Similarities and Differences Between the Sperm Quality Index and Sperm Mobility Index of Broiler Breeder Semen[1,2]

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ABSTRACT The sperm quality index (SQI) and sperm mobility index (SMI) both predict sperm motility. Previous research was conducted comparing the SMI with the SQI; however, semen was diluted improperly for the SQI (40-fold). For an accurate comparison, semen must be diluted 10-fold for the SQI. Therefore, the overall objective of this study, utilizing 4 experiments, was to examine the relationship of the SQI with the SMI when: 1) active, heat-inactivated, and boiled sperm were incubated and analyzed over 60 min, 2) motile and immotile sperm were combined, 3) dead sperm (boiled or frozen) and live sperm were combined, and 4) sperm were rendered immotile by adding Zn. In experiment 1, the SQI was stable throughout incubation for active sperm and zero for heat-inactivated and boiled sperm. The SMI from active sperm steadily increased over incubation. However, the SMI from heat-inactivated sperm increased drastically resulting in values higher than active sperm after 35 min of incubation. The SMI from active sperm was higher than boiled sperm throughout incubation. For experiment 2, a cubic increase in the SQI occurred as active sperm increased from 0 to 100%. However, there was no difference in SMI readings until samples contained 80% active sperm. In experiment 3, for both boiled and frozen sperm, as the percentage of viable sperm increased, there was a logarithmic increase in the SQI. The SMI values were similar for 0 and 100% viable sperm when using boiled sperm, and all SMI readings were similar to 0% viable for frozen sperm. For experiment 4, both the SQI and SMI values from semen incubated with Zn were lower than that of saline. The SQI from sperm incubated in saline was higher than that of Zn throughout incubation, yet there were no differences in SMI values after 90 min of incubation. In conclusion, both the SQI and SMI of sperm decrease in response to Zn. However, it appears that immotile and dead sperm are capable of increasing SMI values but not SQI values.

Key words: sperm quality index, sperm mobility index, sperm motility, sperm mobility, sperm viability

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INTRODUCTION

The sperm quality index (**SQI**) is a single number that provides an estimate of overall semen quality of roosters (McDaniel et al., 1998; Parker et al., 2000) and toms (Neuman et al., 2002). The SQI is determined by monitoring the disruptions of a light path created only by the movement of motile sperm within an SQI capillary.

In the initial research to validate the SQI with broiler breeder sperm, McDaniel et al. (1998) mixed known percentages of frozen, dead, and immotile semen with viable semen to examine the relationship of the SQI with sperm viability. They found that the SQI was highly correlated with sperm viability when using this method. Because only viable motile sperm are capable of fertilization, research was conducted to determine if a relationship existed between the SQI and sperm motility. McDaniel et al. (1998) combined known percentages of live, motile sperm with live, immotile sperm and found that the SQI was very responsive to changes in sperm motility.

However, because rooster sperm are highly concentrated in undiluted semen, they are unable to move freely within the SQI capillary and therefore yield low SQI readings by infrequently disrupting the light path. As a result, McDaniel et al. (1998) recommended that semen be diluted 10-fold before SQI analysis.

An alternative method used to predict poultry fertility is the sperm mobility index (**SMI**; Froman and McLean, 1996). Sperm mobility is defined as the progressive swimming motion of sperm through a viscous medium, such as Accudenz, when incubated at the hen's body temperature (41°C; King and Donoghue, 2000). After 5 min of incubation at 41°C, the SMI is determined by measuring the absorbance of light by sperm that penetrate a layer of Accudenz.

To predict the fertilizing capabilities of roosters and toms, semen must be diluted properly before analysis for

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the SQI and SMI. For example, semen must be diluted 10-fold for the SQI (McDaniel et al., 1998; Parker et al., 2000, 2002; Parker and McDaniel, 2002, 2003, 2004), whereas semen is diluted to a constant 5×10^8 sperm/mL for evaluation of SMI (Froman and McLean, 1996).

Because both the SMI and SQI have been used to predict fertility, research was conducted to compare the SMI with the SQI (Froman et al., 2003). In that study, semen was diluted 40-fold before SQI analysis. However, a 40-fold dilution has recently been shown to be excessive for chicken sperm, resulting in altered sperm motility, fertility, and metabolism (Parker and McDaniel, 2003, 2006). It is well known that the SQI is very predictive of semen quality, fertility, and hatchability when semen is diluted 10-fold before SQI analysis (McDaniel et al., 1998; Parker et al., 2000, 2002; Parker and McDaniel, 2002, 2003, 2004). However, Parker and McDaniel (2003) reported significantly lower correlation coefficients for the relationships of sperm viability and fertility with the SQI from semen diluted 50-fold as opposed to the SQI from semen diluted 10-fold. Because semen was diluted excessively in the study conducted by Froman et al. (2003) comparing the SMI with the SQI, this comparison should be reexamined using a 10-fold dilution for the SQI.

Therefore, the overall objective of this study was to explore the similarities and differences between the SMI and the SQI, when broiler breeder semen was diluted 10-fold for the SQI. Four experiments were conducted to explore this overall objective. In experiment 1, the objective was 2-fold: 1) to compare how each method responded to active, motile, and heat-inactivated immotile sperm incubated over time, and 2) to examine the response of the SQI and SMI to motile and boiled, nonviable sperm over incubation. In experiment 2, the objective was to compare the response of each method when known percentages of motile and heat-inactivated immotile sperm were combined. The objective for experiment 3 was to compare the response of each method when mixing known percentages of live and dead sperm. For experiment 4, the objective was to examine how each method responded to sperm motility over 2 h of incubation when a known motility inhibitor, $ZnSO_4$, was added to semen (Bakst, 1985; Barber et al., 2005).

MATERIALS AND METHODS

Housing and Environment

Twenty Ross broiler breeder males, 82 wk of age, were obtained from a local integrator. Roosters were divided randomly into 2 rooms and housed in individual cages. Broiler breeder males were fed a standard breeder diet (3,080 kcal of ME/kg, 13.9% CP, and 1% Ca) and feed-restricted according to the primary breeder's recommendations. All males received 16 h of light per day throughout the experiment. Additionally, 18 Ideal 236 White Leghorn males, 24 wk of age, were housed in individual cages. The Leghorn males were fed a standard layer diet (2,860 kcal of ME/kg, 14.5% CP, and 4% Ca) ad libitum and received 16 h of light per day. All males were treated in accordance with the Guide for Care and Use of Agricultural Animals in Agricultural Research and Training.

