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Correlation of CASA Velocity and Linearity Parameters With Sperm Mobility Phenotype in Turkeys

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ABSTRACT: Since all domestic turkeys are produced through artificial insemination, a measurable sperm characteristic that would be predictive of fertility would allow for the culling of poor males, resulting in improved reproductive efficiency. The sperm mobility test (SMT), which quantifies sperm penetration into an Accudenz solution, has been shown to correlate highly with fertilization potential of individual turkeys. Since this sire-selection test is based on the differences in sperm mobility between whole ejaculates from individual males, the objective of this study was to determine whether specific sperm velocity parameters would correlate with the SMT and to determine whether these characteristics could account for phenotypic differences in sperm mobility observed between males. The SMT was used to rank males within a flock (n = 110) in triplicate and to classify them into high, average, and low sperm mobility phenotypes on the basis of the sperm mobility index. Several sperm velocity parameters were evaluated for each male by a computer-aided sperm analysis (CASA) system, the Hobson Sperm Tracker. The types of measurements taken of 200 sperm tracks/ejaculate included the following: curvilinear velocity (VCL), average path velocity (VAP), straight-line velocity (VSL), linearity (LIN), beat-cross frequency (BCF), and mean angular displacement (MAD). Significant positive correlations were found between VSL, LIN, BCF, and sperm mobility, and a significant negative correlation was seen between MAD and sperm mobility. Subpopulations of sperm that had penetrated the Accudenz solution were isolated from each mobility phenotype and were analyzed by CASA, and significant correlations were again observed between VSL, LIN, BCF, and sperm mobility. We conclude that sperm velocity and linearity contribute to overall sperm mobility phenotype and are important characteristics of turkey sperm function.

Key words: Motility, computer, spermatozoa.

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Sperm motility is a critical factor in the maintenance of fertility. In birds, the vaginal portion of the hen's oviduct regulates sperm entry (Steele and Wishart, 1992; Brillard, 1993), and only motile sperm are able to traverse the vagina and enter into the hen's sperm storage tubules (SST) (Allen and Grigg, 1958; Steele, 1992). The mechanisms behind such regulation are unknown at this time. SST are specialized structures found in the distal half of the oviduct of all avian species studied to date. These structures contain and store sperm, which are slowly released over time while the hen is in egg production to ensure a resident population of sperm at the site of fertilization (Bakst, 1993). Filling the SST with sperm helps ensure maximal fertility between inseminations. However, approximately 87% of inseminated sperm are expelled from the turkey hen's cloaca within 1 hour of artificial insemination (Howarth, 1971), and less than 1% of inseminated sperm are found inside the SST (Brillard and Bakst, 1990). This indicates that most of the inseminated sperm are lost from the hen and do not reach the SST. With proportionally few sperm entering the SST, the factors or traits that influence the sperm's ability to enter and fill the SST and subsequently fertilize eggs are significant.

One obvious factor critical to fertilization is sperm motility. Historically, it has been difficult to use sperm motility assessment as a predictor of fertility potential in poultry, possibly because of the subjectiveness of the methods used. However, an objective sperm motility assay, the sperm mobility test (SMT), has been developed in the rooster and modified for turkeys (Froman and McLean, 1996; Donoghue et al, 1998). The assay is performed at avian body temperature (41°C) and is a measure of the ability of sperm to penetrate into a dense solution of Accudenz. The concept of sperm mobility is meant to connote a directional forward progression of sperm, as opposed to general movement, described as motility. Sperm from males with a high–sperm mobility phenotype maintain higher fertility than do sperm from average- or low-mobility phenotype males (Froman and McLean, 1996; Froman et al, 1997; Donoghue et al, 1998; Froman and Feltmann, 1998; Froman et al, 1999). Individual males with low-mobility sperm were compromised in their fertilizing ability, as assessed by decreased sperm hydrolysis of the perivitelline layer (Donoghue et al,

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1999). Additionally, males with low-mobility sperm produced fewer poults following heterospermic inseminations with semen from high- and low-mobility or average- and low-mobility phenotype males (Donoghue et al, 1999). These results emphasize the interrelationship between sperm mobility, sperm storage in the hen, and fertility.

