



ELSEVIER

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Modernizing the stallion breeding soundness evaluation

K.M. Janson Whitesell*, S.M. McDonnell, R.M. Turner

Section of Reproduction and Behavior, Department of Clinical Studies, University of Pennsylvania New Bolton Center, 382 West Street Road, Kennett Square, PA 19348, USA

1. Introduction

Breeding Soundness Evaluation (BSE) of stallions was formally introduced in 1975. [1] Classification of a stallion as a Satisfactory Prospective Breeder requires a minimum of one billion progressively motile, morphologically normal sperm (PMMNS) in the second of two ejaculates collected one hour apart. Since 1975, major technological advancements have been introduced to improve objectivity and accuracy when evaluating sperm concentration, motility, and morphology. [2] These include fluorescence-based measures of sperm concentration, computer-assisted sperm motility analysis (CASA), and differential interference contrast (DIC) microscopy for evaluation of sperm morphology. However, little work has addressed how these advanced techniques affect stallion BSE classification or how criteria should be adjusted to account for obvious differences in estimates of various contributing semen measures. Our primary objective is to compare BSE classifications of stallions evaluated with 1975-era (traditional) semen evaluation technology with classifications using current advanced technology. Our hypothesis is that application of fluorescent sperm concentration analysis, CASA and DIC optics to stallion semen evaluation results in a more critical analysis of the number of PMMNS, such that a smaller percentage of stallions meet the traditional standards for classification as a Satisfactory Prospective Breeder. Pending these results, a secondary objective was to compare end-of-season fertility data to the BSE results using current, advanced technology with a goal of refining the criteria for classification as a Satisfactory Prospective Breeder to more accurately reflect fertility. Our second hypothesis is that the traditional minimum of 1 billion PMMNS should be lowered to accurately classify a stallion as Satisfactory or Not Satisfactory (Questionable or Unsatisfactory) when current advanced technology is used.

2. Materials and methods

A traditional BSE was performed for sixteen stallions with predicted books of 20 – 80 mares. [1] Semen analysis was performed in triplicate using either traditional or advanced methods of semen analysis with either CASA1 [3] or CASA2 [4] settings. Traditional methods included determination of sperm concentration by Densimeter® (Animal Reproduction Systems, Chino, CA), and use of phase contrast optics for visual estimation of total and progressive sperm motilities and percentage of morphologically normal and abnormal sperm. Advanced methods included determination of sperm concentration by Nucleocounter® NC-100™ (Chemometec, Denmark), use of CASA (IVOS, Hamilton-Thorne, Inc., USA) for assessment of total and progressive sperm motilities and use of DIC optics for visual estimation of percentage morphologically normal and abnormal sperm. Different sets of CASA settings were used for each semen analysis to determine the effects that each of these widely used settings might have on classification. Testicular measurements were obtained using ultrasonography and calipers. Testicular volume was calculated as described by Love et al. [5]

A stallion was classified as a 'Satisfactory Prospective Breeder' if it 1) produced a minimum of 1 billion PMMNS in the second of two ejaculates collected one hour apart and 2) had two normal testicles with a minimum total scrotal width of 8.0 centimeters and 3) produced total sperm numbers normal for its testicular volume. [5] A stallion was classified as Not Satisfactory if it failed to meet any of these 3 criteria. Values obtained using traditional semen analysis methods were compared to those obtained using each of the two advanced methods to determine if there were significant differences among the values measured. Additionally, the proportions of stallions classified as Satisfactory or Not Satisfactory was compared among the methods. Values are reported as mean \pm standard deviation.

