Use of a Sperm Analyzer for Evaluating Broiler Breeder Males. 1. Effects of Altering Sperm Quality and Quantity on the Sperm Motility Index


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ABSTRACT A new instrument for assessing mammalian semen attributes, the Sperm Quality Analyzer®, was evaluated as a potential tool for determining rooster sperm quality. The Sperm Quality Analyzer® measures the “activity” of sperm in a semen sample as the sperm motility index (SMI). The SMI is defined as the number and amplitude of deflections in a light path per second as a result of sperm movement within a capillary tube. In the present study, effects of sperm concentration, viability, and motility on the SMI were evaluated. Peterson broiler breeder males (n = 40) were used as semen donors. In the initial experiment, semen was diluted from 2- to 25-fold and SMI readings were obtained. The SMI was very low in neat semen samples but increased when semen was diluted up to threefold. However, at dilutions greater than fivefold, the SMI decreased. Apparently, sperm concentration in undiluted semen is so great that sperm are unable to move freely within the capillary tube. Maximum SMI values were obtained at sperm concentrations of approximately 1 billion sperm per milliliter. When thawed, dead sperm were mixed with incubated, live sperm, the SMI decreased with decreasing sperm viability even though sperm concentration was constant. Obviously, fewer sperm move across the light beam as sperm mortality increases. When motile, aerobically incubated sperm were mixed at different rates with immotile, anaerobically incubated sperm samples, the SMI increased with increasing concentrations of motile sperm, whereas total sperm concentration was static. In addition, the SMI was strongly correlated with motility scores obtained by microscopic analysis. The Sperm Quality Analyzer® provides an estimate of the overall quality of sperm from broiler breeder males by reflecting sperm concentration, viability, and motility in a single value, the SMI.

(Key words: sperm motility, sperm viability, sperm concentration, fertility, broiler breeder)


INTRODUCTION

The fertilizing potential of the rooster is dependent upon the quality and quantity of sperm produced by the testes. Because each broiler breeder rooster is responsible for mating several hens, sperm characteristics can have an immense impact on the fertility of a flock (Wilson et al., 1979).

Three sperm parameters that are most often evaluated when determining a rooster’s fertilizing potential include the following: sperm concentration, viability, and motility. These attributes can be evaluated by several different methods (for a review see Bakst and Cecil, 1997). In general, each of these techniques provide an estimate for only one or two sperm characteristics. For example, the fluorometric method of Bilgili and Renden (1984) assesses the sperm concentration and sperm viability of a semen sample but does not estimate sperm motility. Because sperm concentration, viability, and motility influence the total number of sperm available to fertilize an egg, it is often important to consider each of these parameters for a single semen sample. In addition, many of the procedures that are currently utilized to determine sperm quality and quantity require toxic chemical compounds, elaborate equipment, or several minutes of sample preparation and analysis time.

Recently, a new instrument for assessing mammalian sperm quality and quantity, the Sperm Quality Analyzer®, has been evaluated for its use with avian semen samples (McDaniel et al., 1997; Wishart and Wilson, 1997). The Sperm Quality Analyzer® examines the amount of sperm movement in a semen sample and

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Abbreviation Key: MEM = minimum essential medium; SMI = sperm motility index.
displays its output as the sperm motility index (SMI). As defined by the Sperm Quality Analyzer®, the SMI is a single value that reflects the number and amplitude of deflections per second that sperm make in a capillary tube through their disruption of a light beam (Bartoov et al., 1991). In fact, the SMI is proportional to the concentration of motile sperm in human semen (Bartoov et al., 1991; Johnston et al., 1995). Therefore, at least for human semen, the SMI appears to indicate in a single value the amount of sperm “activity” in a semen sample (i.e., intensity of movement and motile sperm concentration as affected by sperm concentration, viability, and motility). In addition, when human females are inseminated with sperm samples that have a low SMI value, conception rate is depressed as compared to inseminations with semen with a high SMI value (Matilsky et al., 1993).