Semen Evaluation

Before all experiments, semen was collected from each rooster every other day for a total of 3 collections to evacuate residual sperm from the bird's body, using the method of Burrows and Quinn (1937). Immediately after collecting semen and before dilution, sperm concentration and viability were determined. In all 4 experiments, sperm concentration was measured using an IMV microreader (IMV International, Maple Grove, MN; King and Donoghue, 2000). Across all experiments semen concentration varied only slightly from 5.8 to 6.6 billion sperm/mL. Sperm viability was determined using the method of Bilgili and Renden (1984). The SQI was obtained using the Sperm Quality Analyzer (Medical Electronic systems, Ltd., Migdal, Haemek, Israel). To obtain the SQI, semen was ultimately diluted 10-fold with 0.85% saline before analysis (McDaniel et al., 1998). For measuring the SMI, the procedure of Froman (1997) was used and these values were obtained utilizing the IMV microreader at 540 nm (King and Donoghue, 2000). To determine the SMI, each sample was diluted to a constant 5×10^8 sperm/mL with 50 m*M* N-Tris [hydroxyl methyl] methyl-2-amino-ethanesulfonic acid (**TES**) containing 128 m*M* NaCl and 2 m*M* $CaCl_2$ (Froman et al., 1999); 60 μL of the diluted sample was overlaid onto 600 μL of prewarmed 6% Accudenz (Accurate Chemical and Scientific Corp., Westbury, NY). In every experiment except experiment 1, the SMI cuvettes were incubated for 5 min at 41°C after overlaying the Accudenz with diluted semen, and then absorbance readings were obtained (Froman, 1997).

Experiment 1

In the first part of this experiment, semen pools obtained from 10 Ross males were divided into 2 equal halves so that one-half of the pool would be active and motile, whereas the other half would be heat-inactivated and immotile. For the active sample, semen was incubated at 21°C on a rotary shaker for 20 min; the other half of the semen pool was heat-inactivated by incubating semen at 56°C for 20 min to immobilize sperm (Froman and McLean, 1996). After incubation, a light microscope was used to confirm that the heat-inactivated sperm were immotile. The objective of this experiment was to compare the SQI and SMI from the same semen sample over incubation time. Therefore, for this experiment only, 2 Accudenz cuvettes of active and 2 of heat-inactivated, immotile sperm were incubated and analyzed over 60 min at 41°C, not just at the recommended 5 min of incubation for the SMI (Froman, 1997). To obtain the SQI, semen from the active sample and the heat-inactivated, immotile sample were diluted 10-fold with TES into a master vial and maintained at 41°C throughout incubation. For each SQI measurement, a new capillary was used and semen was not further diluted with 0.85% saline before testing because the samples were

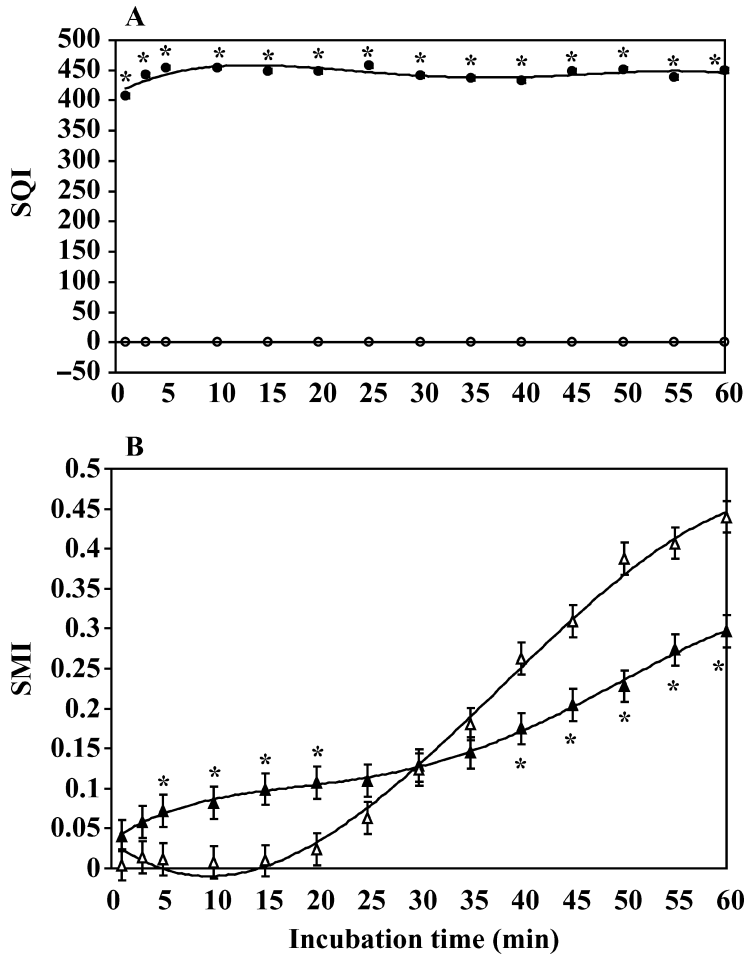

Figure 1. The relationship of active and heat-inactivated immotile sperm over incubation in experiment 1 for A) sperm quality index (SQI), and B) sperm mobility index (SMI). The SQI from heat-inactivated immotile sperm remained at zero throughout the incubation period. Each point represents the mean of 2 replicates from 2 pools of semen. A quartic relationship existed for both the SQI (●) and SMI (▲) from active sperm ($y = -8E{-}05x^4 + 0.01x^3 - 0.5x^2 + 8.2x + 415$, $r^2 = 0.7$, $P < 0.019$, and $y = -7E{-}24x^4 + 0.000001x^3 - 0.0004x^2 + 0.0075x + 0.04$, $r^2 = 0.99$, $P < 0.0001$, respectively). For the SMI from heat-inactivated immotile sperm (△) there was a cubic relationship ($y = -5E{-}06x^3 + 0.0005x^2 - 0.0087x + 0.03$, $r^2 = 0.99$, $P < 0.0001$). However, no relationship existed for the SQI from heat-inactivated immotile sperm (○). *Indicates a significant difference between the active and heat-inactivated immotile sperm for each incubation period (SQI SEM = 3.84; SMI SEM = 0.02).

already diluted 10-fold. Two SQI and SMI readings were obtained from the active and heat-inactivated immotile semen samples at 1, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 min of incubation. This part of the experiment was replicated in duplicate with semen from the remaining 10 males.