One of our interests is to determine the characteristics of individual mobile sperm that may account for phenotypic differences in sperm mobility observed in whole ejaculates. Computer-aided sperm analysis (CASA) technology has been used to investigate sperm motility parameters from several species: rats (Moore and Akhondi, 1996), rabbits (Farrell et al, 1993), boars (Holt et al, 1997), bulls (O'Connor et al, 1981; Amann, 1989), turkeys (Bakst and Cecil, 1992), and humans (Holt et al, 1985, 1989; Barlow et al, 1991; Davis et al, 1991; Liu et al, 1991; Barratt et al, 1993; MacLeod and Irvine, 1995). Significant correlations of CASA motility measurements with fertility have been found in most studies; however, it is not known whether CASA can be used to predict fertility in individual males.

Because the SMT is predictive of fertility in individual turkeys, the objectives of the current study were as follows: 1) to determine whether sperm velocity measurements obtained from individual sperm by CASA would correlate with the sperm mobility phenotypes of ejaculates measured by the SMT, 2) to determine which specific sperm velocity parameters contribute to overall sperm mobility phenotype, and 3) to determine whether the velocity characteristics of the subpopulation of sperm that penetrate the Accudenz solution differ by phenotype.

Methods

Animals and Semen Collection

Male BUTA (British United Turkeys of America, Lewisburg, W Va) Big 6 turkeys (*Meleagris gallopavo*), aged 30 to 56 weeks, were used in this study. Animals were maintained on a 14:10 light:dark photoperiod and housed in pens in groups of 8 to 10. Feed and water was provided ad libitum. Semen was collected from individual males (n = 110) by the abdominal massage method (Burrows and Quinn, 1935). Males were prescreened prior to use, and those who gave little or no semen or who had thin or yellow semen were removed from the study. Semen was used in a split-ejaculate study to compare the Sperm Mobility Test with CASA (multiple parameters), using 3 ejaculates per male as a repeated measure. Ejaculates were collected within a 15-day time frame.

Sperm Mobility Test

The Sperm Mobility Test (SMT) was performed as described for roosters and modified for turkeys (Froman and McLean, 1996; Donoghue et al, 1998). Briefly, 1 aliquot of semen from each male was diluted to 1×10^9 sperm/mL with motility buffer (50 mM TES buffer, pH 7.4, 120 mM NaCl, 10 mM glucose, 2 mM $CaCl_2$). A 300 μL volume of diluted semen was layered upon 3 mL of 2% (wt/vol) Accudenz solution (Accurate Chemical and Scientific Corporation, Westbury, NY), density 1.01 g/mL, that had been prewarmed to 41°C. The sperm suspension was incubated for 5 minutes in a disposable cuvette in a 41°C waterbath, and the percentage of transmitted light was measured 1 minute after the cuvette was loaded into a Densimeter (Model 534B-Mod1, Animal Reproduction Systems, Chino, Calif). The sperm mobility index is expressed as 100 minus the percentage of transmitted light. Semen from each male was analyzed within 10 minutes of collection. Each semen sample was analyzed by replicate readings from 2 separate cuvettes, with an intrasample variation of 0.1–6%. Males were ranked within the flock after 3 assessments, and the high- and low-mobility phenotypes were each at least 1 standard deviation away from the mean (average) sperm mobility index of the flock.

Computer-Aided Sperm Analysis (CASA)

One aliquot of semen from each male was diluted to 25×10^6 sperm/mL with motility buffer and placed into an eppendorf tube in a heat block at 41°C for 5 minutes. Approximately 3 μL of each sample was then loaded onto each side of a 20-μm MicroCell chamber slide (Conception Technologies, San Diego, Calif). The slides were placed on a warmed (41°C) microscope stage, and a sperm motility analyzer (Hobson Sperm Tracker, Model 7V1B, Hobson Tracking Systems Ltd., Sheffield, United Kingdom) was utilized to record 100 sperm tracks from each side of the slide, for a total of 200 tracks per sample. Sample-tracking times averaged 1 minute per 100 sperm tracks, for a total of 2 minutes per sample. The sperm remained in the calibrated frame for at least 3 seconds (minimum track time) before their tracks were accepted. The search radius was 14.50 μm, the framing rate was 60 Hz, and a ×10 objective with phase 4 contrast was used for microscopy.

