

# Temporal Trends in Equine Sperm Motility and Semen Volume: A Retrospective Analysis from a Single UK Breeding Facility

Imogen Thea Harris<sup>1</sup>, Kathryn Nankervis<sup>1</sup>, Alison Pyatt<sup>2</sup>, David Stuart Gardner<sup>3</sup>, Pamela Humphreys<sup>4</sup>, and Rebecca Nicole Blanchard<sup>3,\*</sup>

<sup>1</sup>Hartpury University, Gloucester, GL19 3BE, United Kingdom

<sup>2</sup>International Office, Veterinary Medicines Directorate, Addlestone KT15 3LS, United Kingdom

<sup>3</sup>School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, Loughborough, LE12 5RD, United Kingdom

<sup>4</sup>Stallion AI Services Ltd & Cryogenetics Ltd, Whitchurch, Shropshire, SY13 4BP, United Kingdom

\* Author to whom any correspondence should be addressed; email: [rebecca.blanchard1@nottingham.ac.uk](mailto:rebecca.blanchard1@nottingham.ac.uk)

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## Abstract

The equine industry preferentially selects sires based on pedigree, performance, and conformation, with little concern given to fertility. Increasing evidence supports the theory of geographic-sensitive declines in a range of semen quality parameters, yet the horse is underrepresented within this field. Data presented here retrospectively investigates trends in semen quality from a population of stallions at a single UK breeding facility (from 2001 to 2020). Data on stallion sperm motility (10,686 ejaculates, 984 stallions) and semen volume (11,122 ejaculates, 1,030 stallions) were collected from records during the years 2020 and 2021. Data were analyzed as isolated variables in a linear mixed model (REML). Fixed effects included significant covariates (year of collection, age, and abstinence period). Random effects included stallion and sample numbers. Overall trends indicated that motility has declined over the past 20 years ( $p < 0.001$ ; overall decline: 12.19%). Motility declined similarly in both prime and senescent stallions, confirming trends are not age-specific. Trends in volume ( $p < 0.001$ ) varied over time but typically increased (5.70 mL overall; 0.28 mL annually). Results suggest stallions could be at risk of perturbed reproductive health and function in the future, with serious implications for the economic status of breeding stallions and the health and welfare of breeding stock.

## Keywords

Equine; fertility; horse; motility; sperm; semen volume; temporal trends

## 1. Introduction

Reproductive function, including male factor fertility, is fundamental for the sustainability of a population. Epidemiological studies in humans indicate that sperm quality parameters have declined over recent years [1,2]. In southern India, sperm motility of infertility patients declined significantly between 1993 and 2005 [3]. In a population of human fertile sperm donors from France, where semen analysis methods were consistent, progressive motility declined between 1976 and 2009 [4]. Sperm concentration

and normal morphology have also been shown to decline by 13.1% and 46.2%, respectively, between 1970 and 1985 in a population of Holstein bulls [5]. Retrospective research in the dog sentinel model collected from one laboratory indicates a 30% decline in sperm motility between 1988 and 2014 [6].

The equine industry preferentially selects sires based on pedigree, performance, and conformation, with little concern given to fertility [7,8]. A systematic review and meta-regression analysis in global equine populations indicates that sperm progressive motility has declined significantly between 1984

and 2019, with stronger trends reported in western regions [9]. Results from meta-analyses are often heavily scrutinized due to differences in laboratory protocols between semen collection facilities [10,11]. Knowledge of trends in equine sperm quality from a single population is limited. Previous research in France analyzing secular trends in Breton Draught stallions (in the period 1981 to 1996) reported a yearly 1.8% decline in semen volume, although no change was observed in sperm count, and there was a yearly 2.8% increase in sperm concentration [12]. Equivalent findings have been reported in Anglo-Arab Thoroughbred stallions between 1985 and 1995 [12]. An increase in concentration and decrease in volume could be due to their inverse relationship, given that sperm count remained consistent. Subsequent research has reported mean equine seminal volumes below the recommended artificial insemination referencing range of 60–120 mL [12,13], which raises concern over the reproductive health and function of this population.

Semen quality in equine studies to date is impacted by a range of factors, which are not accounted for within previous analyses that determine reproductive trends [12]. Many stallion factors including age [14], inbreeding [13,15], genetics [16], discipline, and exercise intensity [17] impact stallion semen quality. Additional factors include seasonality [18], testicular heat stress [19], abstinence period [20], and nutrition [21]. To develop a robust understanding of semen quality trends, confounding factors must be accounted for within statistical models.

The research presented here builds upon the current methodological limitations associated with evidence syntheses that are reported in previous literature [11], by aiming to explore select equine semen quality parameters through controlled methodological approaches within a single UK-based population. Here, retrospective data on semen quality (motility and volume) was collated during the years 2020 and 2021 to assess reproductive trends within a large population, while accounting for alternative variables such as age and abstinence period that could impact sperm parameters. It is hypothesized that sperm motility and volume will have declined over time, as observed in alternative species.

