# Use of a Sperm Analyzer for Evaluating Broiler Breeder Males. 2. Selection of Young Broiler Breeder Roosters for the Sperm Quality Index Increases Fertile Egg Production[1,2]

H. M. Parker, J. B. Yeatman, C. D. Schultz, C. D. Zumwalt, and C. D. McDaniel[3]

*Poultry Science Department, Mississippi State University, Mississippi State, Mississippi 39762*

**ABSTRACT** Previous research has shown that the sperm quality index (SQI) of rooster semen is indicative of overall semen quality. The objectives of the present experiments were to determine the correlation of the SQI with semen characteristics and fertility and to determine if selection of young males for the SQI would improve fertility. In Experiment 1 semen was collected from 35 Peterson males and was analyzed individually for sperm concentration and viability. To determine fertility, 100 $\mu$L of diluted semen was inseminated into 10 hens for each rooster. Positive correlations of the SQI with total and live sperm concentrations as well as fertility were found. A negative correlation of the SQI with the percentage of dead sperm was observed. In Experiment 2, four semen samples were collected at 2- to 3-d intervals from each of 142, 27-wk-old Peterson roosters to determine their SQI. Males were then allocated to six treatment groups based on their average SQI readings as follows: 0 to 150, 151 to 200, 201 to 250, 251 to 300, 301 to 350, and >350. For each SQI group, semen was collected weekly for 8 wk, pooled, and used at a rate of 50 $\mu$L/hen to inseminate 40 hens. The percentage of fertilized eggs increased linearly across the SQI groups, from a minimum of 65% for the 0 to 150 SQI group to a maximum of 98% for the >350 SQI group. The SQI groups of 301 to 350 and >350 produced the slowest decline in fertility over days postinsemination. Therefore, selection of males for the SQI at an early age appears to improve flock fertility.

(*Key words*: sperm quality, fertility, semen, broiler breeder, hatchability)

2000 Poultry Science 79:771–777

## INTRODUCTION

Fertilization potential of the broiler breeder rooster is dependent upon several semen quality characteristics, including sperm concentration, viability, and motility. A fault in any one of these three semen characteristics can result in a subfertile rooster. Individually, however, none of these semen characteristics provide an overall view of a male's true fertilizing potential. If many semen characteristics were considered collectively as an overall index of semen quality, the correlation of this index with fertility could be stronger than any single semen characteristic. The sperm motility index appears to be such an indicator of male fertility (McDaniel et al., 1998a).

The sperm motility index, as evaluated by the Sperm Quality Analyzer,®[4] is a single number that provides an estimate of overall sperm quality and quantity in rooster (Wishart and Wilson, 1997; McDaniel et al., 1998a,b) and mammalian semen (Bartoov et al., 1991; Johnston et al., 1995). The Sperm Quality Analyzer® measures the sperm motility index by passing a light beam through a semen sample and quantifying the amplitude and frequency of disruption of that light beam by sperm movement. The sperm motility index can be obtained very quickly (requires only 40 s) and easily (requires only semen dilution) for a semen sample (Bartoov et al., 1991; Johnston et al., 1995; McDaniel et al., 1998a,b). Recently, the sperm motility index revealed the degree of sperm movement in rooster semen samples (McDaniel et al., 1998b). McDaniel and colleagues have shown that, after rooster semen is pooled and then diluted, frozen, or incubated anaerobically, the sperm motility index decreases as sperm concentration, viability, and motility decrease, respectively. This research indicates that sperm concentration, viability, and motility all contribute to the sperm motility index. Because this index is influenced by more than just sperm motility, a more appropriate name for the sperm motility index would be the sperm quality index (SQI). Therefore, in subsequent discussion this term will be used.

Received for publication July 7, 1999.

Accepted for publication January 28, 2000.

[1]This is Journal Article No. J9613 from the Mississippi Agriculture and Forestry Experiment Station supported by MIS-2994.

[2]Use of trade names in this publication does not imply endorsement by the Mississippi Agriculture and Forestry Experiment Station of the products nor of similar ones not mentioned.

[3]To whom correspondence and reprint requests should be addressed: cmcdaniel@poultry.msstate.edu.

[4]Introtech, San Diego, CA 92122.

