



# Relationships among stallion fertility and semen traits using estimated breeding values of German Warmblood stallions



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## ARTICLE INFO

### Article history:

Received 11 April 2016

Received in revised form 7 September 2016

Accepted 10 October 2016

### Keywords:

Horse

Stallion

Semen

Correlation

Estimated breeding value

Fertility

## ABSTRACT

A high quality of stallion semen is of particular importance for maximum reproductive efficiency. In the present study, we estimated the relationships among estimated breeding values (EBVs) of semen traits and EBVs for the paternal component of the pregnancy rate per estrus cycle (EBV-PAT) for 100 German Warmblood stallions using correlation and general linear model analyses. The most highly correlated sperm quality trait was total number of progressively motile sperm ( $r = 0.36$ ). EBV-PAT was considered in three classes with stallions 1 SD below ( $<80$ ), around ( $80$ – $120$ ), and above ( $>120$ ) the population mean of 100. The general linear model analysis showed significant effects for EBVs of all semen traits. EBVs of sperm quality traits greater than 100 to 110 were indicative for EBV-PAT greater than 120. Recommendations for breeding soundness examinations on the basis of the assessments of sperm quality traits and estimation of breeding values seem to be an option to support breeders to improve stallion fertility in the present and future stallion generation.

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## 1. Introduction

Evaluation of stallion semen before use in breeding and artificial insemination (AI) is of crucial importance in horse breeding. The detection of ejaculates likely to result in poor conception rates is most important for maximum reproductive efficiency of stallions. Sperm quality traits including progressive motility, total sperm motility, and morphology may explain some variation in stallion fertility [1–3]. In addition, stallions are selected according to the recommendations for sperm quality traits [4]. Stallion fertility should be measured by the pregnancy rate (PR) per cycle [5–10] or nonreturn rate [11,12] to avoid limitations through the influences of repeated breedings of a mare. Jasko et al. [13] performed a study in 43 Thoroughbred and 56 Standardbred stallions on semen traits and percent

pregnant per cycle (PC). They found significant correlations between the percentages of motile ( $r = 0.40$ ), progressively motile ( $r = 0.46$ ), morphologically normal ( $r = 0.36$ ) sperm, and computer-aided analysis of percentage of motile spermatozoa ( $r = 0.34$ ) with PC. Variation in these characteristics accounted for only 20% of the total variation in PC. Love [3] evaluated sperm quality traits in 88 stallions and their relationships with stallion fertility measured by the seasonal PR, PC, and percent pregnant per first cycle (FCP). Correlations among progressive motility and stallion fertility estimates were moderately positive and showed significant results for PC and FCP ( $r = 0.52$  and  $r = 0.56$ ). Mean values for progressive motility tended to be higher in stallions reaching a PC and FCP rate  $\geq 46\%$ . The results of this study identified the total sperm motility ( $r = 0.59$ ) and percent of morphologically normal sperm ( $r = 0.39$ ) as the sperm quality traits most highly correlated with FCP. Prediction of the first season stallion fertility of 3-year-old Dutch Warmblood stallions revealed a significant positive correlation among morphologic normal sperm and first-cycle nonreturn rates [14]. The relationship between the

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quality of cool-shipped stallion semen and fertility has been studied in 459 ejaculates from 130 stallions that were used for insemination of 196 donor mares in 496 estrus cycles [15]. Embryo recovery rates increased for those estrus cycles when all sperm measures including motility, viability, DNA quality, normal morphology, concentration, and total number of sperm increased. In addition, this study provided evidence that to the total number of progressively motile sperm, other sperm quality traits might be important to estimate the outcome of an insemination.

Nevertheless, studies regarding the relationship among estimated breeding values (EBVs) for semen quality traits and fertility of stallions expressed by EBVs for the paternal component of the pregnancy rate per estrus cycle (EBV-PAT) are missing. This relationship may indicate the usefulness of sperm quality assessments for breeding decisions on stallions.

The objective of the present study was to evaluate the relationships among EBVs for semen quality traits and EBV-PAT in 100 fertile German Warmblood stallions regularly used in AI.