## 2. Materials and Methods

### 2.1. Ethics

The research was approved by the Hartpury University Ethics Committee (ETHICS2019-52). For data collection from breeding records, a site permission form was signed by the facility's owner before data collection. All stallion data were fully anonymized throughout the research process.

### 2.2. Semen Collection and Analyses

Retrospective data on fresh stallion semen quality was collected from one DEFRA (Department for Environment, Food and Rural Affairs) approved breeding facility in the UK between the years 2020 and 2021. All samples were collected as part of routine breeding management practices ( $n = 11,722$  ejaculates). Stallions utilized within the study were those for fresh collection in standing livery for the stud season between the years 2001 and 2020. Stallion feed and forage were designed to help gain the best semen quality, and stallions were housed in indoor 15ft  $\times$  15ft rubberized stallion boxes, with access to stallion paddocks for daily turnout.

Semen collection was carried out using a Missouri Complete artificial vagina (Elite Reproduction Supplies, UK), fitted with a nylon mesh filter to separate the gel-free sperm-rich fraction of the ejaculate from the gel-containing sperm-poor fraction. A teaser mare, phantom, or via ground collection approach was undertaken, as required, to obtain an ejaculate sample, with the collection method factored into the statistical model. Stallion handling and semen analysis methodology were standardized across the study period by one out of two managerial personnel at the facility.

Although the data presented here was collected retrospectively, the approach for analysis was as follows: following the successful collection of the sperm-rich fraction of each ejaculate, which contains the most spermatozoa of the ejaculate, semen was immediately analyzed at the same facility within 30 minutes. The volume of fresh ejaculate (sperm-rich fraction) was calculated by weighing the samples, using the standard conversion of one gram to one milliliter [22]. Before 2012, ejaculate was analyzed neat; however, post 2012, ejaculate was diluted using approximately 20 mL pre-warmed commercial extender, INRA 96 (Stallion AI Services, Shropshire, UK), accepting most stallions have an average concentration of 150-200 million sperm/mL, resulting in an average density of  $10 \times 10^6$  million sperm/mL. A 10  $\mu$ l sample of extended semen (sperm-rich fraction) was placed onto a pre-warmed slide (37°C) and covered with a 22  $\times$  22 mm cover slip before being analyzed by phase contrast microscopy for motility (microscope;  $\times 100$  magnification), subjectively assessing the percentage of sperm progressively moving forwards. While fertility assessment is multifactorial, only sperm motility and volume were assessed here. Supplementary data presents information on sperm concentration. Although sperm concentration data was available, the analysis method changed from a SpermaCue (2001–2012) to a NucleoCounter SP-100 (2013–2020). Low sample numbers, as seen in **Supplementary Table 1**, also supported this data not being presented in this report; however, the figure can be viewed in the supplementary data for reader interest (**Supplementary Figure 1**).

### 2.3. Categorization of Variables

Stallions were allocated a numerical code and an ejaculate number as separate variables to account for multiple collections per stallion. Data regarding the date of collection, stallion date of birth, breed, collection method, country of birth, and discipline were collected from corresponding stallion breeding documents and competition records. Breeds were grouped as warmbloods, hotbloods, coldbloods, mixed breeds, and pony types, a recognized method of categorization in equine reproductive research [23]. The reproductive history of the stallions used in this study was not known. Raw data on the 'date of collection' was used to calculate the 'season of collection' and was used in tandem with the 'stallion date of birth' to calculate the 'age at collection.' Samples displaying haemospermia or urospermia were excluded from the dataset due to the detrimental impacts of blood and urine on sperm quality [24,25]. Extreme data outliers were defined as those greater than three times the interquartile range and were removed from the datasets [26]. Data on 11,722 samples from 1,041 stallions of mixed ages and breeds were obtained from records between the years 2001 and 2020. Following outlier removal, the datasets for sperm motility and volume

consisted of 10,686 and 11,122 samples from 984 and 1,030 stallions, respectively. The overall sample numbers for stallions and ejaculates included for each year of collection are provided in **Table 1**.

**2.4. Statistical Analysis**

Data were analyzed using GenStat 17th edition (VSN International Ltd, Hemphstead, UK) and graphically interpreted on GenStat and GraphPad Prism 9 (GraphPad Prism version 9.0, GraphPad Software, California, CA, USA). Data were analyzed using a linear mixed model (restricted maximum likelihood; REML). The model assumed that missing data were randomly distributed, enabling the inclusion of all data and preventing bias in estimated values. Stallion and ejaculate codes were included as random effects for all analyses. For fixed effects within the REML model, significance was sequentially investigated. Variable significance and the Akaike information criterion (AIC) value were interpreted to determine inclusion within the final refined model. If  $p < 0.05$  and the AIC value did not change appreciably by the variables removal, then the parameter was included in the model [27]. Variables included for each parameter and significance within the statistical model are presented in **Table 2**.

