Measurement of Sperm Concentration in Stallion Ejaculates Using Photometric or Direct Sperm Enumeration Techniques

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A highly significant correlation was detected among photometric and direct sperm enumeration techniques for evaluating sperm concentration. Depending on the instrument used, the photometric techniques tended to overestimate or underestimate sperm concentration when compared to a hemacytometer standard. Precautions should be taken when using techniques other than a hemacytometer to measure sperm concentration, especially for stallions with large mare books where the ejaculate will be split among multiple mares for insemination. Authors' address: Department of Large Animal Medicine & Surgery, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4475.

1. Introduction

Accurate measurement of sperm concentration in ejaculates is important diagnostically because total sperm number is derived as a product of sperm concentration and semen volume. Imprecise estimation of sperm concentration produces a corresponding inaccurate calculation of total sperm number in an ejaculate. Erroneously high measures of sperm concentration could result in reduced pregnancy rates for stallions with large mare books, where ejaculates are split among multiple mares. Conversely, false-low measures of sperm concentration could lead to reduced breeding efficiency. An inaccurate measure in either direction could also result in impaired judgment regarding a stallion's breeding potential.

Sperm concentration in gel-free semen can be determined by using methods involving counts of individual spermatozoa, such as with a hemacytometer or a computerized spermatozoal analysis system; however, these methods of measurement are somewhat time-consuming or expensive. Alternatively, photometric techniques which have been calibrated with these direct sperm enumeration measurements are often used for quick measures of sperm concentration. Potential disadvantages of photometric measurements of sperm concentration include inaccurate estimates intrinsic to the systems, or false-high estimates of sperm concentration in ejaculates mixed with semen extenders or contaminated with cellular debris, blood, urine, purulent material, or premature germ cells.[1] Nonetheless, the ability to determine sperm concentration quickly and easily

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has prompted the development of several commercial photometric instruments for this purpose. This study compared measurement of sperm concentration in ejaculates using 4 different photometric techniques and 3 direct sperm enumeration techniques. Mean concentration of ejaculates measured with each system and the correlation between each photometric technique and each direct sperm enumeration technique are reported.

2. Materials and Methods

One hundred ejaculates (10 from each of 10 stallions) were collected using an artificial vagina[a] equipped with an in-line nylon micromesh filter[b] to permit collection of gel-free semen. Photometric determination of sperm concentration for each ejaculate (3 replicates × 100 ejaculates) was measured using each of 4 different commercially-available photometric systems.[c,d,e,f] Direct sperm enumeration techniques used in the study included: a hemacytometer (4 replicates × 100 ejaculates); a computerized spermatozoal-motility analyzer[g] in dark-field mode (2 replicates × 100 ejaculates); and a computerized system[h] using fluorescence imaging of non-motile sperm (2 replicates × 100 ejaculates). For measurements using the HTM IVOS computerized system, spermatozoa were loaded in disposable chambers with a 20-μm fixed depth.[i]

Spermatozoal concentration (10^6/ml) of each ejaculate was grouped into 1 of 5 concentration ranges based on the concentration determined using a hemacytometer. The concentration ranges were as follows: 1) 0–100 × 10^6/ml; 2) 101–150 × 10^6/ml; 3) 151–200 × 10^6/ml; 4) 201–300 × 10^6/ml; and 5) >300 × 10^6/ml. For each concentration range, the difference between the hemacytometer-derived value and the values derived from each of the other instruments were compared using a paired t test with $p < 0.05$ considered statistically significant. The repeatability of each technique within ejaculate was reported as a coefficient of variation. The correlation coefficients among the various techniques were also calculated.[j]

Table 1. Mean Values (±SD) for Coefficient of Variability (CV) for Spermatozoal Concentration of 100 Ejaculates Determined for 7 Sperm Enumeration Techniques

Sperm Enumeration System	CV
Hemacytometer	9.6 ± 6.0
IVOS—H33342	5.3 ± 4.3
IVOS—dark field	5.7 ± 4.9
Densimeter	3.6 ± 1.8
SpermaCue	3.0 ± 2.2
Micro-Reader 1	4.1 ± 3.9
Model 10 Sperm Counter	6.2 ± 4.3

Table 2. Correlation Matrix Listing Correlation Coefficients for Mean Sperm Concentration Measured Using 4 Photometric and 3 Direct Sperm Enumeration Techniques

	Hemacytometer	IVOS H33342	IVOS Dark Field	Densimeter	SpermaCue	Micro-Reader 1	Model 10 Sperm Counter
Hemacytometer	—	0.98	0.96	0.92	0.92	0.94	0.91
IVOS—H33342		—	0.95	0.93	0.92	0.94	0.92
IVOS—dark field			—	0.91	0.89	0.94	0.91
Densimeter				—	0.92	0.96	0.96
SpermaCue					—	0.95	0.93
Micro-Reader 1						—	0.97
Model 10 Sperm Counter							—

