Objective Measurement of Sperm Motility Based Upon Sperm Penetration of Accudenz®

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ABSTRACT When a suspension of rooster sperm was overlaid upon 6% (wt/vol) Accudenz®, immotile sperm did not enter but motile sperm entered rapidly. The absorbance of the Accudenz® layer increased as a result. These phenomena were used to measure sperm motility objectively at body temperature. The intra-assay coefficient of variation (CV) was 2.6% (n = 3). When roosters (n = 36) were ejaculated repeatedly and sperm motility data analyzed by two-way ANOVA, a male effect was observed (P ≤ 0.001). When roosters were ranked by mean motility scores (n = 3 evaluations per male) and representative males selected as semen donors, a difference in fertility (P < 0.001) was observed between males characterized by minimal and maximal sperm motility. Frequency analysis with data from a second flock of roosters (n = 100) revealed a normal distribution. Roosters categorized by average sperm motility (n = 18) or sperm motility greater than one standard deviation above the mean (n = 17) were selected for further analysis by repeated measurements. A split-plot ANOVA revealed a difference between categories (P ≤ 0.0001) and variation among males within a category (P ≤ 0.0001). In contrast, sperm motility was independent of time and there was no interaction between category and time. Thereafter, five roosters from each group were ejaculated weekly and interassay CV estimated with semen pooled by category (n = 3 observations per category). During this interval, sperm motility of average roosters was 55 ± 5.9% of that of roosters within the high motility category. Interassay CV were 18.1 and 9.2% for roosters originally categorized by average and high sperm motility, respectively. The assay described has potential for: 1) selecting males based on sperm motility, and 2) standardizing the measurement of poultry sperm motility.

(Key words: Accudenz®, chicken, motility, poultry, sperm)

INTRODUCTION

The motility of poultry sperm has been measured objectively by four principal means: spectrophotometry, videomicroscopy, digital image analysis, and sperm movement from one medium into another. Wall and Boone (1973), Atherton et al. (1980), and Wishart and Ross (1985) described modifications of a spectrophotometric technique originally used by Timourian and Watchmaker (1970) for measuring the motility of sea urchin sperm. Spectrophotometry has been used predominantly to evaluate sperm motility in pooled semen samples (Wishart, 1984a,b; Ashizawa and Wishart, 1987, 1992; Wishart and Ashizawa, 1987; Thomson and Wishart, 1988, 1991; Ashizawa et al., 1989a,b, 1990; Froman and Thursam, 1994). In contrast, videomicroscopy has been applied principally towards the study of axonemal function (Ashizawa et al., 1989c, 1992a,b, 1993, 1994a,b; Ashizawa and Hori, 1990; Ashizawa and Sano, 1990). The limited use of digital image analysis (Bakst and Cecil, 1992a,b) is most likely attributable to the cost of instrumentation.

As reviewed recently by Suttiyotin and Thwaites (1993), sperm migration from one medium into another has been used as a measure of sperm motility for numerous mammalian species; however, this phenomenon has received little attention as a means of measuring the motility of poultry sperm (Birrenkott et al., 1977; McLean and Froman, 1996). The latter researchers sought a sperm attribute that would account for the subfertility of roosters homozygous for the rose comb allele. In comparison to fertile heterozygotes, subfertile homozygotes were characterized by decreased sperm motility when sperm suspensions from individual roosters were overlaid upon 6% (wt/vol) Accudenz®. Furthermore, sperm motility was measured at body temperature with standard laboratory equipment, the assay was rapid, and the results were easily interpreted and analyzed. Therefore, the objectives of the present research were to: 1) determine intra- and interassay
coefficients of variation for the sperm penetration test, 2) test for a difference in sperm motility among normal, fertile males, and 3) determine whether a cause and effect relationship could be demonstrated between in vitro sperm motility and fertility.