**Table 1:** Overview of the number of stallions and subsequent ejaculates per parameter, per year.

| Sperm Motility         |        |        |        |         |        |
|------------------------|--------|--------|--------|---------|--------|
| Year of collection     | 2001   | 2002   | 2003   | 2004    | 2005   |
| Stallion/sample number | 4/17   | 31/375 | 4/23   | 16/69   | 52/409 |
| Year of collection     | 2006   | 2007   | 2008   | 2009    | 2010   |
| Stallion/sample number | 82/660 | 88/660 | 94/789 | 75/559  | 54/390 |
| Year of collection     | 2011   | 2012   | 2013   | 2014    | 2015   |
| Stallion/sample number | 82/850 | 71/581 | 60/591 | 89/684  | 81/762 |
| Year of collection     | 2016   | 2017   | 2018   | 2019    | 2020   |
| Stallion/sample number | 74/696 | 93/783 | 94/788 | 100/823 | 30/177 |
| Volume                 |        |        |        |         |        |
| Year of collection     | 2001   | 2002   | 2003   | 2004    | 2005   |
| Stallion/sample number | 4/17   | 33/388 | 30/206 | 54/464  | 56/433 |
| Year of collection     | 2006   | 2007   | 2008   | 2009    | 2010   |
| Stallion/sample number | 82/664 | 89/657 | 93/760 | 73/548  | 54/394 |
| Year of collection     | 2011   | 2012   | 2013   | 2014    | 2015   |
| Stallion/sample number | 81/832 | 70/559 | 58/571 | 90/674  | 81/734 |
| Year of collection     | 2016   | 2017   | 2018   | 2019    | 2020   |
| Stallion/sample number | 73/685 | 92/757 | 95/785 | 100/815 | 30/179 |

**Table 2:** Final variables included within the fixed effects of the REML model for sperm motility and volume.

| Parameter      | Fixed model   |
|----------------|---|
| Sperm motility | Year of collection; abstinence period; age; extender; breed; season of collection; country of birth |
| Volume         | Year of collection; abstinence period; age; country of birth; season of collection                  |

The mean ( $\pm$  SEM) was predicted for each year of collection, accounting for covariates included in the statistical model. Predicted means were plotted for each parameter, and a simple linear regression produced to determine the slope. The equation  $y = mx + c$  was utilized to determine the overall decline over time. The yearly decline was then calculated by dividing the overall decline by the number of collection years [28]. While means predicted from the REML model accounted for age, further analyses of age-restricted time trends ensured that results were not reflective of aging stallions across the study period. Means plots for motility and volume, against age, were produced. Graphs showed age-based trends in semen quality for each parameter, which were visually interpreted. Based on the variability or trend observed, stallions were grouped as reproductively prime or senescent on an individual parameter basis. For the age range at which the semen quality parameter remained consistent, stallions were grouped as reproductively prime. For those presenting more variability, or a decline in the semen quality parameter based on increasing age, stallions were subsequently defined as reproductively senescent. Isolated REML analyses were then utilized to determine differences in time trends between age groups. A  $p$ -value of  $< 0.05$  was considered statistically significant, and a 95% confidence interval was assumed for all analyses.

**3. Results**

The predicted mean ( $\pm$  SEM) outputs for motility and semen volume, for each year of collection, are presented in **Table 3**. These data were predicted from the REML model.

**Table 3:** The predicted mean ( $\pm$  SEM) outputs for sperm motility and semen volume, for each year of collection. Data were predicted from the REML statistical model.

| Sperm Motility (%) |                  |                  |                  |                  |                  |
|--------------------|------------------|------------------|------------------|------------------|------------------|
| Year of collection | 2001             | 2002             | 2003             | 2004             | 2005             |
| Predicted mean     | 47.64 $\pm$ 2.98 | 48.84 $\pm$ 2.63 | 49.29 $\pm$ 4.95 | 51.54 $\pm$ 2.75 | 47.76 $\pm$ 2.23 |
| Year of collection | 2006             | 2007             | 2008             | 2009             | 2010             |
| Predicted mean     | 51.49 $\pm$ 2.18 | 51.95 $\pm$ 2.13 | 54.57 $\pm$ 2.11 | 58.14 $\pm$ 2.09 | 55.79 $\pm$ 2.17 |
| Year of collection | 2011             | 2012             | 2013             | 2014             | 2015             |
| Predicted mean     | 52.01 $\pm$ 2.05 | 50.36 $\pm$ 2.03 | 47.25 $\pm$ 2.02 | 45.17 $\pm$ 1.98 | 41.55 $\pm$ 1.97 |
| Year of collection | 2016             | 2017             | 2018             | 2019             | 2020             |
| Predicted mean     | 42.02 $\pm$ 1.96 | 41.98 $\pm$ 1.93 | 37.25 $\pm$ 1.93 | 37.43 $\pm$ 1.92 | 42.83 $\pm$ 2.17 |
| Volume (mL)        |                  |                  |                  |                  |                  |
| Year of collection | 2001             | 2002             | 2003             | 2004             | 2005             |
| Predicted mean     | 38.98 $\pm$ 5.04 | 43.17 $\pm$ 3.57 | 40.68 $\pm$ 3.47 | 35.17 $\pm$ 3.16 | 36.93 $\pm$ 3.14 |
| Year of collection | 2006             | 2007             | 2008             | 2009             | 2010             |
| Predicted mean     | 35.43 $\pm$ 3.08 | 39.08 $\pm$ 3.00 | 38.68 $\pm$ 3.01 | 33.21 $\pm$ 2.98 | 33.62 $\pm$ 3.17 |
| Year of collection | 2011             | 2012             | 2013             | 2014             | 2015             |
| Predicted mean     | 39.36 $\pm$ 2.93 | 44.33 $\pm$ 2.90 | 46.10 $\pm$ 2.89 | 44.17 $\pm$ 2.83 | 50.96 $\pm$ 2.82 |
| Year of collection | 2016             | 2017             | 2018             | 2019             | 2020             |
| Predicted mean     | 42.56 $\pm$ 2.80 | 45.57 $\pm$ 2.74 | 46.32 $\pm$ 2.75 | 44.06 $\pm$ 2.74 | 39.03 $\pm$ 3.36 |