**Abbreviation Key:** SQI = sperm quality index.

Two experiments were conducted to evaluate the relationship of the SQI with broiler breeder male fertility. In the first experiment, research was conducted to determine the correlation of the SQI from individual males with semen characteristics (i.e., sperm viability and concentration) and fertilized egg production. In addition, because the SQI can be obtained for a male within 40 s and requires no laborious steps or toxic chemicals (McDaniel et al., 1998b), we reasoned that if the SQI is correlated with rooster fertility, it may be a useful tool for selecting broiler breeder males in the poultry industry for their fertilizing potential. Currently, roosters are selected for their fertilizing potential based upon their physical appearance. Research has shown that the physical appearance of a rooster is not always correlated with his fertilizing potential (Wilson et al., 1979; Amann, 1999). Therefore, the objectives of the second experiment were threefold. The first objective of Experiment 2 was to determine the distribution of the SQI in a population of young broiler breeder roosters. The second goal was to determine if selection of young broiler breeder roosters for the SQI would improve semen quality and the percentage of fertilized eggs produced. Our third and final objective for Experiment 2 was to evaluate the rate of decline in fertilized egg production following insemination with semen from males with different SQI values.

## MATERIALS AND METHODS

### Experiment 1

***Housing and Environment.*** Thirty-five Peterson broiler breeder males, ranging from 25 to 55 wk of age, were obtained from a local integrator and housed in individual cages. Three hundred fifty Dekalb Hy-Line 77 White Leghorn hens, 50 wk of age, were also housed in individual cages. Males were fed the Mississippi State University male breeder diet (3,080 kcal ME/kg, 13.9% CP, and 1% Ca) and feed was restricted according to the primary breeder's recommendations. Hens consumed the Mississippi State University layer diet ad libitum (2,860 kcal ME/kg, 14.5% CP, and 4% Ca) and were exposed to light from 0500 to 2100 h. All birds were caged in houses with conventional environmental controls.

***Semen Evaluation.*** Individual ejaculates were collected from each male. Semen samples were collected and analyzed in one afternoon, starting at 1300 h and ending at 1700 h. Sperm viability and concentration for each male's undiluted semen sample was evaluated twice by using the fluorometric method of Bilgili and Renden (1984). Because previous research has shown that undiluted rooster semen produces inaccurate SQI readings (McDaniel et al., 1998b), each semen sample was then diluted 10-fold with minimum essential media (Howarth, 1981). Two SQI readings were obtained with the use of the Sperm Quality Analyzer® for each diluted sample before artificial insemination (CV = 12.3%). Ejaculates were analyzed and inseminated within 30 min of collection.

***Insemination and Fertilization Evaluation.*** To evaluate the occurrence of embryonic development, 10 hens per male were artificially inseminated from 1400 to 1700 h with 100 $\mu$L of the diluted semen. For direct comparison, the same diluted ejaculate from each male was analyzed for SQI and used for insemination. Eggs were collected, labeled, and recorded 2 d after insemination. Approximately nine eggs were evaluated for fertility from each group of 10 hens. Eggs were incubated for 7 d and then broken to determine the occurrence of fertilization based upon macroscopic embryonic development.

***Statistical Analyses.*** Linear correlation analyses were used to examine the relationship of the SQI with sperm viability, total sperm concentration, live sperm concentration, and the percentage of fertilized eggs produced. Fertility was also linearly correlated with sperm viability and sperm concentration (Steel and Torrie, 1980).

### Experiment 2

***Housing and Environment.*** At 21 wk of age, 142 Peterson roosters were obtained from a local integrator and individually caged. Two hundred forty Hy-Line 77 Leghorn hens, 20 mo of age, were also individually caged. Males and hens received the same diet and lighting program as described in Experiment 1.

***Semen Evaluation.*** When the roosters were 27 wk of age, semen was collected from each male on 4 different d (Monday, Wednesday, Friday, and Monday) and was analyzed for the SQI. Each semen sample was diluted 10-fold with 0.85% NaCl prior to duplicate analysis for the SQI (McDaniel et al., 1998b). To provide the most accurate measure of each male's true SQI, the average SQI over the 4 d of semen collection, was obtained. Males were then allocated to six treatment groups based on the following SQI averages: 0 to 150, 151 to 200, 201 to 250, 251 to 300, 301 to 350, and >350. Ten males were selected at random from each treatment group or SQI selection group and were used for weekly inseminations. Each pool of semen was analyzed for sperm concentration and viability using the fluorometric method of Bilgili and Renden (1984).

***Insemination and Fertilization Evaluation.*** Weekly for 8 wk, semen was pooled within each group, analyzed for sperm concentration and viability, and then inseminated at a rate of 50 $\mu$L/hen. Each of the six pools of undiluted semen was used to inseminate 40 White Leghorn hens that were in four replicates of 10 hens each. Each week, eggs were collected from Days 2 to 8 postinsemination and were incubated for 7 d to determine the occurrence of fertilization. After insemination on the 8th wk of the experiment, duration of fertility was calculated for each hen group as the number of days of fertilized egg production until 2 consecutive d of infertile eggs.