## 2. Materials and methods

### 2.1. Animals and semen records

For the present study, semen quality data of 241 fertile German Warmblood stallions regularly used in AI at the Lower Saxon National Stud Celle and the North Rhine-Westphalian National Stud Warendorf were used. Data were recorded at the national studs in the years 2001 to 2014 and included 63,972 veterinary reports on fresh semen. Semen samples were collected once daily on six consecutive days every week in the months February to August using a phantom and an artificial vagina (Hanover model). A total of 25,379 semen samples and 254 semen samples per stallion were examined for the present study. Semen traits included gel-free volume, sperm concentration, total number of sperm (TNS), progressive motility, and the total number of progressively motile sperm (TNM). The sperm concentration ( $10^6/\text{mL}$ ) was determined using a SpermaCue photometer (Minitube, Tiefenbach, Germany). The total number of sperm ( $\times 10^9$ ) was calculated by multiplying gel-free volume and sperm concentration. The progressive motility was assessed by experienced observers through subjective visual estimation using a phase-contrast microscope with a heated stage at  $\times 200$  to  $\times 400$  magnification (Olympus CH-II; Olympus Optical, Hamburg, Germany). The use of computer-assisted sperm analysis provides a more objective analysis of sperm motility [3]. A limitation of the present study may be the use of subjective evaluations of progressive sperm motility. However, the large number of semen samples examined may largely minimize errors caused by subjective evaluations. Fresh semen doses to be used in AI had to contain  $300 \times 10^6$  motile sperm. A more detailed description of this data can be found elsewhere [16].

### 2.2. Statistical analysis

A total of 100 German Warmblood stallions with EBVs for semen quality traits and EBV-PAT were available for the

present analysis. All these stallions had no history of reduced history and were only used in AI. Heritabilities estimated were moderate with the highest estimates for gel-free volume ( $h^2 = 0.28$ ) and the lowest estimates ( $h^2 = 0.13$ ) for TNM [16]. All EBVs were standardized onto a mean of 100 and an SD of 20. For all semen traits, EBVs were estimated with multivariate animal models and (co-)variance component estimates of a multivariate analysis described elsewhere [16]. The reference population for standardization of EBVs included 241 fertile German Warmblood stallions with semen records. EBVs greater than 100 for semen traits indicate stallions transmitting higher abilities for the respective trait than the average of the 241 stallions.

EBV-PAT was estimated using an animal threshold model for the trait PR per estrus cycle and the random additive genetic effects of the stallion and the embryo. In addition, systematic environmental effects were regarded including age of the stallion, breeding season, period within breeding season, and insemination center. Trait analyzed was the PR per estrus. When an AI was successful in an estrus cycle, the trait value was encoded 1, otherwise the trait value was 0. The success rate of AIs was verified through the reports of the breeders on the pregnancy of the mare and/or a foaling and/or an abortion. All estrus periods with consecutive AIs in the same breeding season were treated as unsuccessful. In addition, estrus periods reported by the breeder as unsuccessful (missing pregnancy of the mare) were also encoded with a trait value 0. Only stallions from the National State stud Celle of Lower Saxony were contained in the data set, and these stallions were exclusively used in AI. Fertility data included the breeding seasons 1997 to 2005 of the National State stud Celle of Lower Saxony with a total of 19,897 broadmares, 246 stallions used in AI, 199,000 AI records, and 96,114 estrus cycles. Each mare was bred for a mean of 2.1 per estrus cycle with the same ejaculate of the respective stallion. There was no mixing up of estrus cycles from broadmares with natural service stallions. On average, 391 estrus cycles per stallion were recorded. Heritability for the paternal component of the PR per estrus was 1.1%. All EBVs were standardized onto a mean of 100 and an SD of 20. The random additive genetic effect of the stallion was defined as the paternal component of the PR per estrus (EBV for stallion fertility, EBV-PAT). EBVs greater than 100 mean higher conception rates of the stallions than the population average. The mean reliabilities of the EBVs were at 0.7. A more detailed description of the model and data used can be found elsewhere [17].

Relationships among EBVs of semen traits and EBV-PAT were determined using correlation and general linear model analyses. Correlations among EBVs of semen traits and EBV-PAT were analyzed using the procedure CORR of SAS, version 9.4 (Statistical Analysis System; SAS Institute, Cary, NC, USA). For the general linear model analysis, EBV-PAT were considered in three classes with class 1 including stallions with EBV-PAT less than 80 ( $n = 15$  and on average 627 estrus cycles per stallion), class 2 regarding stallions with EBV-PAT of 80 to 120 ( $n = 62$  and on average 502 estrus cycles per stallion), and class 3 including stallions with EBV-PAT greater than 120 ( $n = 23$  and on average 530

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