Table 3. Spermatozoal Concentration (10^6/ml) of 100 Stallion Ejaculates Measured Using a Hemacytometer and Divided into 5 Groups from Dilute (0–100 × 10^6/ml) to Concentrated (>300 × 10^6/ml)

Concentration (10^6/ml)	N	Hemacytometer	IVOS Dark Field	IVOS H33342	Densimeter	SpermaCue	Micro-Reader 1	Model 10 Sperm Counter
0–100	12	70	58 (−17%)*	51 (−27%)*	82 (+17%)*	88 (+26%)*	53 (−24%)*	57 (−19%)*
100–150	29	121	99 (−18%)*	88 (−27%)*	138 (+14)*	135 (+12%)*	110 (−9%)*	95 (−21%)*
150–200	29	177	147 (−17%)*	129 (−21%)*	193 (+9%)*	172 (−3%)*	169 (−5%)*	148 (−16%)*
200–300	13	249	222 (−11%)*	177 (−29%)*	266 (+7%)*	226 (−9%)*	245 (−2%)	204 (−18%)*
>300	17	404	327 (−19%)*	269 (−33%)*	441 (+9%)*	305 (−25%)*	368 (−9%)*	305 (−25%)*

Mean spermatozoal concentration in each group determined using the hemacytometer was compared with mean concentration determined using other sperm enumeration techniques and photometric techniques. Values in parenthesis represent the difference in the means.

N = number of ejaculates in group.

*$p < 0.05$ when comparing the difference in the means between the hemacytometer and the other techniques within each row.

3. Results

The hemacytometer yielded the largest coefficient of variation over all techniques tested (Table 1). The photometric techniques were highly correlated ($p < 0.0001$) with the direct sperm enumeration techniques (Table 2). The Densimeter yielded higher mean values for spermatozoal concentration than the hemacytometer for all concentration ranges. Conversely, the Micro-Reader 1 and the Model 10 Sperm Counter yielded lower mean values for spermatozoal concentration than the hemacytometer in all concentration ranges. Compared to the hemacytometer, the SpermaCue yielded higher mean values for concentration in more dilute ejaculates (up to 150×10^6/ml) and lower mean values for concentration in more concentrated ejaculates (Table 3).

4. Discussion

Although the hemacytometer is often regarded as the gold standard for determining spermatozoal concentration of ejaculates and is the system by which some of the photometric techniques are calibrated, the coefficient of variability was greatest for the hemacytometer. In instances where precise determination of spermatozoal concentration is desired, increasing the number of replicates is recommended. The HTM IVOS computerized analyzer consistently yielded lower measures of sperm concentration than the hemacytometer. It remains to be determined whether this difference may be related to variation in the depth of the hemacytometer chamber (10-μm depth) compared with the manufactured fixed depth (20 μm) of the disposable chambers used in this study. Further investigation is needed to determine whether computerized analysis or the traditional hemacytometer method yields more accurate results for estimating sperm concentration. As has been previously reported,[1] there was a tendency for greater disparity between the photometric techniques and the hemacytometer when sperm concentration in ejaculates was high or low. In summary, precautions should be taken when using techniques other than a hemacytometer to measure sperm concentration, especially for stallions with large mare books where the ejaculate will be split among multiple mares for insemination.

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Reference and Footnotes

1. Varner DD, Schumacher J, Blanchard TL, Johnson L. Breeding soundness examination. In: Pratt PW, ed. *Diseases and management of breeding stallions.* Goleta, CA: American Veterinary Publications, 1991;61–96.

[a]Missouri-model, Nasco, Ft. Atkinson, WI, USA.
[b]Animal Reproduction Systems, Chino, CA, USA.
[c]Densimeter Model 534B MOD 1; Animal Reproduction Systems, Chino, CA.
[d]SpermaCue; Minitube of America, Inc., Verona, WI.
[e]Model 10 Sperm Counter; Hamilton Research, Hamilton, MA.
[f]Micro-Reader I; I.M.V., Minneapolis, MN.
[g]HTM IVOS; Version 10.8; Hamilton Thorne Research, Beverly, MA.
[h]HTM IVOS, H33342; Hamilton Thorne Research, Beverly, MA.
[i]Standard Count; Spectrum Technologies, Healdsburg, CA.
[j]SAS/STAT™ Users Guide, Release 6.03 Edition. Cary, NC: SAS Institute Inc, 1988.