***Statistical Analyses.*** Regression analyses were used to test the relationship of SQI selection with sperm concentration, sperm viability, live sperm concentration, and the number of sperm inseminated as well as fertility and the duration of fertility. To calculate regression equations,

**TABLE 1. Linear correlation coefficients of semen characteristics with the sperm quality index (SQI) and fertility in Experiment 1**

| Semen characteristic | n | SQI | Fertility (%)[1] |
|---|---|---|---|
| Total sperm concentration | 35 | 0.88** | 0.57** |
| Dead sperm (%) | 35 | –0.57** | –0.48** |
| Live sperm concentration | 35 | 0.91** | 0.61** |
| SQI | 35 | . . . | 0.73** |

[1]For each of 35 males, 10 hens were inseminated with 100 $\mu$L of 10-fold diluted semen. Two days after insemination, approximately nine eggs for each group of 10 hens were incubated and evaluated for the occurrence of fertilization.

**$P < 0.01$.

each SQI category was coded 1 to 6 according to SQI rank as follows: 1 = 0 to 150, 2 = 151 to 200, 3 = 201 to 250, 4 = 251 to 300, 5 = 301 to 350, and 6 = >350 (Steel and Torrie, 1980).

## RESULTS

### Experiment 1

Total sperm concentration, sperm viability, and live sperm concentrations were correlated ($P < 0.01$) with the SQI (Table 1). The SQI increased as the percentage of dead sperm decreased. In addition, the SQI increased with increasing concentrations of total and live sperm. Table 1 also shows the correlation of semen characteristics with fertility. As with the SQI, fertility increased with a decrease in the percentage of dead sperm and increased as total and live sperm concentration increased. Also as shown in Table 1, the SQI was positively correlated with rooster fertility. The correlation coefficient for the SQI-fertility relationship was larger than all other coefficients obtained for relationships of fertility with semen characteristics.

### Experiment 2

The SQI mean for the entire male population was 286 with an SEM of 5.71 (Figure 1). The population was skewed to the right, indicating that there was a large percentage of males with low SQI readings of <250. After grouping males by SQI values, most of the males (42%) were in the 301 to 350 SQI selection category (Figure 2). The lowest three SQI groups, 0 to 150, 151 to 200, and 201 to 250, in total, represented 22% of the population. Therefore, 78% of the population was included in the top three SQI groups, 251 to 300, 301 to 350, and >350, and the top two SQI groups, 301 to 350 and >350, represented 53% of the population.

Increased selection of males for the SQI resulted in a linear increase in total and live sperm concentration as shown in Table 2. Males that were selected in the >350 SQI group had the highest sperm concentration (5.75 billion sperm/mL) and the lowest percentage of dead sperm (9%). On the other hand, the sperm concentration from the lowest SQI group was 3.36 billion sperm/mL with 39% dead sperm. The sperm concentrations ranged from 4.34 to 4.44 billion sperm/mL, and the percentage of dead sperm ranged from 32 to 10% for the SQI groups 151 to 200, 201 to 250, 251 to 300, and 301 to 350. Linear increases were also noted for the amount of total and live sperm inseminated as SQI selection increased (Table 2). Again, the >350 SQI group had the most total and live sperm inseminated, and the 0 to 150 SQI group represented the least total and live sperm inseminated. All hen groups were inseminated with more than 103 million live sperm/wk. The SQI groups 151 to 200, 201 to 250, 251 to 300, and 301 to 350 had similar total sperm insemination doses, ranging from 217 to 222 $\times 10^6$ total sperm.

As the SQI increased above 150 units, a linear increase in fertilized egg production was noted (Figure 3). There was a very wide range (33% difference) in the percentage of fertilized eggs that were produced as a result of insemination with semen from each of the different SQI groups.