Motility parameters measured by CASA included the following: curvilinear velocity (VCL), velocity over the actual sperm track, which included all deviations of sperm head movement; average path velocity (VAP), velocity over a calculated, smoothed path; straight-line velocity (VSL), velocity over the straight-line distance between the beginning and end of the sperm track; linearity (LIN), the straight-line distance divided by the incremental deviations along the actual path; beat-cross frequency (BCF), the frequency with which the sperm crosses the smoothed path; and mean angular displacement (MAD), the time average of absolute values of the instantaneous turning angle of the sperm head along its curvilinear trajectory.

Sperm Subpopulation Studies

Semen from males of each mobility phenotype (5–6 males/phenotype) was analyzed in triplicate. Males were selected on the basis of extremeness and consistency of mobility phenotype. The SMT was initially performed as described, and then the top 2 mL of sperm suspension, representing the 0.3 mL of diluted semen and 1.7 mL Accudenz solution, were removed from the cuvette. This was done to ensure removal of all solution above the Densimeter's light beam. By removing this quantity of the

sperm suspension from the upper portion of the cuvette, only the mobile sperm that penetrated the Accudenz solution to the level of the light beam of the Densimeter and that contributed to the Mobility Index remained in the cuvette. This remaining sperm solution was mixed and diluted to approximately 5×10^9 sperm/mL (high- and average-mobility samples) or was used undiluted (low-mobility samples) and was analyzed by CASA as previously described to determine the velocity characteristics of the subpopulation of sperm that had penetrated the Accudenz solution.

Statistical Analysis

Data were reported as mean ± standard error. Normality of the data was assessed using the univariate procedure, with the Shapiro-Wilkes normality test. The data was analyzed by 1-way analysis of variance (ANOVA), with Fisher's LSD as the posttest (SAS Institute, 1985). Pearson's correlations were performed between the Sperm Mobility Index values and the CASA motility parameters using Prism software (GraphPad, San Diego, Calif). A P value of $<.05$ was considered statistically significant.

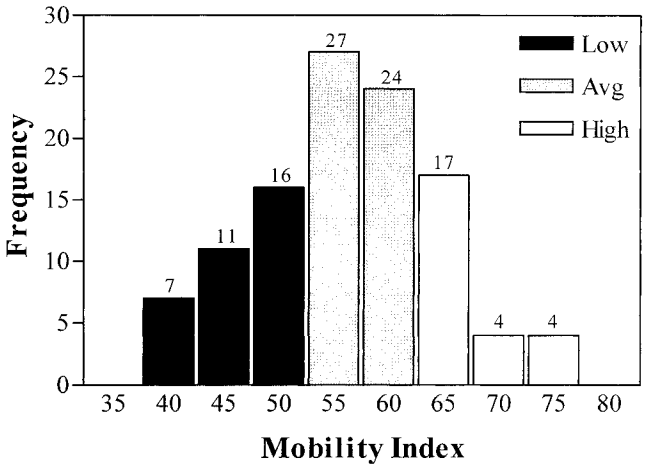

Figure 1. Frequency distribution of turkey sperm mobility phenotype. The label above each column indicates the number of males falling into each distribution category. The flock was divided into high-, average-, and low-mobility phenotypes as indicated.

Results

Sperm Mobility Test

The mean sperm mobility index for the entire flock was 56.9%, with a standard deviation of 8.9% and a coefficient of variation (CV) of 15.6%. Within a mobility class, the high-mobility males had an average mobility index of 69.7%, SD = 3.6%, and a CV = 5.1%. The average-mobility males had an average mobility index of 60.2%, SD = 3.0%, and CV = 5.0%. The low-mobility males had an average mobility index of 47.6%, SD = 5.0%, and a CV = 10.5%. The high–, average–, and low–sperm mobility phenotypes were normally distributed, with a W value of normality = 0.967 for the 110 males analyzed, as shown in Figure 1.

Computer-Assisted Sperm Analysis

There were significant positive correlations found between VSL, LIN, BCF, and sperm mobility of the whole population. For VSL, the Pearson's r value = 0.4075, $P < .0001$; for LIN, the Pearson's r value = 0.3411, $P < .0001$; and for BCF, the Pearson's r value = 0.3250, $P = .0002$. There was a significant negative correlation found between MAD and sperm mobility, with the Pearson's r value = −0.2060, $P = .0187$. These results are shown in Figure 2A through D.