For the second part of this experiment, semen was collected from Leghorn males and pooled. The semen pool was then divided into 2 equal halves so that one-half of the pool would contain active, viable sperm whereas the other contained sperm that would be killed by boiling for 1.5 min. Using the fluorometric method of Bilgili and Renden (1984), sperm were confirmed dead. These samples were analyzed as described in the first part of this experiment.

Experiment 2

Semen was collected from Ross males and pooled. Before treatment, semen pools were divided into 2 equal halves and diluted 1:1 with minimum essential medium (**MEM**; Howarth, 1981). One-half of the semen pools served as active samples and the other half was made immotile by heat-inactivation as described in experiment 1. To maintain the active semen sample, one-half of the 1:1 diluted semen pool was stored at room temperature on a rotary shaker. Active, motile and heat-inactivated, immotile semen samples were combined to attain 0, 20, 40, 60, 80, and 100% active samples. Before testing, a light microscope was used to confirm that the heat-inactivated sperm were immotile. Two SQI and fluorometer readings were taken and samples were added to 2 SMI cuvettes within 1 min of mixing motile and immotile sperm. The SMI readings were obtained after the recommended 5 min of incubation over Accudenz. This experiment was replicated 4 times on different days.

Experiment 3

Semen was collected from Ross males and pooled. Semen pools were divided into 2 equal halves so that one-half of the pool would contain active, viable sperm whereas the other contained boiled, nonviable sperm. In the first

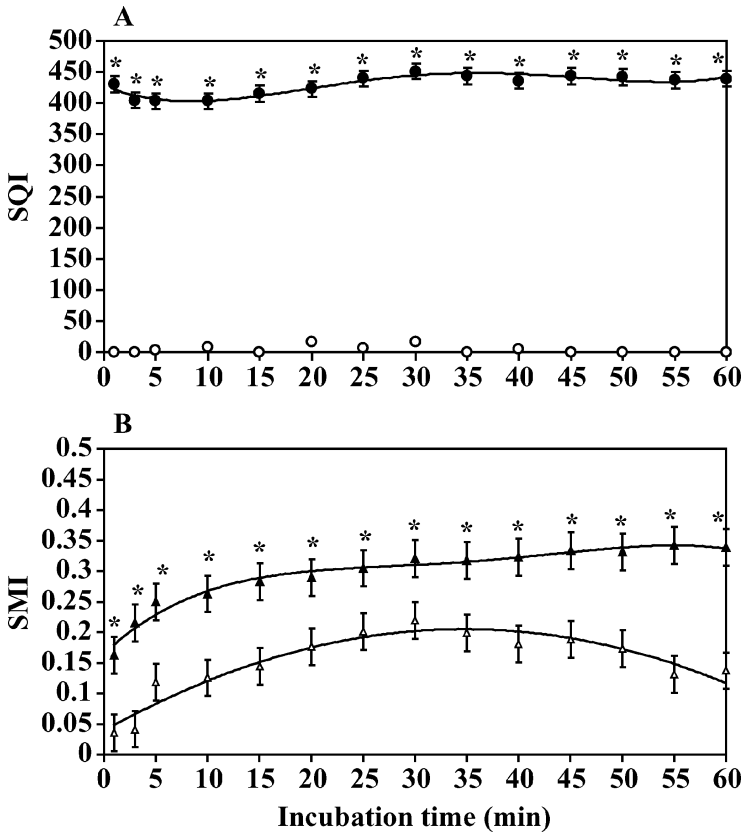

Figure 2. The relationship of active and boiled nonviable sperm over incubation in experiment 1 for A) sperm quality index (SQI), and B) sperm mobility index (SMI). Each point represents the mean of 2 replicates from 3 pools of semen. A quartic relationship existed for both the SQI (●), and SMI (▲) from active sperm ($y = 1E{-}04x^4 - 0.013x^3 + 0.52x^2 - 6.1x + 424.5$, $r^2 = 0.91$, $P < 0.0001$, and $y = -9E{-}08x^4 + 1E{-}05x^3 - 0.0006x^2 + 0.0146x + 0.173$, $r^2 = 0.98$, $P < 0.0001$, respectively). For the SMI from boiled nonviable sperm (△), there was a quadratic relationship ($y = -0.0001x^2 + 0.0094x + 0.0417$, $r^2 = 0.91$, $P < 0.0001$). There was no relationship for the SQI from boiled nonviable sperm (○). *Indicates a significant difference between the active and boiled nonviable sperm for each incubation period.

part of this experiment the viable sperm was diluted 1:1 with MEM and maintained at room temperature on a rotary shaker and the other half of the semen pool was boiled for 1.5 min to create a nonviable sample. Sperm were confirmed dead by using the fluorometer (Bilgili and Renden, 1984). Viable and nonviable samples were combined to attain 0, 20, 40, 60, 80, and 100% viable sperm. The SQI, SMI, and fluorometer readings were obtained as described in experiment 2. This experiment was replicated 4 times on different days.

For the second part of this experiment, semen was collected from Ross males, pooled, and equally divided so that there would be a viable and nonviable sample. The viable sample was diluted 1:1 with MEM and stored at 37°C on a rotary shaker, whereas the sperm in the nonviable sample were lysed by repeated freezing and thawing for 2 h. Semen was thawed and refrozen at 20, 40, 60, 90, and 120 min, and sperm were confirmed to be dead by using the fluorometric method of Bilgili and Renden (1984). Thawed semen was then diluted 1:1 with MEM. Viable and nonviable semen samples were combined to attain 0, 20, 40, 60, 80, and 100% viable sperm. These samples were analyzed as described in experiment 2. The experiment was replicated 4 times on different days.