With this previous human research in mind, it was reasoned that the Sperm Quality Analyzer® and the SMI may be valuable tools for measuring avian sperm quality. Therefore, the objective of the present study was to determine the effects of rooster sperm concentration, viability, and motility on the SMI. Once these effects are known, the SMI could be used as an overall measure of avian sperm quality.

**MATERIALS AND METHODS**

**Housing and Environment**

On September 26, 40 Peterson broiler breeder males ranging from 25 to 55 wk of age were obtained from a local integrator and housed in individual cages to create four groups of 10 roosters each. Roosters were fed a restricted diet of 370 kcal ME per bird per d and exposed to light from 0400 to 2100 h. All birds were caged in houses with conventional environmental controls. For 1 wk after housing, males were trained for semen collection by using the method of Burrows and Quinn (1937).

**Effects of Semen Dilution and Sperm Concentration on the SMI**

After the training period, semen was collected from each of the 40 males, pooled by group of roosters, and diluted with minimum essential medium (MEM, Howarth, 1981) to provide the following dilution factors: 2, 3, 5, 9, 13, 17, 21, and 25 (1 part semen: 24 parts MEM). Sperm concentration and viability for the neat semen sample and each dilution were obtained by using the fluorometric method of Bilgili and Renden (1984). The SMI was determined twice on the neat semen sample and each dilution by using the Sperm Quality Analyzer®. Semen was drawn into a capillary tube with the following internal dimensions: depth of 0.3 mm, width of 3 mm, and length of 50 mm. The outer surface of the capillary tubes were wiped clean with a tissue before being placed into the Sperm Quality Analyzer®. Four 10-s readings were made on each sample to give the average SMI in deflections of the light path per second (i.e., changes in optical density per second). All measurements were made at room temperature, approximately 24 C.

**Effect of Sperm Viability on the SMI**

Five weeks after males were housed, semen was collected from each of the 40 birds, mixed well, and pooled into a single vial. Half of the semen sample was frozen at −47 C, for 2 h, to kill the sperm. The other half of the sample was incubated at 37 C for 2 h so the sperm would remain viable. Results from preliminary research revealed that when semen was diluted threefold or more, sperm were able to move freely within the capillary tube. Therefore, the semen was thawed, and both the thawed sample and incubated sample were diluted 10-fold with MEM. Two fluorometric readings were obtained for each of the samples so that the percentage of dead sperm would be known. Thawed (dead) semen was mixed with incubated (live) semen to create samples containing approximately 10, 20, 30, 40, 50, 60, 70, 80, and 90% dead sperm. Because both the thawed semen sample and the incubated semen sample came from the same initial pool of semen, sperm concentration for each semen mixture remained the same. Three SMI readings were taken at room temperature for each of the nine semen sample combinations.

**Effect of Sperm Motility on the SMI**

Twenty-one weeks after housing, semen was collected from the 40 males, pooled, and diluted 10-fold with MEM. Two fluorometric measurements were taken on the diluted sample to assess sperm viability and concentration. To render the sperm immotile, a 20-mL vial was completely filled with diluted semen and capped to create an anaerobic environment. A second portion of the diluted sample was placed in an uncapped vial to create an aerobic environment that would allow the sperm to remain motile (Wishart, 1984). Both samples were incubated at 37 C on an Adams Nutator® (Model 1105) and rotated at 24 rpm for 3 h. Sperm viability was determined following incubation by obtaining two fluorometric readings for each sample, aerobic (motile) and anaerobic (immotile). To create samples that contained approximately 100, 90, 80, 70, 60, 50, 40, 30, 20, and 0% motile sperm, semen that had been incubated aerobically was mixed with semen that had been incubated under anaerobic conditions. Because all semen combinations came from the same original pool of semen, sperm concentration was identical in every sample. The diluted samples were incubated and rotated for 10 min at 37 C under aerobic conditions before measurements were
taken. Sperm motility was measured by the whirl method and assigned a score between five (most motile) and one (least motile) (Cherms, 1968). Each sample was selected at random from the incubator, and SMI readings were obtained simultaneously with motility score. At no time did the individual evaluating samples by the whirl method know the sample composition. Three replicate readings were obtained for each sample.