**MATERIALS AND METHODS**

**Intra-Assay Coefficient of Variation**

Four solutions were required for the sperm penetration assay. First, a 30% (wt/vol) stock solution of Accudenz® was prepared with 3 mM KCl containing 5 mM N-tris-[hydroxymethyl]methyl-2-amino-ethanesulfonic acid (TES), pH 7.4, as the solvent. Second, another TES-based buffer, henceforth designated as motility buffer, contained 111 mM NaCl, 25 mM glucose, and 4 mM CaCl₂ in 50 mM TES, pH 7.4. The osmolality of the motility buffer was 320 mmol/kg. Third, a portion of the motility buffer was diluted to 290 mmol/kg with deionized water. Fourth, a 6% (wt/vol) Accudenz® solution was prepared by diluting the stock solution with diluted motility buffer. The pH and osmolality of the 6% (wt/vol) Accudenz® solution were 7.35 pH units and 323 mmol/kg, respectively.

A 1.5-mL volume of the 6% (wt/vol) Accudenz® solution was pipetted into each of three polystyrene cuvettes held within a 41°C water bath. After the Accudenz® solution had reached thermal equilibrium, semen was procured from each of 10 New Hampshire roosters. Ejaculates were pooled and the semen thoroughly mixed. Sperm concentration was determined fluorometrically according to Bilgili and Renden (1984). Semen was diluted with motility buffer to a concentration of 5 × 10⁸ sperm per milliliter. At 3-min intervals, a 150-μL volume of sperm suspension was overlaid on Accudenz® in a cuvette. Each cuvette was removed from the water bath after a 5-min incubation and then placed within a spectrophotometer. Absorbance at 550 nm was recorded after a 1-min interval. This process was repeated for sperm that had been immobilized by heating at 56°C for 10 min. The intra-assay coefficient of variation (CV) was calculated and the intra-assay CV calculated as above.

Experiment 1 was replicated as follows. The bottom of each of three polystyrene cuvettes was perforated with a red-hot stainless steel probe. A 7-mm length of polyethylene capillary tubing (1.9 mm outer diameter) was attached to the cuvette with Silastic® Medical Adhesive so that the upper end of the tubing protruded 1 mm above the plane of the bottom of the cuvette. The adhesive was allowed to cure overnight. Prior to loading each cuvette with 6% (wt/vol) Accudenz® as above, the lower end of each capillary tube was sealed with a stainless steel sealing plug. Thereafter, cuvettes were placed in a 41°C water bath.

Semen was collected and manipulated as above. At 3-min intervals, a 100-μL volume of sperm suspension was overlaid on Accudenz® in a cuvette. After incubating for 10 min at 41°C, each cuvette was removed from the water bath, the stainless steel plug removed, and the Accudenz® layer collected into a 1.5-mL microcentrifuge tube. Sperm were concentrated by centrifugation at 15,600 × g for 1 min. Each supernatant was removed with a Pasteur pipet, a 40-μL volume of motility buffer was added to the microcentrifuge tube, the pelleted cells were resuspended, and the final volume of the sperm suspension recorded. Likewise, residual sperm suspensions were recovered from cuvettes, volumes recorded, and sperm concentrations measured. Sperm recovered from the Accudenz® layer were expressed as a percentage of the total sperm recovered from each cuvette. A mean percentage was calculated and the intra-assay CV calculated as above.

**Difference in Sperm Motility Among Males**

Repeated measurements were made on individually caged males as follows. Manual ejaculation of 48-wk-old New Hampshire roosters (n = 36) was initiated on an every-other-day basis. Roosters were ejaculated randomly on each of 3 consecutive semen collection d. The following steps were performed sequentially for each rooster. Immediately after ejaculation, sperm concentration was determined as above, the ejaculate diluted to 5 × 10⁸ sperm per milliliter with prewarmed motility buffer, a 300-μL volume of the sperm suspension overlaid upon 3 mL of prewarmed 6% (wt/vol) Accudenz® held in a polystyrene cuvette, the cuvette incubated for 5 min at 41°C, the cuvette placed within a photometer, and a reading made after a 1-min interval. Photometric data were analyzed by two-way ANOVA (Sokal and Rohlf, 1969a).

