ADAPTATION OF THE SPERM MOBILITY TEST FOR IDENTIFICATION OF TURKEY TOMS WITH LOW FERTILIZING POTENTIAL

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Primary Audience: Primary and Commercial Turkey Breeders, Researchers, Flock Supervisors

SUMMARY

Because nearly all the turkeys produced in the United States are the result of artificial insemination (AI), it is important to increase the efficiency of this labor-intensive practice. A semen evaluation test predictive of fertility would be extremely beneficial. Studies have shown that sperm mobility is predictive of fertility. The Sperm Mobility Test (SMT) objectively quantifies sperm mobility by measuring the density of sperm that swim through a solution of Accudenz at body temperature. This test results in a sperm mobility index score which is used to rank toms within a flock. Toms classified as having low sperm mobility have reduced fertility compared to those toms with high mobility. By using the SMT, it is possible to screen semen to determine which toms have the least chance of siring poult, then to cull them from a flock. This study attempted to determine if a reliable laboratory method for the analysis of turkey sperm mobility could be successfully modified for commercial field use in the turkey industry. Both the laboratory method and the proposed field methods yielded equivalent results. Consistently, toms classified with low mobility sperm could be identified utilizing any of the test methods evaluated. We conclude that the SMT is adaptable for on-farm use for the selection of potential sires.

Key words: Semen evaluation, sire selection, sperm, sperm motility, turkey
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DESCRIPTION OF PROBLEM

Significant advances have been achieved in the selection of turkeys for growth and body conformation. Although reproductive performance is critical to efficient production, suitable selection criteria for toms based on semen characteristics have not been identified. Ejaculates are generally evaluated for semen volume and color as well as for sperm viability and subjective sperm motility. Toms are culled if they produce yellow semen, a trait that has

1 The use of any trade names in this publication does not imply endorsement by the Agricultural Research Service, USDA.
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been linked with reduced fertility [1]. Toms are also removed for low semen volume or sperm concentration, and sometimes for low sperm viability [2]. However, no routine semen evaluation procedure that reveals the fertilizing potential of a tom is performed before toms participate in an artificial insemination (AI) program. This situation results in part from the nature of the tests: many of these assays are time consuming or technically difficult. In addition, most of these methods have not been adapted to large-scale application, a necessity for use on commercial farms. Furthermore, semen evaluation tests must be compatible with the existing tom breeder management procedures.

Our study relies on the use of an objective sperm mobility assay, which has recently been developed for the rooster and modified for turkeys [3, 4]. The Sperm Mobility Test (SMT) is designed to test the progressive swimming motion of sperm through a viscous solution at body temperature, similar to the hen’s reproductive tract. The SMT results in a sperm mobility index (100 – % transmission) used to rank toms within a flock. Average mobility semen is considered the mean sperm mobility index of the flock. Toms with High or Low mobility semen are defined as those with sperm mobility indexes one standard deviation above or below the mean, respectively. When toms were selected from a flock based on the upper and lower extremes of sperm mobility, fertility was positively correlated with the mobility phenotype [4]. Additionally, mobility of sperm from individual toms screened before the initiation of fertility trials remained consistent with the mobility phenotype throughout their reproductive lifetime [5].

The SMT is fairly simple to perform and consistently predicts the fertility of toms. However, commercial farms house thousands of breeder toms, and this method must be streamlined for practical use with large groups of toms. This study was designed to compare the reliable laboratory method for the analysis of turkey sperm mobility with simpler modifications. Specifically, we tested several variables: 1) altering the percentage of Accudenz (Accurate Chemical and Scientific Corporation, New York, NY) in the solution to maximize the differences between toms with high and low sperm mobility; 2) answering the question of whether a standard dilution of semen could be used instead of an exact sperm concentration, thereby removing several steps in the procedure; and 3) comparing different instruments to measure the sperm mobility index objectively.

**MATERIALS AND METHODS**

**STUDY 1**

*Effect of Accudenz Concentration on Turkey Sperm Mobility: The SMT for turkeys was originally validated using a 2% Accudenz solution. In order to maximize the differences between individual toms, ejaculates from toms that had been previously ranked and classified as High (one standard deviation above mean) were used to evaluate the effect of increasing Accudenz concentration on sperm mobility. This group of toms was used because sperm with High mobility progress the farthest through the Accudenz solution. Each male was tested twice, with n = 5.*

For all studies, males classified as having High sperm mobility represented toms with sperm mobility indexes one standard deviation above the mean (Average) sperm mobility of the flock. Toms with Low sperm mobility had sperm mobility indexes one standard deviation below the mean.

One liter of 6% Accudenz solution was made up by adding 60 g Accudenz powder to 100 mL TES-buffered KCl (1.15 g TES and 0.22 g KCl added to 1 L distilled water, pH = 7.5) and 840 mL motility buffer (11.46 g TES, 6.49 g NaCl, 4.51 g glucose, and 0.59 g CaCl2 added to 1 L distilled water, pH = 7.5, and osmolarity = 310-320 mmol/kg). To adjust the osmolarity, 50 mL of the solution was removed and replaced with 50 mL distilled water. Concentrations from 2 to 10% Accudenz solutions were made up by adding from 20 to 100 g Accudenz powder to 100 mL TES-buffered KCl and 880-800 mL motility buffer, respectively.