**Statistical Analysis**

Linear regression analyses were utilized to determine the effects of semen dilution, sperm concentration, and sperm viability on the SMI as well as the effect of anaerobic incubation on the motility score of a semen sample. As suggested by Cox (1980), two linear equations were used to describe the relationship of semen dilution and sperm concentration with SMI instead of one curvilinear equation. The effect of anaerobic incubation on the SMI was analyzed with the following logistic model as suggested by Ratkowsky (1983):

\[ Y = \frac{\alpha}{1 + \exp(\beta - \gamma X)} \]

where \( Y = \text{SMI} \), \( \alpha = \text{asymptote;} \) \( \beta = \text{y intercept indicator;} \) and \( \gamma = \text{slope.} \) The relationship between motility score and the SMI was ascertained with a linear correlation analysis. For all linear regression and correlation analyses, replicate means were used as individual data points.

**RESULTS**

**Effects of Semen Dilution and Sperm Concentration on the SMI**

The percentage of dead sperm in each semen sample remained stable at each level of dilution for an overall average of 8%. As shown in Figure 1 for neat semen samples, the SMI was very low, ranging from 36 to 85 for each of the four groups. A linear increase in the SMI was noted with increasing semen dilution up to threefold. However, at dilutions above fivefold the SMI decreased linearly. The maximum SMI obtained for any of the semen samples was 439 after a fivefold dilution. For all samples, the coefficient of variation between the two replicate SMI readings per sample was 16%.

A similar response in the SMI was seen with increasing sperm concentration (Figure 2). As sperm concentration climbed from 0.1 billion sperm per milliliter to 1 billion sperm per milliliter, a linear increase in the SMI was noted. On the other hand, as sperm concentration increased above 1 billion sperm per milliliter the SMI decreased linearly.

**Effect of Sperm Viability on the SMI**

The SMI for the incubated semen sample averaged 261, and only 12.5% of the sperm were dead after incubating for 2 h at 37 C. Mortality of the thawed sperm sample increased to 90%, creating a sample with apparently no sperm activity and a SMI value of 0. The combination of these two aforementioned samples, that initially came from the same pool of semen, resulted in specimens that contained from 12.5 to 90% dead sperm but a constant sperm concentration of 0.3 billion sperm per milliliter after the 10-fold dilution. The SMI decreased linearly as the percentage of dead sperm in the semen samples increased.

**FIGURE 1.** Effect of semen dilution on the sperm motility index (SMI). Each point represents the mean of two replicates from one pool of semen. Four different pools of semen from four groups of 10 roosters each were diluted from 2- to 25-fold with minimum essential medium. A linear increase in the SMI was noted as semen dilution increased up to 3-fold \( (y = 143x - 76, r^2 = 0.76, P < 0.0001) \). At semen dilutions greater than fivefold a linear decrease in the SMI was obtained \( (y = -11x + 399, r^2 = 0.63, P < 0.0001) \).

**FIGURE 2.** Effect of sperm concentration on the sperm motility index (SMI). Each point represents the mean of two replicates from one pool of semen. Four different pools of semen from four groups of 10 roosters each were diluted from 2- to 25-fold with minimum essential medium to obtain various sperm concentrations. A linear increase in the SMI was noted as sperm concentration increased up to 1 billion sperm per milliliter \( (y = 308x + 111, r^2 = 0.63, P < 0.0001) \). At sperm concentrations greater than 1 billion sperm per milliliter, a linear decrease in the SMI was obtained \( (y = -68x + 412, r^2 = 0.68, P < 0.0005) \).
The relationship between sperm viability and the sperm motility index (SMI). Each point represents the mean of three replicates. A linear decrease in the SMI was obtained as the percentage of dead sperm in the semen samples increased ($y = -3x + 307$, $r^2 = 0.96$, $P < 0.0001$).