When grouped by sperm mobility phenotype, the sperm motion parameters VSL, LIN, and BCF were significantly higher in the high-mobility group as compared with both the average- and low-mobility groups. The parameters VCL and VAP were significantly higher in the high-mobility group as compared with those of the low-mobility group. The sperm motion parameter MAD was significantly lower in the high-mobility group as compared with the low-mobility group. This data is presented in Table 1.

Sperm Subpopulation Studies

The concentration of sperm remaining in the cuvette after removal of the top 2 mL of solution was found to directly correlate with the mobility phenotype (Figure 3), with the Pearson's r value = 0.8803, $P < .0001$. Overall, the sperm velocity parameters were lower in the mobile sperm subpopulation studies because the sperm were analyzed in the 2% Accudenz solution, which is slightly more dense than the motility buffer normally used. However, significant differences between mobility phenotypes were still apparent.

Because this study was performed after the SMT and CASA studies, we determined the variation between mobility rankings and found that the Sperm Mobility Index values varied between 1–8% from previous rankings and that the males remained within their mobility class during the entire sampling period. Tom turkeys have been shown to maintain their sperm mobility phenotype for at least 5 months (Holsberger et al, 1998).

There was a significant positive correlation between VSL, LIN, BCF, and sperm mobility in the mobile sperm subpopulations recovered from the solution in the lower portion of the cuvette. For VSL, the Pearson's r value = 0.6384, $P = .0078$; for LIN, the Pearson's r value = 0.7261, $P = .0014$; and for BCF, the Pearson's r value = 0.8068, $P = .0002$. These results are shown in Figure 4A through C.

When grouped by sperm mobility phenotype, the motion parameters VSL, LIN, and BCF were significantly higher in the mobile sperm subpopulation from the high-mobility phenotype as compared with those from the av-