**Sperm Motility and Fertility**

Roosters were ranked according to their mean motility scores. A fertility trial was performed in which 50-wk-old Single Comb White Leghorn hens (n = 45 per treatment group) were inseminated with sperm obtained from roosters categorized as having minimal, average, or maximal sperm motility. Roosters within a category (n = 3) were manually ejaculated, their semen pooled, sperm concentration measured as above, and pooled semen extended to 5 × 10⁸ sperm per milliliter with motility buffer. Each hen was inseminated intravaginally with 5 × 10⁷ sperm. Egg collection, incubation, and data analysis were performed according to Kirby and Froman (1990).

This experiment was replicated as follows. The true difference in fertility between roosters categorized as having minimal or maximal sperm motility was assumed to be 5 percentage units based upon the results of the first fertility trial. The number of eggs per treatment group needed to detect this difference with 90% certainty at a
significance level of $\alpha = 0.05$ was calculated according to Sokal and Rohlf (1969b). Thus, only roosters categorized by maximal or minimal sperm motility were used as semen donors in the replicate fertility trial. Males within one category ($n = 3$) were manually ejaculated and their semen processed as above. Prior to insemination, sperm motility was measured as outlined in the first experiment using a 5-min incubation interval. Then, each of approximately 130 54-wk-old Leghorn hens was inseminated intravaginally with $5 \times 10^7$ sperm. Thereafter, this process was repeated for males within the second category and the hens constituting their corresponding treatment group. Egg collection, incubation, and data analysis were performed as above.

**Analysis of Males Categorized by Sperm Motility**

Repeated measurements were made on males categorized by sperm motility as follows. Males were selected from a second flock of New Hampshire roosters based upon a single measurement of sperm motility and frequency analysis. Individually caged 25-wk-old roosters ($n = 100$) were assigned randomly to be ejaculated on one of 3 consecutive d. Sperm motility was measured photometrically as described above. Data were analyzed by single classification ANOVA (Sokal and Rohlf, 1969c) in order to determine whether observations were independent of a time effect. The Kolmogorov-Smirnov test for goodness of fit was used to determine whether observed frequencies approximated a normal distribution (Sokal and Rohlf, 1969d).

Males were ranked by their sperm motility scores. Males with scores near average ($n = 18$) were categorized as average. Males with scores greater than one standard deviation above the mean ($n = 17$) were categorized as high sperm motility males. Manual ejaculation of categorized roosters was initiated on an every-other-day basis. Roosters were randomized by cage number, and sperm motility was measured photometrically on each of 3 d. Photometric data were analyzed by split-plot design ANOVA (Sokal and Rohlf, 1969e).

**Interassay Coefficient of Variation**

Five representative roosters were selected from each of two sperm motility categories. Each rooster was ejaculated on a weekly basis. Semen was pooled by category as roosters were ejaculated, and duplicate measurements of sperm motility were made by spectrophotometric analysis per pool per week. A different batch of reagents was used each week. Interassay CV were estimated from the sample means of each category.

**RESULTS**

**Intra-Assay Coefficient of Variation**

Sperm rendered immotile by heating to 56 C did not penetrate the Accudenz® layer. In contrast, motile sperm entered the Accudenz® layer rapidly and, as a consequence, absorbance increased as a function of time. A representative plot of absorbance vs time is shown in Figure 1. In preliminary experiments, we found the rate of sperm penetration to be most rapid during the initial 5 min of incubation. Likewise, once cuvettes were placed within the spectrophotometer, results were most consistent when measurements were made after a slight delay. We attributed this effect to physical stabilization of the Accudenz® layer. Therefore, we adopted a 5-min incubation interval and a 1-min delay between cuvette transfer and making a measurement as standard operating procedures. When these conditions were used with a sperm suspension derived from pooled semen containing $5 \times 10^8$ sperm per milliliter, the mean absorbance, standard deviation, and coefficient of variation ($n = 3$) were 0.9385, 0.0244, and 2.6%, respectively. When the repeatability of the assay was estimated in terms of the percentage of sperm recovered from the Accudenz® layer after 10 min of incubation at 41 C, the mean recovery and CV were 8.2% and 6.2%, respectively.