Experiment 4

Semen was collected from Ross males and pooled. Pooled semen was divided into 2 equal halves. One half was diluted 1:1 with 0.85% saline and the other half was diluted 1:1 with 1,300 mg/L of $ZnSO_4$, a known motility inhibitor. Both treatments were incubated at 37°C on a rotary shaker and analyzed over 2 h of incubation. Two SQI, SMI, and fluorometer readings were taken at 0, 15, 30, 45, 60, 75, 90, 105, and 120 min from the same dilution for each treatment as described in experiment 2. The experiment was replicated 4 times on different days.

Statistical Analysis

Data from all experiments were analyzed in randomized complete block designs, with replicates over time as blocks. Means were separated using Fisher's protected least significant difference at $\alpha < 0.05$. Regression analyses were used to reveal the relationship of the SQI and SMI with motile and immotile sperm over incubation time in experiments 1 and 4. Regression analyses were also used in experiments 2 and 3 to determine the relationship of the SQI and SMI with the percentages of active and viable sperm, respectively (Steel and Torrie, 1980).

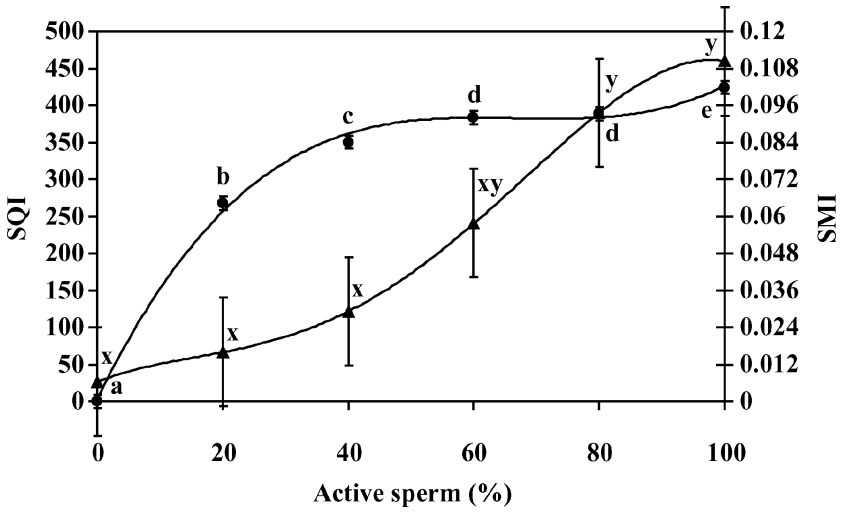

Figure 3. The relationship of sperm quality index (SQI) and sperm mobility index (SMI) with the percentage of active sperm from heat-inactivated immotile sperm in experiment 2. Each point represents the mean of 2 replicates from 4 pools of semen. [a–e]Points with different superscripts are significantly different for the SQI (●); and [x,y]points with different superscripts are significantly different for the SMI (▲; $P < 0.05$). A cubic relationship existed for the SQI with the percentage of active sperm ($y = 0.0013x^3 - 0.263x^2 + 17.4x + 3.4$, $r^2 = 0.99$, $P < 0.0037$) and a quartic relationship existed for the SMI ($y = -5E{-}09x^4 + 8E{-}07x^3 - 3E{-}05x^2 + 0.0008x + 0.0063$, $r^2 = 0.99$, $P < 0.004$).

RESULTS

Experiment 1

The relationship of the SQI from active and heat-inactivated immotile sperm over incubation is presented in Figure 1a. At each incubation period, the SQI from active sperm was significantly higher than that of heat-inactivated immotile sperm. For active sperm, there was a slight numeric increase in SQI values during the first 5 min of incubation, and then the SQI stabilized. The SQI from heat-inactivated immotile sperm remained at zero throughout incubation. There was a quartic relationship over incubation for the SQI from active sperm. However, no relationship with incubation time existed for the SQI from heat-inactivated, immotile sperm.

The response of the SMI from active and heat-inactivated immotile sperm over incubation is presented in Figure 1b. There was a steady increase in SMI values from active sperm over incubation resulting in a quartic relationship. For heat-inactivated, immotile sperm, the SMI was initially similar to that of active sperm up to 3 min of incubation. From 5 to 20 min of incubation, the SMI from heat-inactivated sperm decreased yielding values that were significantly lower than active. However, a drastic increase was noted for the SMI from heat-inactivated immotile sperm after 15 min of incubation. As a result, SMI values from heat-inactivated immotile sperm were numerically higher

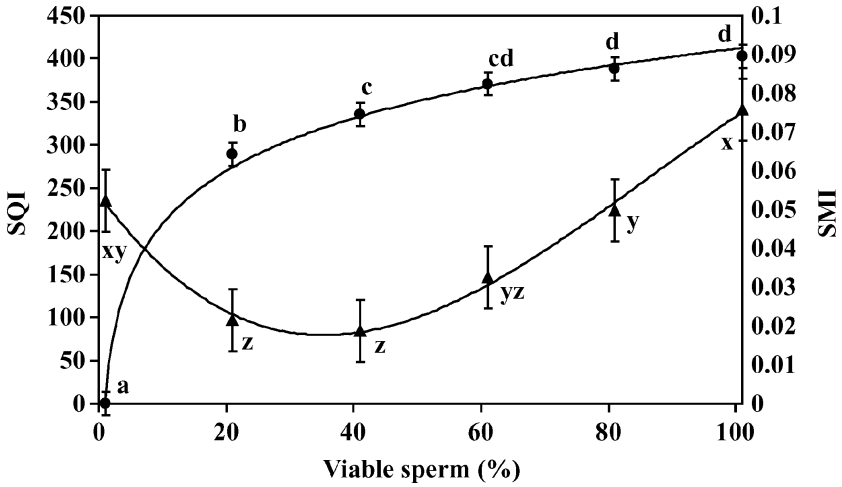

Figure 4. The relationship of sperm quality index (SQI) and sperm mobility index (SMI) with the percentage of viable sperm from boiled sperm in experiment 3. Each point represents the mean of 2 replicates from 4 pools of semen. [a–d]Points with different superscripts are significantly different for the SQI (●) and [x–z]points with different superscripts are significantly different for the SMI (▲; $P < 0.05$). A logarithmic relationship existed for the SQI with the percentage of viable sperm ($y = 88.15\text{Ln}(x) + 5.65$, $r^2 = 0.99$, $P < 0.0001$) and a cubic relationship existed for the SMI ($y = -2E{-}07x^3 + 4E{-}05x^2 - 0.0022x + 0.054$, $r^2 = 0.99$, $P < 0.007$).