The relationship between sperm motility scores and the percentage of motile sperm in semen samples. Each point represents the mean of three replicates. A linear increase in motility score was obtained as the percentage of motile sperm in the semen samples increased ($y = 0.05x + 0.32$, $r^2 = 0.92$, $P < 0.0001$).

The relationship between the sperm motility index (SMI) and the percentage of motile sperm in semen samples. Each point represents the mean of three replicates. A logistic increase in the SMI was obtained as the percentage of motile sperm in the semen samples increased ($y = 300/(1 + \exp(3.29 - 0.078x))$, $r^2 = 0.99$, $P < 0.0001$).

(In Figure 3). In addition, the coefficient of determination for this linear relationship indicated that 96% of the variation in the SMI could be accounted for by sperm viability alone.

Effect of Sperm Motility on the SMI

Before incubation, the original pool of semen utilized to assess the effects of sperm motility on the SMI contained 3.2 billion sperm per milliliter and 10% dead sperm. After incubation, the semen samples incubated under aerobic and anaerobic conditions contained similar amounts of dead sperm (12 vs 10%, respectively). The semen sample incubated for 3 h under aerobic conditions displayed excellent sperm motility as was evidenced by an average motility score of 4.3 or almost 100% motile sperm. However, incubation under anaerobic conditions for 3 h resulted in total inhibition of sperm motility (motility score = 0). As expected when motile and immotile samples were mixed, the motility score increased in a linear fashion with increasing percentages of motile sperm (Figure 4).

A somewhat similar pattern was observed for the SMI. A logistic relationship was noted between the percentage of motile sperm in each sample and the SMI (Figure 5). In general, as the percentage of motile sperm increased so did the SMI. However, when samples contained very low or very high percentages of motile sperm, the differences in the SMI between samples were less than when samples contained from 30 to 70% motile sperm. The minimum and maximum SMI values were obtained in the 0% aerobic semen sample (SMI = 0) and the 100% aerobic semen sample (SMI = 324), respectively.

Additionally, a very strong linear correlation ($r^2 = 0.97$) was observed between motility scores and the SMI of each sample (Figure 6). Semen samples that contained much sperm activity, as determined by microscopic analysis, also produced very high SMI values. On the other hand, samples with very little sperm activity produced very low SMI values.

DISCUSSION

Results from this study indicate that the Sperm Quality Analyzer® provides a very fast and complete measure of overall avian sperm quality, the SMI. However, because sperm concentration, viability, and motility all affect the SMI, it would be difficult to ascertain which of these three factors was responsible for a low SMI reading, unless at least two of these sperm characteristics were known for a semen sample. On the
are able to interact with the light path, thereby decreasing the SMI. It is also possible that at the higher levels of semen dilution sperm motility may also be decreasing. Excessive dilution (greater than 11-fold) of avian semen can result in a temporary increase in sperm motility and metabolic activity accompanied by a decrease in the life span of sperm in vitro (for review see Sexton, 1979). Because the percentage of dead sperm in each of the diluted samples of the present study remained stable, the semen dilution effect on the SMI must be due to the shear number of sperm in each sample and not the number of viable sperm in each sample.

However, sperm viability does drastically affect the SMI of avian semen. Because the total sperm concentration in every live-dead mixture of semen in the present study was the same and both live and dead semen samples came from the same initial pool of semen, only effects due to sperm viability were evaluated. The decrease in the SMI with increasing sperm mortality, again, can be explained by fewer sperm interacting with the path of light passing through the capillary tube. Of course, only live sperm would be motile and able to swim repeatedly across the light path to increase the SMI.