**Difference in Sperm Motility Among Males**

When sperm penetration into Accudenz® was measured with a photometer (Figure 2), a pattern comparable to that obtained with a spectrophotometer (Figure 1) was observed. When sperm motility was tested repeatedly for

![FIGURE 1. Absorbance of 6% (wt/vol) Accudenz® (Δ) after overlay with a sperm suspension containing motile sperm. Time zero denotes the time at which a 150-μL volume of sperm suspension, containing $5 \times 10^8$ rooster sperm per milliliter, was overlaid upon a 1.5-ML volume of Accudenz® prewarmed to 41 C in a polystyrene cuvette and the initial reading made. Thereafter, the cuvette was returned to the water bath. Subsequent measurements were made after 2, 4, 6, 8, 10, 20, and 30 min incubation at 41 C. When this procedure was repeated with sperm immobilized by preheating to 56 C, the absorbance remained at zero over the time course shown.](https://academic.oup.com/ps/article-abstract/75/6/776/1588757)
OBJECTIVE MEASUREMENT OF SPERM MOTILITY

TABLE 1. Summary of two-way ANOVA following repeated measurements of rooster sperm motility

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>2</td>
<td>8,002</td>
<td>4,001</td>
<td>1.0959</td>
</tr>
<tr>
<td>Rooster</td>
<td>35</td>
<td>507,096</td>
<td>14,488</td>
<td>3.9682***</td>
</tr>
</tbody>
</table>

Each of 36 New Hampshire roosters was ejaculated on an every-other-day basis. Three consecutive measurements were made per rooster.

***P ≤ 0.001.

In each of 36 New Hampshire roosters, the effect of time was nonsignificant. However, a difference (P ≤ 0.001) in sperm motility was found among roosters. The ANOVA is summarized in Table 1. When males were ranked by mean scores, the maximal sperm motility score was five times greater than the minimal score.

In Vitro Sperm Motility and Fertility

Data from the initial fertility trial are summarized in Table 2. No difference in fertility was observed among treatment groups when hens were inseminated with sperm from males categorized a priori by minimal, average, or maximal sperm motility. Fertility averaged 49, 53, and 54% for these treatment groups, respectively. In contrast, a difference in fertility (P ≤ 0.001) was observed in the second trial between hens inseminated with sperm from males categorized by minimal or maximal sperm motility (Table 3). Graphical analysis of this data set (Figure 3) revealed that the difference was attributable to lower initial fertility in the case of hens inseminated with sperm characterized by minimal motility. In the second fertility trial, differential sperm motility was confirmed spectrophotometrically prior to insemination. After a 5-min incubation interval at 41°C, the absorbance of the 6% (wt/vol) Accudenz® was 0.4423 and 0.8470 for males categorized a priori as having minimal and maximal sperm motility, respectively.

Analysis of Males Categorized by Sperm Motility

Measurements of sperm motility from individual roosters made over a 3-d interval were independent of time. Therefore, data were pooled and a frequency analysis performed (Figure 4). The hypothesis that observed frequencies approximated a normal distribution was not rejected. The solid line in Figure 4 represents the shape of the predicted distribution using 283 and 131.4 as estimates of μ and σ, respectively. When males were ranked by motility scores, the maximum was 30.5-fold greater than the minimum. Analysis of repeated measurements from males categorized by average or high sperm motility demonstrated highly significant differences (P ≤ 0.0001) between categories and among males within categories (Table 4). In contrast, neither time nor a category by time interaction exerted an effect on sperm motility.

Interassay Coefficient of Variation

Mean absorbance, standard deviation, and interassay CV (n = 3) for roosters categorized by photometry as having average sperm motility (see Figure 4) were 0.5614, 0.10133, and 18.0%, respectively, when the sperm motility of pooled semen was evaluated by spectrophotometry. Likewise, these statistics were 1.0082, 0.09256, and 9.2% for roosters categorized by high sperm motility. Sperm from average roosters penetrated the Accudenz® layer to only 55 ± 5.9% of the extent to which sperm from high sperm motility roosters did.