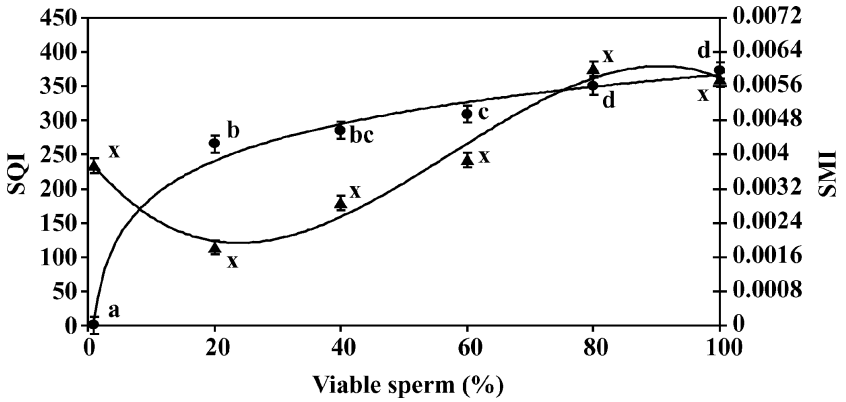

Figure 5. The relationship of the sperm quality index (SQI) and sperm mobility index (SMI) with the percentage viable sperm from frozen sperm in experiment 3. Each point represents the mean of 2 replicates from 4 pools of semen. [a–d]Points with different superscripts are significantly different for the SQI (●; $P < 0.05$). A logarithmic relationship existed for the SQI with the percentage of viable sperm ($y = 78.4\text{Ln}(x) + 4.06$, $r^2 = 0.98$, $P < 0.0001$) and a cubic relationship existed for the SMI (▲; $y = -3\text{E}{-}08x^3 + 5\text{E}{-}06x^2 - 0.0002x + 0.0037$, $r^2 = 0.98$, $P < 0.036$).

than that of active sperm after 30 min of incubation. In fact, the SMI values from heat-inactivated immotile sperm were significantly higher than the active values after 35 min of incubation. Over incubation, there was a cubic relationship for the SMI from heat-inactivated immotile sperm.

At each incubation period, the SQI from active sperm was significantly higher than that of boiled nonviable sperm (Figure 2a). There was a slight increase in SQI from active sperm over incubation time yet the SQI from boiled, nonviable sperm was zero or close to zero throughout incubation. Over incubation, a quartic relationship existed for the SQI from active sperm, yet no relationship existed for boiled, nonviable sperm over incubation.

The SMI from active sperm was significantly higher than that of boiled nonviable sperm at each incubation period (Figure 2b). The SMI from active sperm steadily increased over incubation resulting in a quartic relationship. However, for boiled, nonviable sperm there was an initial increase in SMI values during the first 30 min of incubation, followed by a descent, yielding a quadratic relationship over incubation.

Experiment 2

The relationship of the SQI and SMI with the percentage of active sperm is presented in Figure 3. For the SQI, there was a cubic relationship resulting in a significant increase in values from semen samples containing 0 through 60% active sperm and samples containing 80 to 100% active sperm. However, a quartic relationship existed for the SMI. As a result, there were no statistical differences in the SMI from semen samples with 0, 20, 40, and 60% active sperm. In fact, differences in the SMI did not occur until the semen samples contained 80% active sperm.

Experiment 3

Using boiled semen, there was a logarithmic increase in the SQI as the percentage of viable sperm increased (Figure 4). As a result, the SQI was zero and significantly lower for 0% viable sperm compared with 20, 40, 60, 80, and 100% viable sperm. However, for the SMI there was an initial decrease in values from 0 to 40% viable sperm followed by an increase at 60 through 100% viable sperm yielding similar SMI values for 0 and 100% viable sperm. A cubic relationship existed for the SMI with the percentage of viable sperm from boiled semen.

The relationship of the SQI and SMI with the percentage of viable sperm from combined viable and nonviable semen samples utilizing nonviable sperm from frozen semen is presented in Figure 5. As the percentage of viable sperm increased, there was a logarithmic increase in the SQI. As a result, the SQI was again zero and significantly lower for 0% viable sperm as opposed to 20, 40, 60, 80, and 100% viable sperm. In experiment 3, SQI and SMI values were less than in the previous experiment. A cubic relationship was noted for the SMI with the percentage of viable sperm from frozen semen. As a result, Fisher's protected least significant difference test yielded no significant difference in the SMI values for any percentage of viable sperm.

Experiment 4

The relationship of the SQI from semen incubated in 0.85% saline with that of semen incubated in $ZnSO_4$ is shown in Figure 6a. At each incubation period, the SQI from semen incubated with 0.85% saline was significantly higher than that of $ZnSO_4$. For saline, the SQI values increased in the first 15 min of incubation and were stable afterward, yielding a cubic relationship. The SQI from semen incubated with $ZnSO_4$ increased slightly in the first 30 min of incubation. After 30 min of incubation, the SQI decreased significantly throughout incubation producing a quadratic relationship for semen incubated with $ZnSO_4$.