Three mL of Accudenz solution were added to each cuvette, and filled cuvettes were placed in a 41°C waterbath. Semen concentration was determined using an IMV Micro-Reader I (Minneapolis, MN), using an optical density of 381 nm. Semen was diluted to 1×10^5 sperm/mL using warm (41°C) motility buffer. To each cuvette, 300 μL of diluted sperm was carefully layered on top of the 3 mL warm (41°C) Accudenz. Cuvettes were incubated for 5 min in the waterbath. At the end of incubation, cuvettes were carefully removed.
from the waterbath, wiped dry, and read in the ARS Densimeter Model 534B Mod 1 (Chino, CA) using the motility program included with the densimeter.

After using from 2 to 10% Accudenz, the concentrations from 2 to 6% Accudenz were selected for further study based on at what point the mean sperm mobility index appeared to plateau. Sperm from toms that had been previously ranked as having High, Average, or Low mobility using 2% Accudenz were tested with Accudenz concentrations from 2 to 6% to determine if differences between the phenotypes would still be apparent. Each male was tested twice, with n = 4 to 8 per group.

STUDY 2

Comparison of a Standard 1:8 Dilution vs. Dilution by Semen Concentration in the Sperm Mobility Test: Determining sperm concentration is a time-consuming procedure and adds several steps to the SMT. We were interested in determining how a standard 1:8 dilution of ejaculates compared to using a known concentration of 1×10⁹ sperm/mL to identify low mobility toms in a flock. Using the optimized Accudenz concentration (determined in Study 1) of 6%, a flock of 81 toms was screened and classified as High, Average, or Low mobility phenotype. This study and portions of Study 3 were performed in the tom barn to test the feasibility of evaluating potential sires in a commercial production setting. Semen concentration from individual ejaculates was determined using an IMV Micro-Reader I as described in Study 1. A portion of the ejaculate was diluted to 1×10⁹ sperm/mL in warm (41°C) motility buffer. To evaluate a sample standardized on volume, 50 μL neat semen was added to 350 μL warm (41°C) motility buffer. The standardized and the known sperm concentration samples were incubated and evaluated concurrently, as described in Study 1.

STUDY 3

Comparison of Sperm Mobility Readings on the Densimeter vs. a Spectrophotometer: Since the ARS Densimeter was used in the initial development and validation of the SMT in chickens, we were interested in determining how universal the mobility index scores would be when using different instruments. The spectrophotometer has also been used to perform the SMT in roosters [3]. Semen from toms used in Studies 1 (n = 4 to 8 per group) and 2 (n = 81) was used to compare the ARS Densimeter and the IMV Micro-Reader in both the laboratory and the barn settings. In Study 1, the semen was tested in 2 to 6% Accudenz, read on the Densimeter, and then immediately read on the spectrophotometer. Performance of the ARS Densimeter and the IMV Micro-Reader were also compared when using the standard 1:8 semen dilution (Study 2).

STATISTICAL PROCEDURE

Data were analyzed within flock for deviations from Gaussian distribution using the Kolmogorov-Smirnov test. For the comparison of sperm mobility assessment methods, intraclass correlations were used. For comparison of instruments, Pearson's correlation coefficient with a two-tailed P-value was calculated because the instruments give different readings. Significance implies P < .05.

RESULTS AND DISCUSSION

Increasing the percentage of Accudenz in solution from 2 to 10% resulted in a linear decline in sperm mobility (Figure 1). Because the mean sperm mobility indexes for 7 to 10% Accudenz were on the lower end of the mobility index scale, Accudenz concentrations from 2 to 6% were used with the semen from High, Average, and Low mobility toms.

The most significant difference (P < .01) between High, Average, and Low mobility toms resulted from the 6% Accudenz (Figures 2 and 3), and was utilized for subsequent studies. This concentration of Accudenz is also used for evaluation of sperm mobility using New Hampshire [3] and broiler breeder [6] roosters.

Sperm mobility indexes obtained from 1×10⁹ sperm/mL compared to a standard 1:8 dilution of ejaculates correlated positively with both instruments. There was an 83.3% agreement between the two SMT methods on identification of the Low mobility males when using the Densimeter (Figure 4). When using the IMV Micro-Reader, there was an 80.0% agreement between the two SMT methods on identification of the Low mobility males (Figure 5).

When performing the SMT with 1×10⁹ sperm/mL, the Densimeter and the IMV correlated positively and significantly (P < .0001, R = 0.815, Figure 6). The overall
FIGURE 1. Sperm mobility of High mobility phenotype toms with increasing concentrations of Accudenz buffer. Toms were previously ranked by mobility phenotype with 2% Accudenz. Mobility was measured by the Sperm Mobility Index (100 - % transmitted light). Values are expressed as mean±SEM, n = 5.