### 3.1. Time Trends in Mean Sperm Motility between 2001 and 2020

Motility varied over time ( $p < 0.001$ ), declining between 2001 and 2020, with a more substantial decrease detected from 2009. Applying a trend line, sperm motility declined by 12.19% over the entirety of the study period, with a yearly decline of 0.61% (Figure 1a). Focusing on the trends from 2009 to 2019 where there was a rapid decline, motility fell from 56.20% (2009) to 35.20% (2019), suggesting an overall decline of 1.90% per year (20.93% over the 11 years). Prime and senescent age groups were defined based on the variability of data (Prime: 2–17 years;  $n = 8,374$  samples;  $n = 735$  stallions and 'Senescent': 18–31 years;  $n = 1,211$  samples;  $n = 79$  stallions). This resulted in two categorization groups for sperm motility. Stallions aged between 2–17 were analyzed as prime, and those aged 18–31 analyzed as senescent. Overall, motility declined in both age groups (prime: 14.34%; senescent: 24.13%) with a yearly decline of 0.72% (prime) and 1.21% (senescent) between the years 2001 and 2020 (Figure 1b).

### 3.2. Time Trends in Mean Semen Volume between 2001 and 2020

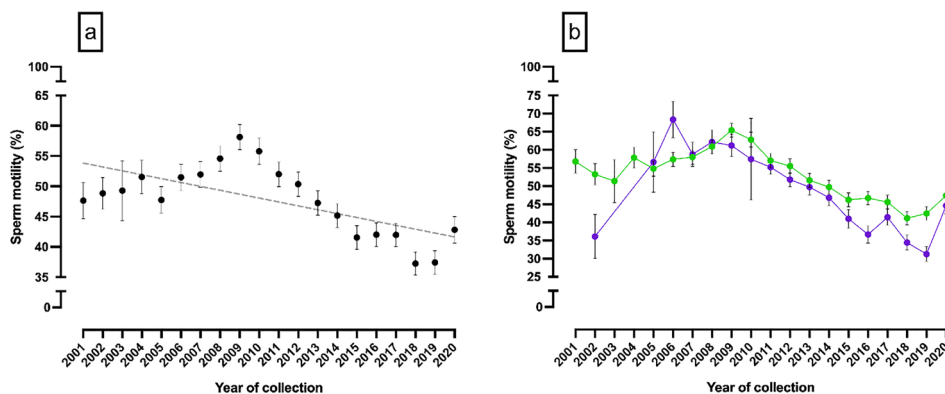
Means ( $\pm$  SEM) predicted from the model indicated that semen volume increased by 5.70 mL between 2001 and 2020, with an annual increase of 0.28 mL (REML;  $p < 0.001$ ; Figure 2a). Following the analysis of time trends in volume, age-restricted trends were determined. Prime and senescent age groups were defined based on the variability of data. This resulted in the following categories for volume whereby stallions aged between 2–25 were analyzed as prime ( $n = 9,757$  samples; 818 stallions), and those aged 26–31 were analyzed as senescent ( $n = 79$  samples; 7 stallions). When assessing the overall trend, volume increased for prime stallions between the years 2001 and 2020 (Figure 2b; 10.13 mL overall). While volume declined for senescent stallions (17.85 mL overall), distinct variability in this parameter was noted across the study period.