The relationship of the SMI from semen incubated in 0.85% saline with that of semen incubated in $ZnSO_4$ over incubation is presented in Figure 6b. During the first 60 min, the SMI from semen incubated with 0.85% saline was significantly higher than semen incubated with $ZnSO_4$. For the SMI from semen incubated with 0.85% saline, there

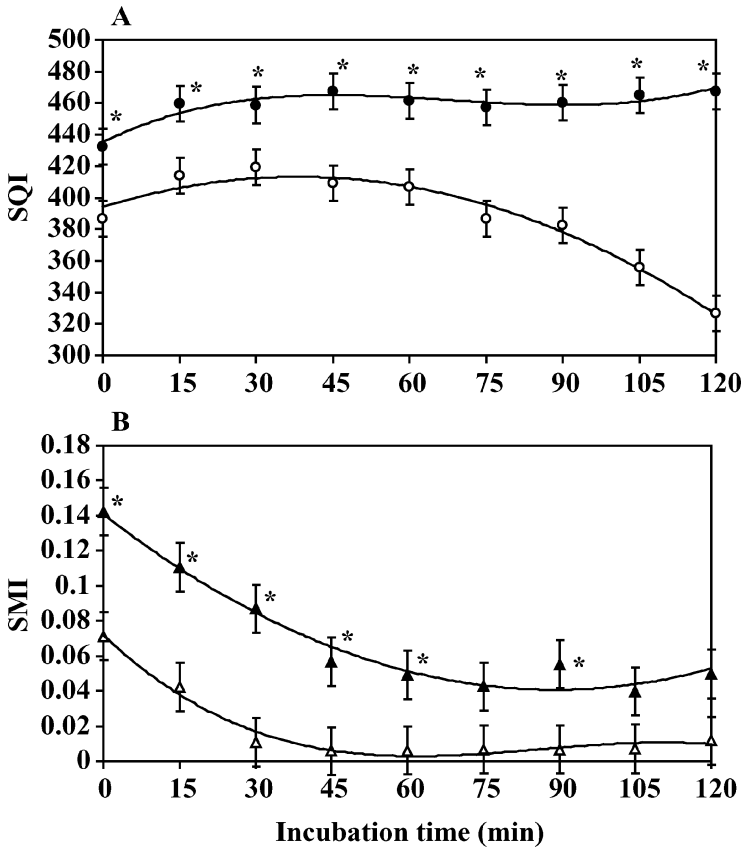

Figure 6. The relationship of semen incubated with 0.85% saline and $ZnSO_4$ over incubation in experiment 4 for A) sperm quality index (SQI), and B) sperm mobility index (SMI). Each point represents the mean of 2 replicates from 4 pools of semen. A cubic relationship existed for both the SQI (●) of saline and SMI of $ZnSO_4$ (△) ($y = 0.0001x^3 - 0.03x^2 + 1.6x + 435$, $r^2 = 0.88$, $P < 0.009$, and $y = -1E{-}07x^3 + 3E{-}05x^2 - 0.003x + 0.07$, $r^2 = 0.98$, $P < 0.0001$, respectively). For both the SQI from $ZnSO_4$ (○) and the SMI from saline (▲), a quadratic relationship existed ($y = -0.01x^2 + 1.0x + 394$, $r^2 = 0.96$, $P < 0.0001$, and $y = 1E{-}05x^2 - 0.0022x + 0.14$, $r^2 = 0.97$, $P < 0.0001$, respectively). *Indicates a significant difference between the active and $ZnSO_4$-inactivated sperm for each incubation period.

was a steady decrease during the first 75 min of incubation, and then the values stabilized. However, the SMI values from semen incubated with $ZnSO_4$ fell rapidly during the first 30 min of incubation. After 30 min, these values stabilized. For the SMI from semen incubated with 0.85% saline, a quadratic relationship existed, whereas a cubic relationship existed for the SMI from semen incubated with $ZnSO_4$.

DISCUSSION

Both the SQI and SMI are indicative of sperm motility (Froman and McLean, 1996; McDaniel et al., 1998). However, the principle for measuring sperm motility is different for each technique. For example, the SMI is obtained by measuring the density of sperm as it penetrates a viscous solution, whereas sperm penetration is not utilized to determine the SQI. Instead, the SQI is generated by monitoring the disruptions of a light path, which are created by the movement of motile sperm.

To determine the SMI, semen must be overlaid on Accudenz and incubated for 5 min (Froman and McLean, 1996). However, in experiment 1, the SMI was obtained by reading the same cuvettes incubated up to 60 min. This was done to investigate if changes occur in the SQI and SMI from active, motile and heat-inactivated, immotile sperm over incubation as well as viable and nonviable sperm over incubation. In experiment 1, the SQI from active, motile and heat-inactivated, immotile sperm remained almost constant throughout incubation, but this was not true for the SMI. Sperm motility, as determined by the SQI, was constant over incubation because the SQI remained around 450 units throughout the incubation period. The SQI of active sperm did increase very slightly in the first 5 min of incubation, most likely due to the availability of oxygen in the diluent (Parker and McDaniel, 2006). However, there was a steady increase in the SMI from active sperm as incubation increased. Apparently, more sperm were able to penetrate the Accudenz suspension over time. When using 25 m*M* of glucose in their motility buffer, Froman and McLean (1996) reported a sudden increase in SMI during the first 8 to 10 min of incubation followed by a very slight increase after 10 min of incubation. However, this sudden increase in the SMI was not observed in the present study. Perhaps the SMI responded differently in this trial due to the lack of glucose in the motility buffer (Froman, 1997; Froman et al., 1999). Unlike the present study, Froman and McLean (1996) reported zero for the SMI from heat-inactivated sperm. The differences in the SMI from the heat-inactivated sperm may also be due to different strains of roosters used for semen donation.