Columns with different letters are significantly different (P < .05).

FIGURE 2. Sperm mobility of High, Average, and Low mobility phenotype toms with increasing concentrations of Accudenz buffer. Toms were previously ranked by mobility phenotype with 2% Accudenz. Mobility was measured by the Sperm Mobility Index (100 - % transmitted light), using the ARS Densimeter. Values are expressed as mean±SEM, n = 4 to 8 per group.

**Columns with different letters are significantly different (P < .05).**
FIGURE 3. Sperm mobility of High, Average, and Low mobility phenotype toms with increasing concentrations of Accudenz buffer. Toms were previously ranked by mobility phenotype with 2% Accudenz. Mobility was measured by absorbance, using the IMV Micro-Reader I. Values are expressed as mean±SEM, n = 4 to 8 per group. * Columns with different letters are significantly different (P < .05).

FIGURE 4. Correlation of $1 \times 10^9$ sperm/mL with a standard 1:8 dilution of semen in the Sperm Mobility Assay, using the ARS Densimeter. Mobility in both cases was measured by the Sperm Mobility Index (100 - % transmitted light). Each point represents one tom, n = 71. Open squares represent toms that fall into the Low mobility category using both methods.
FIGURE 5. Correlation of $1 \times 10^9$ sperm/mL with a standard 1:8 dilution of semen in the Sperm Mobility Assay, using the IMV Micro-Reader I. Mobility in both cases was measured by absorbance. Each point represents one tom, $n=71$. Open squares represent toms that fall into the Low mobility category using both methods.

FIGURE 6. Correlation of the ARS Densimeter and the IMV Micro-Reader I in the Sperm Mobility Assay, performed using $1 \times 10^9$ sperm/mL. Sperm Mobility Index ($100 - \%$ transmitted light) is plotted vs. absorbance. Each point represents one tom, $n=71$. Open squares represent toms that fall into the Low mobility category using both methods.
association between the two instruments was 81.5%. When using a standard 1:8 semen dilution, the Densimeter and the IMV again correlated positively and significantly (P < .0001, R = 0.949, Figure 7), with an overall association between the two instruments of 94.9%.

Variations in association between the two instruments may be due to the differences in light path position of the two instruments. The Densimeter light path is approximately 2.5 cm from the bottom of the cuvette, while the IMV light path is approximately 1 cm from the bottom. Thus, readings of high sperm density by the Densimeter may not be reflected as such by the IMV, where the sperm must travel farther down into the medium. However, the correlations are still excellent, especially in the lower mobility range.

These findings demonstrate that by using the modifications of the SMT described here (i.e., semen dilution, instrumentation), toms with low sperm mobility can be identified. This is the first functional test that quickly and easily detects males with low fertility potential. In order to screen a flock, High, Average, and Low sperm mobility must be defined. Low mobility was defined as one standard deviation below the mean mobility index of the flock. The modifications to the test resulted in agreement or association of 80% or greater for the identification of Low mobility toms. The other 20% of the toms were ranked in the lower range of Average, but less than one standard deviation below the mean. These "borderline" toms still represent the lower spectrum of toms in a flock. It is also important to note that these results were obtained with a flock of 81 toms. When these results are extrapolated to the larger flocks used in commercial settings, the differences between the sperm mobility groups will become even more obvious. Overall, these results describe a very simple method for culling the least fecund toms from a breeder flock. Since sperm mobility is a primary determinant of fertility [6], the Sperm Mobility Test is a means by which to identify and remove toms that do not contribute to progeny production. Toms could be ranked by sperm mobility early in production to maximize the fertility potential of a flock.

![Sperm Mobility Index vs. Absorbance](image)

**FIGURE 7.** Correlation of the ARS Densimeter and the IMV Micro-Reader I in the Sperm Mobility Assay, performed using a standard 1:8 dilution of semen. Sperm Mobility Index (100 - % transmitted light) is plotted vs. absorbance. Each point represents one tom, n = 71. Open squares represent toms that fall into the Low mobility category using both methods.
CONCLUSIONS AND APPLICATIONS

1. A 6% Accudenz solution used in the Sperm Mobility Test revealed the greatest differences between toms classified as having High or Low sperm mobility.

2. Since there was a significant agreement rate on identification of toms with Low sperm mobility when using $1 \times 10^9$ sperm/mL and a standard 1:8 dilution of semen, either method would be suitable for use. To simplify and increase the efficiency of the procedure, a standard 1:8 dilution of semen should be employed.

3. Both the ARS Densimeter and the IMV Micro-Reader perform well in the field and produce highly correlative sperm mobility results. Therefore, either instrument (and perhaps others not tested here) can be used in the Sperm Mobility Test.

4. The updated and improved Sperm Mobility Test described here is a simple and consistent procedure for identifying toms with decreased fecundity. It offers an easy method of sire selection in commercial settings.

REFERENCES AND NOTES


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