* Presenting author

3. Results

Table 1. Summarizes the classification results for the 16 stallions by each of the three methods. A smaller proportion of these 16 stallions were classified as Satisfactory by Advanced CASA1 than by either Traditional or Advanced CASA2 methods (Fisher's Exact Test, $p < 0.05$). Mean estimates of sperm concentration were significantly higher with the Densimeter® ($369 \pm 203 \times 10^6/\text{mL}$ and $208 \pm 83 \times 10^6/\text{mL}$) than with the Nucleocounter® ($336 \pm 187 \times 10^6/\text{mL}$ and $196 \pm 91 \times 10^6/\text{mL}$; one-tailed paired t-test, 15 df, $p < 0.05$) for ejaculates 1 and 2, respectively. As a result, calculated total sperm numbers were significantly higher using the Densimeter® estimates of concentration ($18.1 \pm 9.0 \times 10^9$ and $8.6 \pm 3.9 \times 10^9$) than using the Nucleocounter® estimates ($16.4 \pm 8.1 \times 10^9$ and $7.9 \pm 3.8 \times 10^9$; one-tailed paired t-test, 15 df, $p < 0.05$) for ejaculates 1 and 2, respectively. The percentage of morphologically normal sperm was significantly lower with DIC optics (64.4 ± 9.7 and 64.1 ± 14.5) than with phase contrast optics (72.1 ± 13.1 and 72.7 ± 11.9 ; one-tailed Wilcoxon Signed Rank Test, 15 df, $p < 0.05$) for ejaculates 1 and 2, respectively, largely due to an increase in the percentage of abnormal heads identified with DIC microscopy. Total motility (TMOT; %) for traditional (74.6 ± 14.3 and 78.1 ± 7.7) and CASA1 (74.5 ± 13.2 and 76.5 ± 13.3) were not different for either ejaculate 1 or ejaculate 2, respectively (two-tailed Wilcoxon Signed Rank Test, 15 df, $p > 0.05$). However, estimation of TMOT was significantly lower for CASA2 (70.1 ± 14.9 and 71.2 ± 13.2), than for either visual or CASA1 estimations for both ejaculates 1 and 2 (one-tailed Wilcoxon Signed Rank Test, 15 df, $p < 0.01$). Progressive motility (PMOT; %) for traditional (53.7 ± 19.4 and 56.9 ± 16.9), CASA1 (26.2 ± 13.5 and 29.4 ± 12.6), and CASA2 (41.7 ± 14.0 and 41.3 ± 10.3), all differed significantly from one other for both ejaculates 1 and 2 (one-tailed Wilcoxon Signed Rank Test, 15 df, $p < 0.01$). For both ejaculates, visually estimated PMOT was significantly higher than either of the CASA methods. Additionally, CASA2 settings resulted in significantly higher estimations of PMOT than did CASA1. As a result of the above differences, the number of PMMNS was significantly lower with both the CASA1 ($2.6 \pm 1.7 \times 10^9$ and $1.5 \pm 1.1 \times 10^9$) and CASA2 ($4.6 \pm 3.4 \times 10^9$ and $2.1 \pm 1.4 \times 10^9$) methods for ejaculates 1 and 2, respectively compared to traditional methods ($7.2 \pm 4.8 \times 10^9$ and $3.8 \pm 2.4 \times 10^9$; one-tailed paired t-test, 15 df, $p < 0.01$). Lowest numbers resulted from the

Table 1
Stallion Classification

Analysis Method	Satisfactory	Not Satisfactory
Traditional	13/16	3/16
Advanced CASA1	7/16	9/16
Advanced CASA2	13/16	3/16

combination of the Nucleocounter®, DIC optics, and CASA1 settings.

4. Discussion

Previous work has shown that the percentage of total motile sperm is more highly correlated with fertility than is the percentage of progressively motile sperm. [5] Our data support estimation of total motility as a more reliable and (based on its similarity to CASA measures), a more precise measurement, particularly when only visual estimation of motility can be performed. In this regard, the number of total motile, morphologically normal sperm may be more predictive of fertility than the number of progressively motile morphologically normal sperm. The significant differences in the estimated number of PMMNS among all three methods suggest that method-specific classification criteria should be established and validated.

In summary, these results support the hypothesis that advanced semen analysis techniques are more stringent than traditional techniques, likely resulting in fewer stallions classified as Satisfactory Prospective Breeders when applying the traditional cutoff value of 1 billion PMMNS. End-of-season fertility data, when available, will be used to evaluate seasonal pregnancy rate, percent pregnant/cycle, and percent pregnant/first cycle to assess which of the three methods most accurately related to actual fertility of these 16 stallions. [5] These data then will be used to consider adjustment of the current classification criteria with a long-term goal of suggesting a set of industry standards that could be adopted to better standardize the BSE.

Acknowledgements

This work was funded by The Raymond Firestone Research Fund. Dr. Whitesell's travel was funded in part by the Dorothy Russell Havemeyer Foundation.

References

- [1] Kenney RM. Clinical fertility evaluation of the stallion. Proc Annu Meet Am Assoc Equine Pract 1975;21:336–55.
- [2] Varner DD. Developments in stallion semen evaluation. Therio 2008; 70:448–62.
- [3] Loomis PR, Graham JK. Commercial semen freezing: Individual male variation in cryosurvival and the response of stallion sperm to customized freezing protocols. Anim Reprod Sci 2008;105:119–28.
- [4] Waite JA, Love CC, Brinsko SP, Teague SR, Salazar Jr JL, Mancill SS, et al. Factors impacting equine sperm recovery rate and quality following cushioned centrifugation. Therio 2008;70:704–14.
- [5] Love CC, Garcia MC, Riera FR, Kenney RM. Evaluation of measures taken by ultrasonography and caliper to estimate testicular volume and predict daily sperm output in the stallion. J Reprod Fertil Suppl 1991;44:99–105.
- [6] Love CC. Relationship between sperm motility, morphology and the fertility of stallions. Therio 2011;76:547–57.