DISCUSSION

The objective analysis of poultry sperm motility has been hampered by a number of factors. These factors...
include the very nature of poultry semen, the lack of proximity of semen donors to analytical equipment, and the cost of computer-assisted analysis. As inferred from a recent review of sperm interaction with the oviduct (Bakst et al., 1994), the motility of sperm populations within the oviduct, the vagina in particular, should be viewed in terms of millions of sperm dispersed over a ciliated epithelial surface in oviducal fluid at body temperature. Based upon alternative methods reviewed for this report, the spectrophotometric method originally adapted by Wall and Boone (1973) for poultry sperm has been used most frequently. In this assay, sperm motility is determined in a volume of diluent containing 5 to 20 million sperm per milliliter. Furthermore, in 50% of the reports cited, sperm motility was measured at ≤ 30°C. Meaningful information may be derived from the measurement of sperm motility under nonphysiological conditions. However, it may be best to measure sperm motility at body temperature, and the motility of chicken sperm at body temperature is Ca²⁺-dependent (Ashizawa and Wishart, 1987, 1992; Wishart and Ashizawa, 1987; Thomson and Wishart, 1988, 1991; Ashizawa et al., 1989a, 1992, 1994; Ashizawa and Sano, 1990).

The spectrophotometric assay cited most frequently (Wishart and Ross, 1985) can be performed at body temperature if the concentration of extracellular Ca²⁺ is sufficient to overcome temperature-induced loss of motility (Wishart and Ashizawa, 1987). The assay itself is based upon three principles. First, absorbance is proportional to the concentration of sperm within a sperm suspension. Second, if a suspension of motile sperm is passed through a flow cell and the flow is stopped abruptly, then absorbance decays exponentially as a function of time. Third, the extent to which absorbance decreases is proportional to the percentage of motile sperm in the suspension. Therefore, change in absorbance is the key variable to be estimated. This value can be expressed as an index (Wall and Boone, 1973; Atherton et al., 1980) or a parametric estimate (Wishart and Ross, 1985; Froman and Thursam, 1994).

We attempted to use the method of Wishart and Ross (1985) to measure the motility of sperm from roosters characterized by heritable subfertility (McLean and Froman, unpublished data), but found sperm penetration of an Accudenz® solution to be a preferable approach. This method enabled us to demonstrate that subfertile R/R males were characterized by poor sperm motility (McLean and Froman, 1996). Accudenz® is a nonionic, biologically inert cell separation medium, and the absorbance of Accudenz® at 550 nm increased when motile sperm entered the medium from an overlaid sperm suspension. We hypothesized that sperm penetration of Accudenz® could be used to detect differences in sperm motility among normal, fertile males.

![Figure 3. Duration of fertility after a single insemination of Single Comb White Leghorn hens with sperm from New Hampshire roosters categorized as maximal (△) or minimal (○) sperm motility. Sperm motility was measured by sperm penetration into 6% (wt/vol) Accudenz solution from an overlay. Designations were based upon the ranked mean scores of 36 roosters. Each hen was inseminated intravaginally with 5 x 10⁷ sperm. Solid lines represent the functions y(x) = [98]/[1 + e^((-0.586)(x-13.6))] and y(x) = [92]/[1 + e^((-4.279)(x-19.4))], in which 98 and 92 are estimates of the parameter γ, the initial percentage of fertilized eggs. The hypothesis that these values represented estimates of a common parameter was tested by the extra sums of squares F test and rejected (P ≤ 0.05).](https://academic.oup.com/ps/article-abstract/75/6/776/1588757)

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**TABLE 2. Summary of first fertility trial**