## 4. Discussion

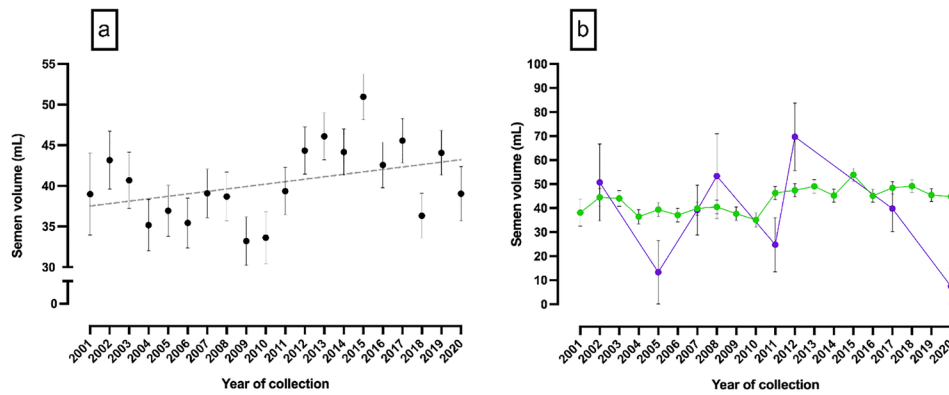
This retrospective study contributes significant data towards the equine breeding sector and supplements the debate on

adverse trends across species. To our knowledge, this is the first retrospective study to present results on fresh equine sperm motility trends, displaying findings that could have distinct implications for the global equine industry. It is essential to account for specific industry and species-specific factors when determining semen quality trends in sentinel species. From an industry practice perspective, horses are used for sporting and competition purposes, the intensity and discipline of which may influence semen quality and other reproductive parameters. Failure to include such variables within analyses is a primary source of critique in human-based evidence syntheses in reproductive trends [10].

Sperm motility is a fundamental parameter for the assessment of the fertilizing capabilities of semen samples in humans [29] and forms part of the stallion breeding soundness examination [30]. Furthermore, sperm motility is reported to be the most correlated kinematic parameter with equine per-cycle pregnancy rate [31]. In cooled semen, the threshold values of sperm motility for embryo recovery rate are  $> 65\%$  [32]. According to earlier research, stallions with high and low fertility have been defined as those with fresh motility values of  $73 \pm 11\%$  and  $63 \pm 17\%$ , respectively [33]. All predicted motility values within the current study were below the threshold for both high and low fertility individuals, raising concern over the fertilizing capabilities of the current and future equine population. Given this study is retrospectively assessed, pregnancy data were not analyzed but future work should assess this association. Given the lack of standardization across the industry, accepted thresholds for stallion semen quality parameters including motility make it challenging to predict the true implications of values presented within this study. While findings show an overall decline in motility, from 2009 this decline was substantial and the 20.90% drop over 11 years presents a distinct cause of concern. The reason behind this increased drop is unknown. Research in other species suggests external factors to be a cause as the observed change is too sudden to be a result of genetic mechanisms [6]. Our hypothesis that sperm motility will have declined over time is supported from these findings.



**Figure 1:** Time trends in mean sperm motility parameters between 2001 and 2020. (a) Sperm motility across all samples (%); (b) Sperm motility, age restricted (%). Each point represents the mean predicted value for that year. Error bars =  $\pm 1$  SEM. The black line in denotes regression slope (simple linear) of predicted means (a). (b) Green points denote 'prime ages' and purple points denote ages classed as 'senescent.' Graphs produced on GraphPad Prism version 9.0, GraphPad Software, California, CA, USA. The equation  $y = mx + c$  determined overall trends; (a)  $y = -0.6421X + 1339$  (2001–2020; graphically plotted),  $y = -2.093X + 4261$  (2009 – 2019); (b)  $y = -0.7553X + 1572$  (prime);  $y = -1.270X + 2605$  (senescent).



**Figure 2:** Time trends in mean ejaculate volume between 2001 and 2020. **(a)** Ejaculate volume across all samples (mL) **(b)** Ejaculate volume, age restricted (mL). Each point represents the mean predicted value for that year. **(b)** Green points denote 'prime ages' and purple points denote ages classed as 'senescent.' Error bars =  $\pm 1$  SEM. The black line denotes the regression slope (simple linear) of predicted means **(a)**. Graphs produced on GraphPad Prism version 9.0, GraphPad Software, California, CA, USA. The equation  $y = mx + c$  determined overall trends; **(a)**  $y = 0.3003X - 563.4$  **(b)**  $y = 0.5329X - 1028$  (prime);  $y = -0.9925X + 2032$  (senescent).

Research presented here is comparable to the canine sentinel model, which also found declining motility over time in fresh sperm samples. A yearly decline of 1.2% was reported in a UK population of dogs between 1988 and 2014 [6]. Semen quality declines in carnivorous and omnivorous species were considered more prominent compared to herbivorous populations. In human populations, yearly declines in motility are reported between 0.66% and 1.37% [34,35] subject to geographical location.

When considering the method of analysis of motility within the current study, the parameter was assessed utilizing subjective microscopy. While standardization in training for semen analysis was carried out across the study period, subjective analysis methods could introduce a level of variability into the readings provided, a limitation of the current study. Employing a computer-assisted sperm analysis-based approach with standardized settings could have accounted for this potential confounding factor; however, this was outside the scope of this retrospective study. Advancements in semen collection and treatment methods, such as the use of specific semen extenders, may have impacted the results presented, and it is noted that this, in addition to advancing analysis methods, is an inherent limitation of analyzing semen quality trends across time.