Avian sperm motility has a pronounced effect on the SMI. Rooster sperm incubated under anaerobic conditions are not able to utilize endogenous substrates as an energy source; and after several hours of incubation, the sperm, although still alive, are immotile because they are unable to maintain sufficient adenosine triphosphate concentrations (Wishart, 1984). Indeed, in the present study, semen incubated under anaerobic conditions for 3 h became completely immotile as was evident in the visual motility score and SMI value of 0 obtained for this sample.

When aerobically (motile) and anaerobically (immotile) incubated semen were mixed, sperm motility, as measured by the swirl method and the SMI, increased with increasing percentages of motile sperm. Because all samples had the same total sperm concentration but different percentages of motile sperm, it can be reasoned that the SMI also increased with increasing numbers of motile sperm.

Bartoov et al. (1991) illustrated that the SMI is, in fact, dependent upon the motile sperm concentration of human semen. It is interesting to note that Bartoov et al. (1991) obtained a SMI reading of approximately 50 when the motile sperm concentration of a human semen sample was 30 million motile sperm per milliliter. In the present study, a similar SMI reading of approximately 50 was also obtained when the motile sperm concentration of rooster semen was approximately 30 million motile cells per milliliter (Figure 5, 10% motile sperm sample). Bartoov et al. (1991) also demonstrated that the SMI increased only very slightly from 40 to 120 million motile cells per milliliter. In contrast, we have shown that for rooster sperm the SMI increases dramatically as the motile sperm concentration increases from approximately 80 to 170 million motile cells per milliliter. It

FIGURE 6. Correlation of the sperm motility index (SMI) with visual sperm motility scoring. Each point represents the mean of three replicates. A linear correlation of the SMI with visual motility scoring was obtained (y = 69x – 7.3, r = 0.97, P < 0.0001).
appears that the movement of rooster sperm is greater than that of human sperm at these higher motile sperm concentrations. Human sperm motility appears to be inhibited by sperm to sperm contact when the motile sperm concentration exceeds 40 million cells per milliliter (Vantman et al., 1989).

The strong correlation between the SMI and the motility scoring method reveals the dependency of the SMI on the “activity” of sperm in a semen sample. It is most likely that the SMI reflects not only the number of sperm moving in a semen sample but also the intensity of sperm motility.

When compared to other measures of avian sperm motility the SMI, as determined by the Sperm Quality Analyzer®, exhibits some advantages. Possibly the greatest advantage of the SMI over other means of accessing sperm motility is the speed (40 s per sample) and simplicity of operation of the Sperm Quality Analyzer®. Most procedures for determining sperm motility require elaborate semen handling procedures and equipment (for a review see Bakst and Cecil, 1997). The SMI can be determined by simply diluting a semen sample and loading it into a capillary tube and the Sperm Quality Analyzer®. Visual scoring of sperm motility by microscopy requires only a microscope and slides; however, microscopy provides a very subjective measure of motility that can be greatly influenced by the user. By attaching a video camera to the microscope it is possible to determine the percentage of sperm that are motile in a sample and decrease subjectivity. With the use of even more elaborate equipment and methods, sperm motility can be measured with a spectrophotometer equipped with a thermostatically controlled flow cell. This procedure provides a very objective view of collective sperm motility but requires several minutes per sample. In addition, Froman and McLean (1996) have shown that penetration of sperm through a gel like solution (Accudenz®) provides an objective estimate of avian sperm motility that is very repeatable (CV from 9 to 18%). However, this technique also requires a considerable amount of time per sample (6 min) and several steps.

In conclusion, the SMI could be a very useful tool for evaluating the overall sperm quality of avian semen. The SMI is influenced by avian sperm concentration, viability, and motility. In addition, recent research has shown that the SMI is highly correlated with not only avian sperm quality but also the fertilizing ability of rooster sperm (McDaniel et al., 1997). Because the SMI can be obtained within 1 min without the use of complex procedures or toxic chemicals, the sperm quality of several roosters or semen samples can be evaluated quickly.

**REFERENCES**