<table>
<thead>
<tr>
<th>Roosters</th>
<th>Sperm motility¹</th>
<th>Hens²</th>
<th>Eggs³</th>
<th>Fertility⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td></td>
<td>(n)</td>
<td></td>
<td>(%)</td>
</tr>
<tr>
<td>3</td>
<td>Maximal</td>
<td>43</td>
<td>697</td>
<td>54 ± 1.6</td>
</tr>
<tr>
<td>3</td>
<td>Average</td>
<td>43</td>
<td>686</td>
<td>53 ± 2.0</td>
</tr>
<tr>
<td>3</td>
<td>Minimal</td>
<td>44</td>
<td>741</td>
<td>49 ± 2.6</td>
</tr>
</tbody>
</table>

¹A priori categorization based upon sperm penetration of 6% (wt/vol) Accudenz®. Roosters (n = 36) were ranked by mean motility scores, and 3 representative roosters were chosen per category.

²Each hen was inseminated intravaginally with a single dose of 5 x 10⁷ sperm.

³Collected over a 21-d interval.

⁴Each value is a mean ± SEM.
TABLE 3. Summary of second fertility trial

<table>
<thead>
<tr>
<th>Roosters (n)</th>
<th>Sperm motility</th>
<th>Hens (n)</th>
<th>Eggs (n)</th>
<th>Fertility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Maximally</td>
<td>135</td>
<td>2,590</td>
<td>52 ± 1.0A</td>
<td></td>
</tr>
<tr>
<td>3 Minimally</td>
<td>129</td>
<td>2,485</td>
<td>44 ± 1.4B</td>
<td></td>
</tr>
</tbody>
</table>

A,B Means in a column with no common superscript differ significantly (P ≤ 0.0001).

1 A priori categorization based upon sperm penetration of 6% (wt/vol) Accudenz®. Roosters (n = 36) were ranked by mean motility scores, and 3 representative roosters were chosen per category. Differential sperm motility was confirmed prior to insemination. Sperm from “minimal” roosters penetrated Accudenz® to only 51% of the extent to which sperm from “maximal” roosters did.

2 Each hen was inseminated intravaginally with a single dose of 5 x 10⁷ sperm.

3 Collected over a 21-d interval.

4 Each value is a mean ± SEM.

Our first objective was to determine the intra-assay CV. This was done by overlaying a suspension of rooster sperm upon 6% (wt/vol) Accudenz® in a disposable cuvette, incubating the cuvette at 41°C for a predetermined interval, and then measuring the absorbance of the Accudenz® layer. This method yielded an intra-assay CV of 2.6% (n = 3). In a subsequent experiment, the intra-assay CV was based upon sperm recovered from the Accudenz® layer. In this case, the CV was 6.2% (n = 3). We attributed the greater variability to experimental error associated with sperm recovery prior to determination of sperm concentration. In either case, sperm penetration of Accudenz® was found to be a repeatable phenomenon.

Our next objective was to test for differences in sperm motility among males. This was accomplished with two different flocks of New Hampshire roosters. These experiments were performed with a portable photometer rather than a spectrophotometer. The photometer was suitable for use within the building in which roosters were housed. Therefore, the photometer afforded an assessment of sperm motility immediately after ejaculation. As shown in Figures 1 and 2, patterns of sperm penetration were comparable between instruments. Volumes of Accudenz® and sperm suspension differed between instruments due to different locations of the light beam relative to cuvette height. Volumes used for photometry were determined empirically to simulate the pattern of sperm penetration observed with the spectrophotometer. As evidenced by ranked motility scores and ANOVA (Tables 1 and 4), appreciable differences in sperm motility were observed among males. We concluded that the rate at which sperm penetrated Accudenz® served as a basis for making distinctions among normal, fertile males.

Another objective was to determine whether a cause and effect relationship could be demonstrated between in vitro sperm motility and fertility. In the first fertility trial, males characterized by minimal, average, or maximal sperm motility were selected as semen donors (n = 3 males per category). As shown in Table 2, no difference was observed among groups of hens. In view of the work of Wishart and Palmer (1986), who reported a correlation coefficient of 0.82 between fertility and the sperm motility of individual males, we attributed the trial's outcome to inadequate sample size. In the first trial, fertility for the minimal and maximal sperm motility groups differed by only 5 percentage units (Table 2). This difference was viewed as an estimate of the true difference between these two categories of males. We determined that a minimum of 2,100 eggs would be needed per group of hens to detect this difference with a 90% certainty at a significance level of α = 0.05 (Sokal and Rohlf, 1969b).