Poor semen quality can have significant implications for the economic value of sires within the breeding industry and the ability to maintain desirable heritable traits in the gene pool [14]. In certain equine breeds such as the thoroughbred, semen quality may be at risk of the effects associated with inbreeding, given industry selection pressures behind performance and conformation [36]. While breed was factored into the statistical model, including five breed categories based on 71 individual breeds, inbreeding was not directly investigated here. Further research analyzing semen quality trends in stallions accounting for differential inbreeding coefficients is suggested, to determine to what extent this factor could influence the trends presented. While etiological causes of declines remain to be determined, the adverse motility trends

reported here raise substantial concern over the reproductive health and breeding potential of stallions.

The quality assurance and consistency in semen volume analysis is reassuring, indicating that over the past two decades semen volume has increased, rejecting our original hypothesis that semen volume would have decreased over time. Of note, however, is that the artificial insemination referencing range in the equine industry is recommended at 60 to 120 mL [13]. All predicted means for volume fell below the lower bracket of this threshold value. Suboptimal semen volumes, as assessed by volume and not weight, have been reported previously in equine studies [12,13]. Employing the concept that 1 g is equivalent to 1 mL, as undertaken within this report [22], results, together with prior publications, could indicate that reproductive aberrations resulting in low volume exist in the wider equine population. While volume is not a direct measure of testicular function, in humans, low volume can be an indication of androgen deficiency, obstruction to the ejaculatory duct, or poor development of the seminal vesicles [29]; all reflective of poor reproductive health. Given the findings in human studies, low volume could therefore be concerning for equine fertility; however, this remains to be investigated. Continual monitoring of semen volume is required to maintain a current understanding of trends within this parameter. Given that semen volume is impacted by a number of collection factors, research must standardize collections where possible and include a range of covariates within analyses to produce robust indications of trends in semen volume.

While this study included many variables within the statistical approach, there are some inherent limitations. The time of collection and length of time required for the collection of an ejaculate was not collated. In bulls, it is reported that a greater amount of ejaculate is obtained following increased teasing and morning collections [37]. Furthermore, regarding ejaculate collection, a filter approach was undertaken for collection rather than an open-ended artificial vagina. This does mean that it could have been possible for the gel proportion of the ejaculate to mix with the sperm-rich portion, impacting motility. Within this study, we refer to

ejaculate volume; however, this was obtained by weighing the samples. As this study was performed retrospectively, we did not assess the specific gravity of equine sperm. The direct relationship between weight and volume hinges on the density of the semen being 1 g/mL [38]. Human semen studies report that weight should be an accurate index of volume.

While there are limitations and areas for further study, this comprehensive retrospective cohort study provides fundamental data on temporal trends in sperm motility and semen volume specific to a UK based equine population. Given the high economic importance of stallion fertility, the findings from this study are concerning as sperm motility and ejaculate volume were below recommended industry thresholds. The reproductive histories of the stallions used here are unknown, but it must be acknowledged that poor reproductive function could have significant industry implications, influencing the economic status of breeding stock. Reduced fertility potential is likely to result in additional costs associated with managing stallions with poor semen quality, such as the need for an increased number of collections, coverings, and inseminations required to achieve a successful pregnancy. It is the responsibility of the equine breeding sector to implement practices to optimize semen quality, including integrating fertility into selective breeding programs, enhancing standardization of analysis, and investing in further research determining the effects of external factors upon equine reproductive health and function.

### Supplementary Materials

Data on sperm concentration can be found in the [supplementary materials](#).

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### Authors' Contributions

Conceptualization, I.T.H., R.N.B., K.N., and A.P.; Methodology, I.T.H., R.N.B., and P.H.; Formal analysis, I.T.H., R.N.B., and D.S.G.; Data curation, I.T.H. and R.N.B.; Writing—original draft preparation, I.T.H.; Writing—review and editing, I.T.H. and R.N.B.; Supervision, R.N.B., K.N., and A.P. All authors have read and agreed to the published version of the manuscript.

### Data Availability

The raw data supporting the conclusions of this article will be made available by the authors on request.

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### Conflicts of Interest

The authors declare no conflicts of interest.

### Ethical Approval

The study was approved by the Hartpury University Ethics Committee (ETHICS2019-52, 17<sup>th</sup> March 2020).