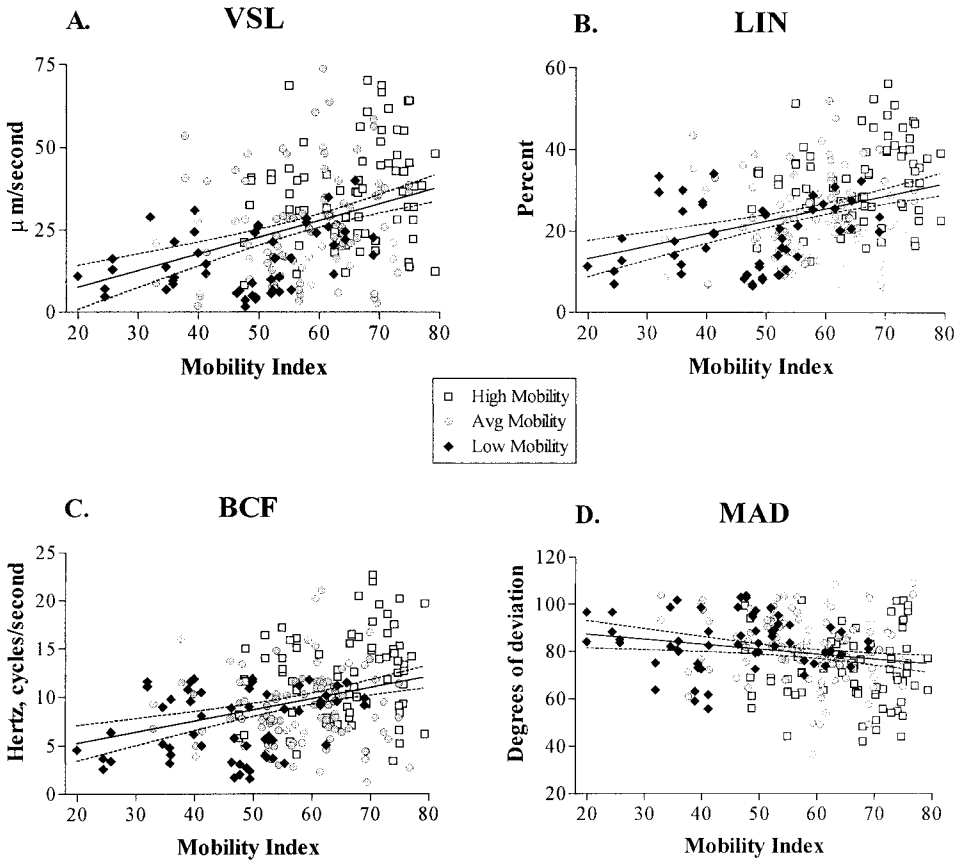

Figure 2. Computer-aided sperm analysis (CASA) parameters of individual turkey sperm from whole ejaculates. The velocity parameters of sperm classified as high, average, or low mobility by the Sperm Mobility Test is shown. Data points represent individual ejaculates, n = 31–67 per mobility group. The linear regression line is shown with the 95% confidence interval. **(A)** Straight-line velocity (VSL), which describes the velocity over the straight-line distance between the beginning and end of the sperm track. **(B)** Linearity (LIN), which describes the straight line distance divided by the incremental deviations along the actual sperm path. **(C)** Beat-cross frequency (BCF), which is the frequency with which the sperm crosses the smoothed path. **(D)** Mean angular displacement (MAD), which describes the time average of absolute values of the instantaneous turning angle of the sperm head along its curvilinear trajectory.

erage- and low-mobility phenotypes ($P < .01$). The motion parameter MAD was lower in the mobile sperm subpopulation from the high-mobility phenotype as compared with the mobile subpopulations from the average- and low-mobility phenotypes. The other velocity parameters, VCL and VAP, were not significantly different between mobile sperm subpopulations in any mobility phenotype. This data is presented in Table 2.

Discussion

The data presented here demonstrate that the sperm mobility phenotype can be attributed to specific sperm velocity parameters of individual sperm as determined by CASA. The motion parameters VSL, LIN, and BCF contribute to the overall sperm mobility phenotype in turkeys, as these were all significantly correlated with sperm mo-

Table 1. *CASA velocity parameters of turkey sperm grouped by mobility phenotype**

Mobility Phenotype	n	VCL, μm/s	VAP, μm/s	VSL, μm/s	LIN, %	BCF, Hz	MAD, degrees of deviation
High	25	94.5 ± 3.1[a]	49.9 ± 3.2[a]	32.6 ± 3.2[a]	29.3 ± 2.1[a]	11.4 ± 0.9[a]	74.1 ± 2.8[a]
Average	51	90.9 ± 2.2[ab]	44.0 ± 2.1[ab]	25.0 ± 2.1[b]	23.5 ± 1.4[b]	9.0 ± 0.6[b]	81.1 ± 1.8[ab]
Low	34	86.0 ± 3.0[b]	41.5 ± 2.8[b]	22.0 ± 3.0[b]	22.1 ± 2.0[b]	8.2 ± 0.8[b]	82.4 ± 2.6[b]

* VCL indicates curvilinear velocity; VAP, average path velocity; VSL, straight line velocity; LIN, linearity; BCF, beat cross frequency; and MAD, mean angular displacement. Values are expressed as mean ± SEM. Columns with different letters are significantly different ($P < .05$).

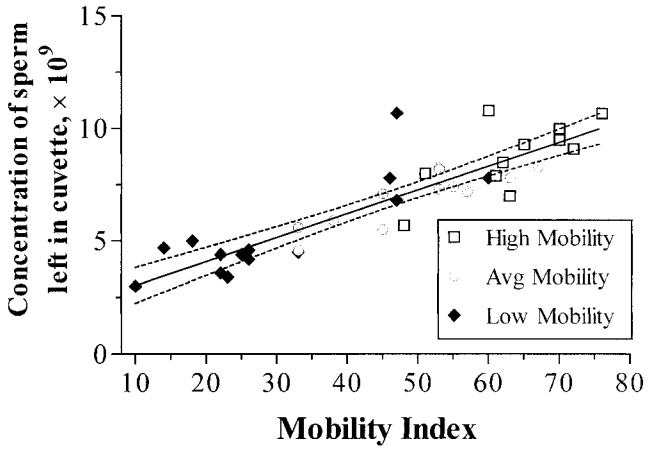

Figure 3. Correlation of turkey sperm mobility phenotype with concentration of mobile sperm subpopulation. The sperm mobility index (100 − %T), as determined by the sperm mobility test, was plotted against the concentration of mobile sperm remaining in the lower 1.3 mL (of 3.3 mL) of solution in the cuvette. The linear regression line is shown with the 95% confidence interval. Data points represent individual samples.