TABLE 4. Summary of split-plot ANOVA following repeated measurements of sperm motility from roosters categorized a priori by average or high sperm motility

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category</td>
<td>1</td>
<td>813,875</td>
<td>813,875</td>
<td>70.54****</td>
</tr>
<tr>
<td>Males within category</td>
<td>33</td>
<td>1,465,375</td>
<td>44,405</td>
<td>3.85****</td>
</tr>
<tr>
<td>Time</td>
<td>2</td>
<td>12,033</td>
<td>6,016</td>
<td>0.52</td>
</tr>
<tr>
<td>Category by time</td>
<td>2</td>
<td>28,010</td>
<td>14,005</td>
<td>1.21</td>
</tr>
</tbody>
</table>

Each of 35 New Hampshire roosters was ejaculated on an every-other-day basis. Three consecutive measurements of sperm motility were made per rooster. Roosters were categorized by average (n = 18) or high sperm motility (n = 17) after the sperm motility of each of 100 New Hampshire roosters had been determined by sperm penetration of 6% (wt/vol) Accudenz®.

****P ≤ 0.0001.
In contrast to the first fertility trial, a difference in fertility was detected between minimal and maximal sperm motility males in the second trial (Table 3). When these data sets were analyzed graphically (Figure 3), the initial level of fertility, as estimated by the parameter \( \gamma \), differed by 6 percentage units, and this disparity increased over the course of a week. In fact, fertility during this interval was 84% (\( n = 842 \) eggs) and 96% (\( n = 868 \) eggs) for males categorized by minimal and maximal sperm motility, respectively. Thus, although insemination doses were equivalent, the effective insemination doses were not. The designations minimal and maximal must be viewed in context, because these terms only refer to the ranked motility scores of the 36 roosters used in the initial test for variability among males. Thus, the roosters categorized by minimal and maximal sperm motility in the second fertility trial may have been comparable to the roosters used to determine interassay CV, which were categorized by average and high sperm motility, respectively. Based upon spectrophotometry, the absorbance observed with “maximal” roosters was 84% of the mean observed for the high sperm motility roosters. Sperm from “minimal” roosters penetrated Accudenz\textsuperscript{®} to only 51% of the extent to which sperm from “maximal” roosters did. Likewise, sperm from average roosters penetrated Accudenz\textsuperscript{®} to only 55 ± 5.9% of the extent to which sperm from high sperm motility roosters did. Therefore, it is likely that the difference in fertility observed in the second fertility trial (Table 3; Figure 3) reflects a comparison of average vs above average sperm motility rather than genuine extremes as implied by the terms minimal and maximal.

In summary, our overall experimental goal was to determine whether we could develop an objective sperm motility assay that would: 1) approximate physiological conditions, 2) require simple, portable equipment, 3) be applicable to individual males, and 4) yield repeatable, biologically significant results. Each of these stipulations were met. Furthermore, we found that the assay, with minor modifications, could be applied to turkey sperm (Figure 5). Even so, measurement of poultry sperm motility by sperm penetration of Accudenz\textsuperscript{®} does not afford information about individual sperm. Indeed, it is an assessment of the mobility of a sperm population. Nonetheless, it is sperm mobility rather than sperm motility \textit{per se} that enables sperm sequestration within the hen’s sperm storage tubules. In conclusion, our experiments have demonstrated the potential for selecting semen donors based upon sperm motility and the establishment of simple, objective criteria for assessing
the quality of semen sold as a commodity. Likewise, in conjunction with the single step technique whereby sperm can be washed by centrifugation through Accudenz® (Froman and Thursam, 1994), our experiments have demonstrated the potential for studying sperm motility in chemically defined environments and the analysis of highly motile or largely immotile subpopulations of sperm derived from a sperm population equivalent in size to that used as an insemination dose.

REFERENCES