## References

- [1] Tiegs AW, Landis J, Garrido N, Scott RT, Hotaling JM. Total motile sperm count trend over time: evaluation of semen analyses from 119,972 men from subfertile couples. *Urology* 2019;132:109–16. <https://doi.org/10.1016/j.urology.2019.06.038>.
- [2] Rolland M, Le Moal J, Wagner V, Royère D, De Mouzon J. Decline in semen concentration and morphology in a sample of 26,609 men close to general population between 1989 and 2005 in France. *Human Reproduction* 2013;28:462–70. <https://doi.org/10.1093/humrep/des415>.
- [3] Adiga S, Jayaraman V, Kalthur G, Upadhy D, Kumar P. Declining semen quality among south Indian infertile men: A retrospective study. *Journal of Human Reproductive Sciences* 2008;1:15–8. <https://doi.org/10.4103/0974-1208.38972>.
- [4] Splingart C, Frapsauce C, Veau S, Barthélémy C, Royère D, Guérif F. Semen variation in a population of fertile donors: evaluation in a French centre over a 34-year period. *International Journal of Andrology* 2012;35:467–74. <https://doi.org/10.1111/j.1365-2605.2011.01229.x>.
- [5] Wahl RL, Reif JS. Temporal trends in bull semen quality: A comparative model for human health? *Environmental Research* 2009;109:273–80. <https://doi.org/10.1016/j.envres.2008.10.012>.
- [6] Lea RG, Byers AS, Sumner RN, Rhind SM, Zhang Z, Freeman SL, *et al.* Environmental chemicals impact dog semen quality in vitro and may be associated with a temporal decline in sperm motility and increased cryptorchidism. *Scientific Reports* 2016;6:31281–31281. <https://doi.org/10.1038/srep31281>.
- [7] Maziero RRD, Guaitolini CR de F, Guasti PN, Monteiro GA, Martin I, Silva JPM da, *et al.* Effect of using two cryopreservation methods on viability and fertility of frozen stallion sperm. *Journal of Equine Veterinary Science* 2019;72:37–40. <https://doi.org/10.1016/j.jevs.2018.10.008>.
- [8] Varner DD, Gibb Z, Aitken RJ. Stallion fertility: a focus on the spermatozoon. *Equine Veterinary Journal* 2015;47:16–24. <https://doi.org/10.1111/evj.12308>.
- [9] Harris IT, Maddock C, Farnworth M, Nankervis K, Perrett J, Pyatt AZ, *et al.* Temporal trends in equine sperm progressive motility: a systematic review and meta-regression. *Reproduction* 2023;165:M1–10. <https://doi.org/10.1530/REP-22-0490>.
- [10] Fisch H. Declining worldwide sperm counts: disproving a myth. *Urologic Clinics of North America* 2008;35:137–46. <https://doi.org/10.1016/j.ucl.2008.01.001>.
- [11] Pacey AA. Are sperm counts declining? Or did we just change our spectacles? *Asian Journal of Andrology* 2013;15:187–90. <https://doi.org/10.1038/aja.2012.165>.
- [12] Multigner L, Magistrini M, Ducot B, Spira A. Secular sperm trends in stallions between 1981 and 1996. *Journal of Andrology* 1999;20:763–8. <https://doi.org/10.1002/j.1939-4640.1999.tb03383.x>.
- [13] Wilson M, Flesner AT. A preliminary comparison of semen quality between competing and non-competing equine Stallions. *Journal of Veterinary Science & Technology* 2017;08. <https://doi.org/10.4172/2157-7579.1000443>.