For the heat-inactivated sample, sperm were confirmed immotile by microscopy. For this treatment, the SQI was zero at each period, indicating that sperm were immotile throughout incubation. However, at the recommended 5 min of incubation (Froman, 1997), the SMI from heat-inactivated sperm was greater than zero, although the SMI from heat-inactivated, immotile sperm was significantly lower than the SMI from active, motile sperm. Also at the recommended 5 min of incubation, the SMI from heat-inactivated, immotile sperm was 25% of the SMI from active, motile sperm. In fact, the SMI of heat-inactivated sperm was higher than active sperm after 30 min of incubation and remained higher throughout the rest of the incubation period. At each incubation period, the cuvettes for both active and heat-inactivated sperm were visually inspected. This inspection revealed that the cuvettes containing active semen were almost clear. However, after 15 min of incubation, cuvettes containing heat-inactivated sperm were cloudy and contained small masses. The samples became even cloudier after 30 min of incubation. Because the percentage of dead sperm was slightly higher for heat-inactivated sperm as opposed to the active samples (15 and 8%, respectively; data not shown), it is possible that these masses were dead sperm or the agglutination of dead sperm. It is also possible that these masses were subcellular fragments released through the broken membranes of dead sperm. Because the heat-inactivated samples contained these masses and the active samples did not, it is possible that, due to gravity, these masses were able to penetrate the Accudenz suspension. As a result of this penetration, these masses were able to interact with the photometer light path. If the masses were able to penetrate the Accudenz suspension at a faster rate than active sperm, this could explain why the SMI from the heat-inactivated sample was higher than that of active sperm samples after 30 min of incubation. It is not uncommon for rooster semen to contain 10 to 15% dead sperm, as was seen in the heat-inactivated sample (Parker and McDaniel, 2003). Perhaps even small increases in the percentage of dead sperm, as was noted in experiment 1, may elevate the SMI.

Active, viable and boiled, nonviable sperm were also incubated over time in experiment 1. In this study, the SQI and SMI from viable samples as well as the SQI from the nonviable sperm responded in a similar manner to that of active and heat-inactivated sperm. However, even though sperm were confirmed dead, the SMI from the boiled semen was greater than 0 at 5 min of incubation and remained above zero throughout incubation. At each time point, the SMI cuvettes were visually inspected. Similar to the cuvettes containing active and heat-inactive immotile sperm, the cuvettes containing viable sperm were almost clear with no evidence of any masses, whereas the cuvettes with boiled nonviable sperm were cloudy and contained large masses and small particles. When comparing the cuvettes containing heat-inactivated immotile samples to that of boiled nonviable samples, the masses appeared larger for the boiled, nonviable samples. Initially, there was a similar increase in the SMI from boiled, nonviable semen when compared with heat-inactivated sperm. However, unlike the SMI from heat-inactivated sperm, which became higher than that of active after 30 min of incubation, there was a decrease in the SMI from boiled, nonviable sperm after 30 min. The visual inspection of the cuvettes containing boiled, nonviable sperm revealed that these cuvettes became cloudier as the incubation period increased. However, after 30 min of incubation, the larger masses found in the boiled, nonviable samples appeared to gravitate to the bottom of the cuvettes at a much faster rate than those observed in the heat-inactivated samples. Because these larger masses gravitated to the bottom of the cuvette there was no interaction between these masses and the photometer light path, whereas the masses found within the heat-inactivated samples remained in the light path. Even though the larger masses from boiled, nonviable semen fell to the bottom of the cuvettes, there were still several small particles that remained and interacted with the light path. Because these particles were able to interact with the light path, the SMI was affected, resulting in values above zero.

As the percentage of active sperm increased in experiment 2, the SMI was less responsive to sperm motility than was the SQI. In fact, there were no significant changes in the SMI from semen containing increasing levels of active sperm until the samples reached 80% active sperm, yet it is apparent that the SQI increased as the percentage of active sperm increased from 0 to 100%. However, the SMI from heat-inactivated, immotile sperm containing 0% active sperm was greater than zero. Upon visual inspection of the SMI cuvettes, micro masses were again observed in samples containing 0 through 60% active sperm; as the percentage of active sperm increased, there was a decrease in the number of micro masses that could interact with the photometer light path. In this study, the percentage of dead sperm in the heat-inactivated sample was higher than that of the active sample (18.6 and 9.6%, respectively; data not shown). Therefore, it is possible that dead sperm and subcellular fragments released from broken cell membranes interacted with the photometer light path resulting in inflated SMI readings. Unlike in experiment 1 in which the SMI cuvettes were read over time, in this trial the SMI cuvettes were only incubated for 5 min. Therefore, the micro masses found in experiment 2 would not affect SMI readings as severely as those reported in experiment 1.

Unlike the SMI, there was a significant rapid increase in the SQI as the percentage of active sperm increased from 0 to 100% active sperm. This increase in the SQI is due to the fact that more motile sperm are available to disrupt the light path, resulting in higher SQI values as the percentage of motile sperm increases (McDaniel et al., 1998; Parker and McDaniel, 2003).

In experiment 3, when using boiled or frozen semen combined with viable semen samples, the response of the SQI was compared with the SMI as the percentage of viable sperm increased. Whether using boiled or frozen sperm, there was a logarithmic increase in the SQI yielding significantly higher values as the percentage of viable sperm increased. The results of this study were similar to the results reported by Parker and McDaniel (2003). They re-

vealed that there was a logarithmic increase in the SQI as the number of live sperm increased.

However, the response of the SMI was different as the percentage of viable sperm increased when using boiled and frozen semen in combination with viable semen samples. For example, the SMI obtained from samples containing boiled semen revealed that there were no statistical differences in values for samples having 0 and 100% viable sperm. In addition, the SMI values obtained using frozen semen were statistically the same as the percentage of viable sperm increased from 0 through 100%.

When using samples containing boiled semen, visual inspection of the SMI cuvettes containing 0 through 60% viable sperm revealed that these cuvettes were turbid and contained small masses. In this study, the 0% viable sample contained the largest quantity of these masses. However, as the percentage of viable semen increased, there was a decrease in the occurrence of small masses. Even though these masses were also observed in the 20 and 40% viable samples, the SMI values were significantly less than that with 0% viable sperm. Because the SMI was higher for 0% viable sperm than for those with 20 or 40% viable sperm, it is likely that these masses were able to interact with the light path and inflate SMI values for the samples with 0% viable sperm. Apparently the masses from the 0% viable samples are able to penetrate the Accudenz suspension at the same rate or faster than the motile samples containing 20 through 80% viable semen as evidenced by the lower SMI readings for 20 through 80% viable semen. For this study, it appears that the SMI is responsive to motility when semen samples contain more than 60% viable sperm. However, it seems that something other than motile sperm are capable of increasing the SMI from semen containing 0 to 40% viable sperm when mixing boiled sperm with active semen.

