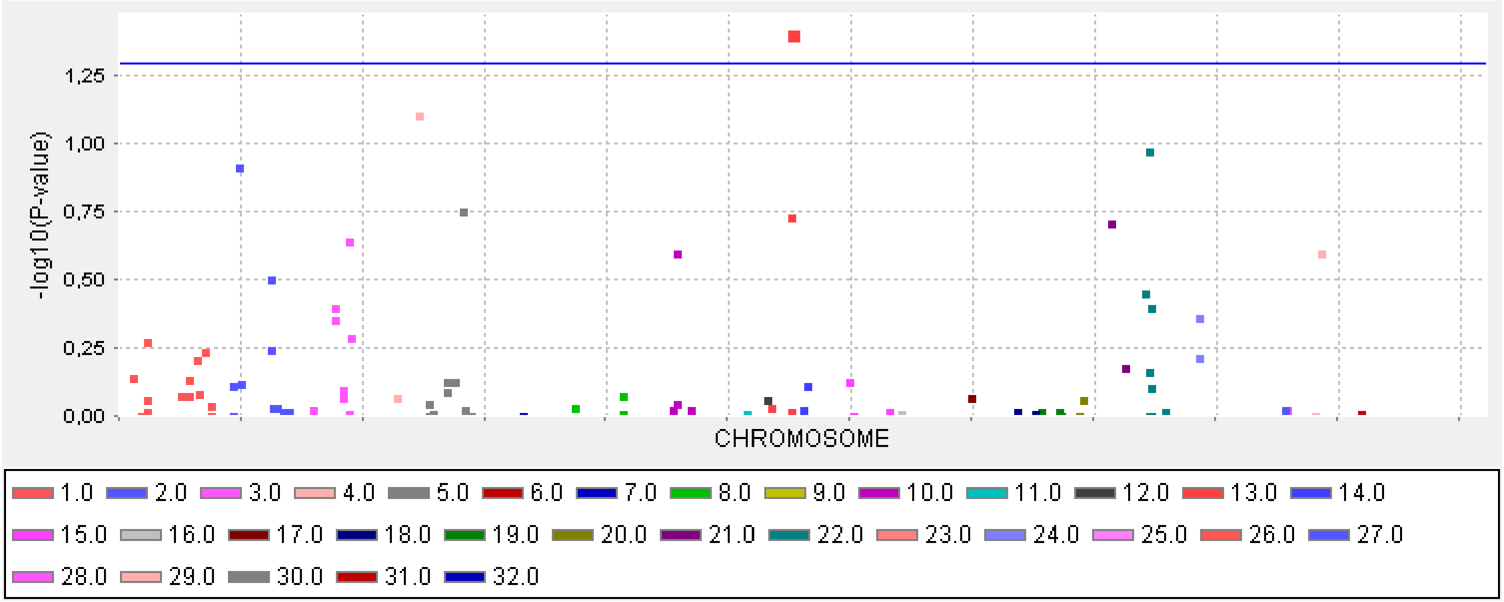
ECA	Position (Mb)	P-value	Effect	Variance (%)
1	55	0.03	-19	20
3	39	0.04	19	13
3	108	0.02	-22	21

GWAS – total no of progressively motile sperm



ECA	Position (Mb)	P-value	Effect	Variance (%)
1	54	0.01	-28	21
8	56	0.02	23	19
9	71	0.02	-24	19
15	5.5	0.02	23	19
22	8.8	0.02	-25	19

GWAS – DFI



ECA	Position (Mb)	P-value	Effect	Variance (%)
13	42	0.04	-15	26

GWAS

- GWAS revealed 16 significantly associated SNPs on 13 different chromosomes

- 8 regions with candidate genes effecting spermiogenesis, sperm motility, sperm morphology, acrosome reaction, zona-pellucida reaction and testis function
    - ECA1: SIRT1, HERC4, POLG, MFGE8
    - ECA9: LRRC6
    - ECA13: SOX8 (dog)
    - ECA15: ATP6V1E2
    - ECA20: CRISP1-3 (stallion, bull), PGK2 (boar)
    - ECA26: CCT8 (bull), SOD1 (bull)
    - ECA28: GLIPR1L1 (bull)

Genomic inbreeding and stallion fertility

- Runs of homozygosity (ROHs) >> genomic inbreeding (ROH/aut-size)

- Genomic inbreeding

  mean ± std: 0.0629 ± 0.0288

  min-max: 0.0011 - 0.2040

- Significant negative correlations with

• EBVs for stallion fertility	-0.15
• Sperm concentration	-0.11
• Progressive motility	-0.08
• Post-thaw motility	-0.15

Conclusions

- Semen quality traits show moderate heritabilities in German Warmblood horses

- Estimated breeding values (EBVs) and their reliabilities indicate whether semen quality in stallions can be improved through genetic selection

- Genome-wide significantly associated regions and candidate genes for semen quality traits were identified

- Inbreeding has negative effects on stallion fertility

Thank you for your attention !