- [14] Darr CR, Moraes LE, Scanlan TN, Baumber-Skaife J, Loomis PR, Cortopassi GA, *et al.* Sperm mitochondrial function is affected by stallion age and predicts post-thaw motility. *Journal of Equine Veterinary Science* 2017;50:52–61. <https://doi.org/10.1016/j.jevs.2016.10.015>.
- [15] Piro Santo Y, Valera M, Molina A, Dorado J, Demyda-Peyrás S. 23 sperm quality of pure Spanish Stallions is affected by inbreeding coefficient and age. *Reproduction, Fertility and Development* 2020;32:137. <https://doi.org/10.1071/rdv32n2ab23>.
- [16] Gottschalk M, Sieme H, Martinsson G, Distl O. Heritability of semen traits in German warmblood stallions. *Animal Reproduction Science* 2016;170:10–4. <https://doi.org/10.1016/j.anireprosci.2016.03.004>.
- [17] Wilson M, Williams J, Montrose VT, Williams J. Variance in stallion semen quality among equestrian sporting disciplines and competition levels. *Animals (Basel)* 2019;9:485. <https://doi.org/10.3390/ani9080485>.
- [18] Aurich C. Reprint of: Seasonal influences on cooled-shipped and frozen-thawed stallion semen. *Journal of Equine Veterinary Science* 2016;43:S1–5. <https://doi.org/10.1016/j.jevs.2016.07.007>.
- [19] Albrizio M, Lacalandra GM, Volpe S, Nicassio M, Cinone M. Heat shock proteins in equine spermatozoa: expression and correlation to kinetic and environmental parameters. *Theriogenology* 2020;155:185–96. <https://doi.org/10.1016/j.theriogenology.2020.05.042>.
- [20] Sieme H, Katila T, Klug E. Effect of semen collection practices on sperm characteristics before and after storage and on fertility of stallions. *Theriogenology* 2004;61:769–84. [https://doi.org/10.1016/s0093-691x\(03\)00251-6](https://doi.org/10.1016/s0093-691x(03)00251-6).
- [21] Brinsko SP, Varner DD, Love CC, Blanchard TL, Day BC, Wilson ME. Effect of feeding a DHA-enriched nutraceutical on the quality of fresh, cooled and frozen stallion semen. *Theriogenology* 2005;63:1519–27. <https://doi.org/10.1016/j.theriogenology.2004.07.010>.
- [22] Whigham A, Blanchard T, Love C, Teague S, Brinsko S, Welsh T, *et al.* Equine semen quality following sperm exposure to seminal plasma stored under different conditions. *Clinical Theriogenology* 2014;6:459–66.
- [23] Ebel F, Vallejos A, Gajardo G, Ulloa O, Clavel E, Enric J. Semen quality and freezability analysis during breeding and non-breeding seasons in heavy draft stallions in southern Chile. *Andrologia* 2020;52:1–7. <https://doi.org/10.1111/and.13797>.
- [24] Ellerbrock R, Canisso I, Feijo L, Lima F, Shipley C, Kline K. Diagnosis and effects of urine contamination in cooled-extended stallion semen. *Theriogenology* 2016;85:1219–24. <https://doi.org/10.1016/j.theriogenology.2015.12.002>.
- [25] Turner CE, Walbornn SR, Blanchard TL, Varner DD, Brinsko SP, LaCaze KA, *et al.* The effect of two levels of hemospermia on stallion fertility. *Theriogenology* 2016;86:1399–402. <https://doi.org/10.1016/j.theriogenology.2016.04.084>.
- [26] Curran-Everett D, Benos DJ. Guidelines for reporting statistics in journals published by the American Physiological Society. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 2004;287:R247–9. <https://doi.org/10.1152/ajpregu.00346.2004>.
- [27] Verbyla AP. A note on model selection using information criteria for general linear models estimated using REML. *Australian & New Zealand Journal of Statistics* 2019;61:39–50. <https://doi.org/10.1111/anzs.12254>.
- [28] Levine H, Jørgensen N, Martino-Andrade A, Mendiola J, Weksler-Derri D, Mindlis I, *et al.* Temporal trends in sperm count: a systematic review and meta-regression analysis. *Human Reproduction Update* 2017;23:646–59. <https://doi.org/10.1093/humupd/dmx022>.
- [29] Björndahl L, Kirkman Brown J. The sixth edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen: ensuring quality and standardization in basic examination of human ejaculates. *Fertility and Sterility* 2022;117:246–51. <https://doi.org/10.1016/j.fertnstert.2021.12.012>.
- [30] Varner DD. Approaches to breeding soundness examination and interpretation of results. *Journal of Equine Veterinary Science* 2016;43:S37–44. <https://doi.org/10.1016/j.jevs.2016.06.075>.
- [31] Love CC. Relationship between sperm motility, morphology and the fertility of stallions. *Theriogenology* 2011;76:547–57. <https://doi.org/10.1016/j.theriogenology.2011.03.007>.
- [32] Love CC, Noble JK, Standridge SA, Bearden CT, Blanchard TL, Varner DD, *et al.* The relationship between sperm quality in cool-shipped semen and embryo recovery rate in horses. *Theriogenology* 2015;84:1587–1593.e4. <https://doi.org/10.1016/j.theriogenology.2015.08.008>.
- [33] Jasko DJ, Little TV, Lein DH, Foote RH. Comparison of spermatozoal movement and semen characteristics with fertility in stallions: 64 cases (1987–1988). *Journal of the American Veterinary Medical Association* 1992;200:979–85. <https://doi.org/10.2460/javma.1992.200.07.979>.
- [34] Wang L, Zhang L, Song X-H, Zhang H-B, Xu C-Y, Chen Z-J. Decline of semen quality among Chinese sperm bank donors within 7 years (2008–2014). *Asian Journal of Andrology* 2017;19:521–5. <https://doi.org/10.4103/1008-682X.179533>.
- [35] Mínguez-Alarcón L, Williams PL, Chiu Y-H, Gaskins AJ, Nassan FL, Dadd R, *et al.* Secular trends in semen parameters among men attending a fertility center between 2000 and 2017: Identifying potential predictors. *Environment International* 2018;121:1297–303. <https://doi.org/10.1016/j.envint.2018.10.052>.
- [36] Dini P, Bartels T, Revah I, Claes AN, Stout TAE, Daels P. A retrospective study on semen quality parameters from four different Dutch horse breeds with different levels of inbreeding. *Theriogenology* 2020;157:18–23. <https://doi.org/10.1016/j.theriogenology.2020.07.017>.
- [37] Umesiobi DO, Iloje M. Effect of sexual teasing and diurnal period of semen collection on reaction time and semen characteristics of Large White boars. *Journal of Sustainable Agriculture and the Environment* 1999;1:231–5.
- [38] Matson PL, Myssowski K, Yovich S, Morrison L, Irving J, Bakos HW. The density of human semen and the validation of weight as an indicator of volume: a multicentre study. *Reproductive Biology* 2010;10:141–53. [https://doi.org/10.1016/s1642-431x\(12\)60056-4](https://doi.org/10.1016/s1642-431x(12)60056-4).

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