For semen containing sperm killed by freezing, there was no statistical difference in SMI readings obtained from 0 through 100% viable sperm. For this experiment, an overall reduction in sperm motility regardless of treatment was noted, as evidenced by a 50-unit decrease in the SQI and a 10-fold reduction in SMI values as compared with previous experiments. These lower values were most likely due to extended semen handling time. For this part of the experiment, semen was frozen for 2 h to kill the sperm. As a result, the viable sample was maintained at room temperature during the freezing process. Because the viable sample was stored for 2 h before mixing with the nonviable sample, it appears that this caused a reduction in sperm motility.

As in the first part of experiment 3, the SMI cuvettes containing the highest percentages of nonviable sperm were cloudy with tiny masses, and the occurrences of these masses decreased as the percentage of viable sperm increased. Irrespective of the percentage of viable sperm, similar SMI readings were noted for samples containing from 0 to 100% viable sperm. This would indicate that the masses from the frozen semen samples were passing through the Accudenz suspension at the same rate as samples containing viable motile sperm.

For the 0% viable sperm samples in experiment 3, the SMI from semen samples containing sperm killed by boiling was approximately 10 times higher than for that containing sperm killed by freezing. It is possible that sperm killed by boiling may agglutinate more than frozen sperm, thus forming bigger masses, which are able to penetrate the Accudenz at a faster rate due to gravity.

In experiment 1, semen samples were incubated for 60 min. As mentioned previously, the increase in SMI values for heat-inactivated sperm was most likely due to the micro masses and subcellular particles as a result of sperm death or agglutination that were found within the SMI cuvettes as the incubation period progressed. However, in experiments 2 and 3, semen samples were incubated for only 5 min, which is perhaps enough time for these micro masses and subcellular particles to interact with the light path and elevate SMI readings. This is supported by the fact that SMI readings were greater than zero for semen samples containing both 0% active and 0% viable sperm. In fact, when comparing the SMI from 0% active to that of 0% viable sperm, the values are higher for 0% viable sperm indicating that dead sperm may have the greatest impact on the SMI.

To determine how the SQI and SMI would respond to chemically reduced sperm motility, semen was incubated with $ZnSO_4$ in experiment 4. For semen incubated with $ZnSO_4$, both the SQI and SMI were able to detect a reduction in sperm motility as demonstrated by readings that were significantly lower than the values from semen incubated with 0.85% saline. However, even though both methods were sensitive to the reduction in sperm motility, the SMI from semen incubated with $ZnSO_4$ was significantly lower only during the first 60 min of incubation, whereas the SQI from semen treated with $ZnSO_4$ was significantly lower for the entire 2-h period. In this trial, the depression in SQI values obtained from semen incubated in $ZnSO_4$ was similar to that reported by Barber et al. (2005).

The SQI from semen treated with 0.85% saline was constant over incubation indicating that there was no change in sperm motility. However, there was a steady decrease in the SMI obtained from semen incubated with 0.85% saline over incubation. To obtain the SMI, semen must be diluted with TES, which contains Ca^{2+}, a known stimulator of sperm motility (Wishart and Ashizawa, 1987). The observed decrease in the SMI is most likely due to the fact that 0.85% saline contains no Ca^{2+} to stimulate motility or any energy source used to maintain motility (Parker and McDaniel, 2006). When incubating semen with TES for up to 30 min, Froman and Feltman (2005) reported that sperm motility was independent of time. The results of the present study suggest that the SMI and sperm mobility are rapidly affected by the length of incubation when semen is stored in 0.85% saline; however, SQI is independent of time.

In each experiment, when comparing the variation in the SQI to that of the SMI, it appears that there is more variation within semen samples when using the SMI. However, there was less variation in the SMI in experiment 3 than in other experiments. In this study, when mixing viable and frozen semen, there were no differences in the

SMI from 0 through 100% viable samples and the readings were 10-fold lower than the readings obtained after mixing viable and boiled semen. The viable sample was stored for 2 h before testing, which could explain why the variation was less for the SMI, as it has been determined that the ability of the SMI to measure sperm motility is dependent on time. Interestingly, the greatest variation in the SMI occurred when mixing known percentages of active semen with heat-inactivated immotile semen in experiment 2. Because the amount of variation was so large, it appears that immotile sperm can also cause erratic SMI values similar to that of dead sperm.

Several possibilities may explain the variation observed using the SMI technique. The first reason is due to the technique itself. For example, SMI readings can be greatly influenced by crucial steps such as diluting semen to a constant number of sperm. If an error occurs when pipetting semen to a constant number of sperm, the diluted samples can have either too many or too few sperm. As a result, the amount of sperm in the diluted sample could affect the number of sperm that would be available for penetration. Also, extreme care must be taken when overlaying the diluted semen samples onto the Accudenz suspension. If care is not taken, SMI values could be erroneous. There are numerous steps to follow to generate the SMI. However, for the SQI, semen is simply diluted to a constant 10-fold volume before loading a capillary that is inserted into the sperm quality analyzer.

In summary, there were differences and similarities when comparing the results of the SQI with those of the SMI. For example, in experiment 1, the SQI for the active and viable sample was stable over incubation whereas the SMI showed a steady increase. For the heat-inactivated, immotile and nonviable sample, the SQI was zero throughout incubation. However, the SMI from heat-inactivated, immotile and nonviable samples did not remain at zero; instead, these values increased with incubation. There was a similar response for the SQI in experiments 2 and 3, but the response was different in each experiment for the SMI. For the SMI in each experiment, the 0% active and 0% viable samples were all above zero after 5 min of incubation and the values from 0% viable samples were similar to that of the 100% viable samples. In experiment 4, both methods responded to reductions in sperm motility when semen was incubated with a known sperm motility inhibitor but they responded differently to semen incubated with 0.85% saline.

In conclusion, both the SQI and SMI are indicative of sperm motility. However, it appears that immotile and dead sperm are capable of increasing SMI but not SQI values. Therefore, when evaluating broiler breeder semen, it appears that the semen samples containing elevated dead or immotile sperm may yield inflated SMI values but accurate SQI values.

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