bility. The parameters VSL, VAP, VCL, LIN, and BCF were also significantly higher for the males classified as high mobility as compared with the low-mobility males. Because VSL, VAP, and VCL are all measures of sperm velocity over specified paths, this indicates that the sperm classified as high mobility swam faster than did those classified as lower mobility. The parameter LIN is a measure of linearity, and the BCF motion parameter indicates the number of times the sperm track crosses the smoothed path, both of which indicate linear progression. The MAD motion parameter is a measure of the deviation of the sperm path from linearity and was inversely related to sperm mobility phenotype. Thus, high-mobility sperm swim faster and straighter than did low-mobility sperm. This may be biologically significant because the sperm mobility phenotype, on the basis of research with whole ejaculates, is predictive of fertility (Froman and McLean, 1996; Froman et al, 1997; Donoghue et al, 1998, 1999; Froman and Feltmann, 1998; Froman et al, 1999). In the current study, we demonstrate that velocity characteristics of individual sperm within these ejaculates differ significantly and contribute to the phenotypes expressed.

We had originally anticipated that the mobile sperm subpopulations from each phenotype would not differ in velocity characteristics. Our assumption was that sperm ranked as low mobility by the SMT would merely contain a lower percentage of sperm that penetrated the Accudenz solution as compared with average- or high-mobility sperm and that the mobile sperm from all phenotypes would move with similar velocity parameters. However, this was not the case. Similar to that seen with whole populations of sperm, the CASA parameters VSL, LIN, and BCF were all positively correlated with sperm mo-

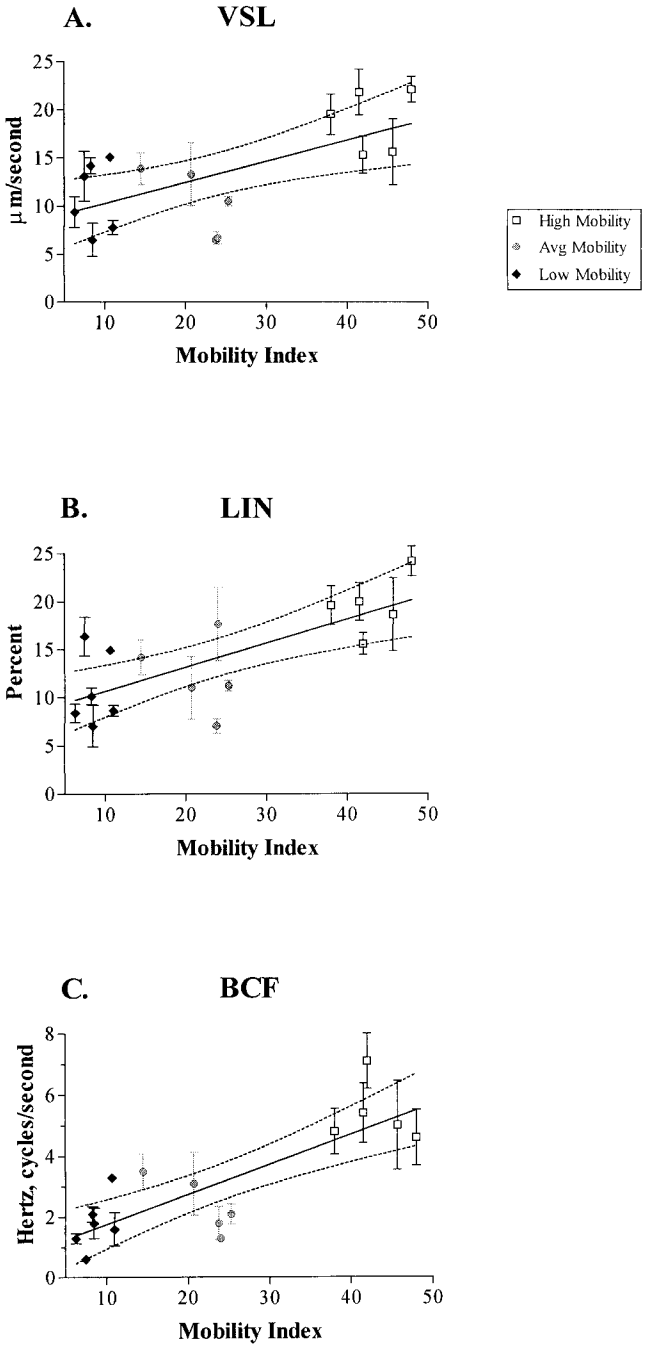

Figure 4. Computer-aided sperm analysis (CASA) parameters of individual turkey sperm from mobile sperm subpopulations. The velocity parameters of sperm classified as high, average, or low mobility by the sperm mobility test is shown. Data points represent mean ± SEM for 3 replicates, n = 5–6/mobility group. The linear regression line is shown with the 95% confidence interval. **(A)** Straight-line velocity (VSL), which describes the velocity over the straight-line distance between the beginning and end of the sperm track. **(B)** Linearity (LIN), which describes the straight-line distance divided by the incremental deviations along the actual sperm path. **(C)** Beat-cross frequency (BCF), which is the frequency with which the sperm crosses the smoothed path.

Table 2. *CASA velocity parameters of mobile turkey sperm subpopulations grouped by mobility phenotype**

Mobility Phenotype	n	VCL, μm/s	VAP, μm/s	VSL, μm/s	LIN, %	BCF, Hertz	MAD, degrees of deviation
High	5	96.8 ± 1.9[a]	44.6 ± 1.3[a]	18.7 ± 1.2[a]	19.5 ± 1.1[a]	5.3 ± 0.4[a]	71.3 ± 1.8[a]
Average	5	98.6 ± 2.4[a]	48.2 ± 2.0[a]	10.2 ± 1.1[b]	12.9 ± 1.5[b]	2.3 ± 0.3[b]	81.0 ± 2.5[b]
Low	6	99.8 ± 1.7[a]	48.8 ± 2.7[a]	10.2 ± 1.1[b]	11.1 ± 1.3[b]	1.7 ± 0.2[b]	79.9 ± 2.1[b]

* VCL indicates curvilinear velocity; VAP, average path velocity; VSL, straight line velocity; LIN, linearity; BCF, beat cross frequency; and MAD, mean angular displacement. Values are expressed as mean ± SEM. Columns with different subscripts are significantly different ($P < .05$).

bility in the mobile subpopulations. The motion parameters VSL, LIN, and BCF were significantly higher in the high-mobility subpopulations, as compared with the average- and low-mobility subpopulations. The MAD values of the mobile sperm in the high-mobility subpopulations were less than that of the average- or low-mobility subpopulations. Even the sperm that penetrated the Accudenz solution in the low-mobility samples proceeded at a slower rate and with more deviation from linearity.

Thus, the low-mobility phenotype is not characterized by a smaller percentage of mobile sperm but by sperm that move according to significantly different patterns overall. Males with low-mobility semen may contain a genetic variation that adversely affects sperm motility. Or there may be a defect in the pathway that controls or provides the energy for the interaction of the axonemal microtubules involved in sperm motility in these males. A recent study has shown that sperm mobility is significantly correlated with adenosine triphosphate (ATP) content of sperm in roosters and that rates of mitochondrial ATP synthesis vary according to sperm mobility phenotype (Froman and Feltmann, 1998). This suggests that sperm with low mobility may not generate adequate ATP for efficient movement.

Sperm velocity parameters, especially VSL, have been highly correlated with fertility in rats (Moore and Akhondi, 1996) and in boars (Holt et al, 1997). Differences between the VSL of sperm from fertile and subfertile men have also been reported (Liu et al, 1991; Leidl et al, 1993; Wainer et al, 1996). One study found no significant differences in VSL between fertile and subfertile roosters, but this was most likely due to the 30% difference in the percentage of motile sperm between the groups (McLean et al, 1997). In the current study, we found significant differences in VSL in both diluted ejaculates and in mobile sperm subpopulations. Sperm velocity and linearity appear to be important characteristics of turkey sperm function. Sperm that were highly mobile (as ranked by the SMT) also displayed increased velocity parameters and decreased deviation from linearity. This suggests that highly mobile sperm might be more efficient at traveling through the reproductive tract and reaching the site of sperm storage and/or fertilization.

In conclusion, the results from this study demonstrate that the sperm mobility phenotype is positively correlated to the sperm velocity parameters VSL, LIN, and BCF and is negatively correlated to MAD. The mobile subpopulations of turkey sperm from the different mobility phenotypes also displayed significant differences in the sperm velocity parameters VSL, LIN, and BCF, and MAD. Sperm velocity and linearity appear to be important determinants in mobility phenotype and may influence the ability of sperm to traverse the female reproductive tract, thereby affecting subsequent fertility.

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