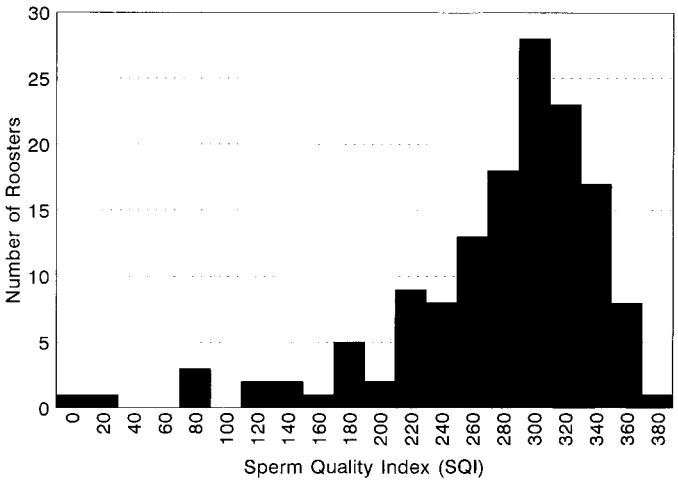


**FIGURE 1.** Distribution of the sperm quality index (SQI) in the 27-wk-old male population of Experiment 2. For ease of presentation, each bar represents the number of males (n = 142) grouped by every 20 units of SQI. Semen was collected from each male four times, and the four readings were averaged to obtain their SQI.

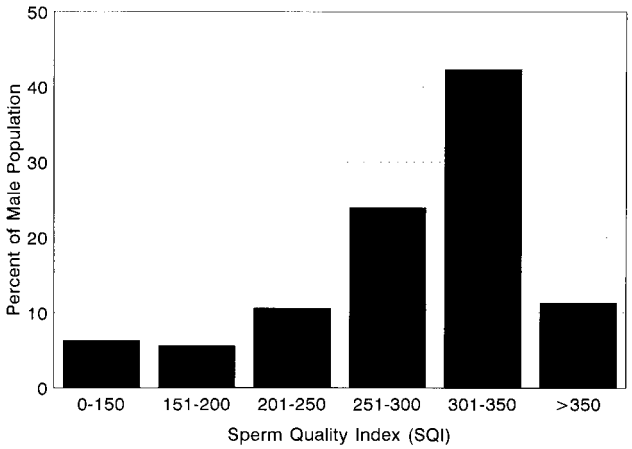


**FIGURE 2.** Distribution of males in each sperm quality index (SQI) group of Experiment 2. Each bar represents the percent of males in each SQI selection category. Semen was collected from each male four times, and the four readings were averaged to obtain their SQI.

**TABLE 2. Linear relationship of sperm quality index (SQI) selection with sperm concentration and the number of sperm inseminated in Experiment 2**

| | Sperm concentration[1] | | Sperm inseminated[2] | |
|---|---|---|---|---|
| SQI Category | Total | Live | Total | Live |
| | (billions/mL) | | (millions/hen) | |
| 0–150 | 3.36 | 2.05 | 168 | 103 |
| 151–200 | 4.34 | 2.93 | 217 | 147 |
| 201–250 | 4.36 | 3.16 | 218 | 158 |
| 251–300 | 4.44 | 3.70 | 222 | 185 |
| 301–350 | 4.34 | 3.91 | 217 | 196 |
| >350 | 5.75 | 5.21 | 288 | 261 |
| $P <$ | 0.03 | 0.01 | 0.03 | 0.01 |
| $r^2$ | 0.71 | 0.94 | 0.71 | 0.94 |
| Linear equation | y = 0.34 × + 3.2 | y = 0.55 × + 1.57 | y = 17.2 × + 1.62 | y = 27.5 × + 79 |

[1]Values represent the mean of semen samples collected and pooled from 10 males during each of 8 wk (n = 8).

[2]Undiluted semen was inseminated at the rate of 50 $\mu$L/hen. Values represent the mean of semen samples collected and pooled from 10 males during each of 8 wk (n = 8).

The weighted average for fertility of the total population was 90%.

There was a significant week of age by SQI category effect ($P < 0.0006$; data not shown). Statistically significant changes across weeks were only found for the 0 to 150 SQI group. This lowest SQI category did indeed have the most variation in fertility throughout this study, being lowest some weeks at 55% and peaking at 78% at 31 wk of age. The other five SQI groups showed no statistically significant changes over the 8 wk of the study. There was some variation in the other SQI categories, but they were able to maintain a fertility rate of 80% and greater. The highest SQI group, >350, maintained the best fertility rate followed by the 301 to 350 SQI group. In general, the fertility of each SQI group maintained its rank with regard to SQI category each week of the study (i.e., fertility of the >350 SQI group was the highest, followed by the 301 to 350 SQI group, etc., throughout the study).

As expected, a linear decline in fertility each day postinsemination was observed (Figure 4). However, this decline in fertility postinsemination was less drastic as SQI selection increased. Over the 8-d postinsemination period, the 301 to 350 and >350 SQI categories lost 5 and 2% fertility, respectively, and remained at 90% and greater fertility. The slopes for these two lines were significantly less than those from the 0 to 150, 151 to 200, 201 to 250, and 251 to 300 SQI groups. The SQI groups of 151 to 200,

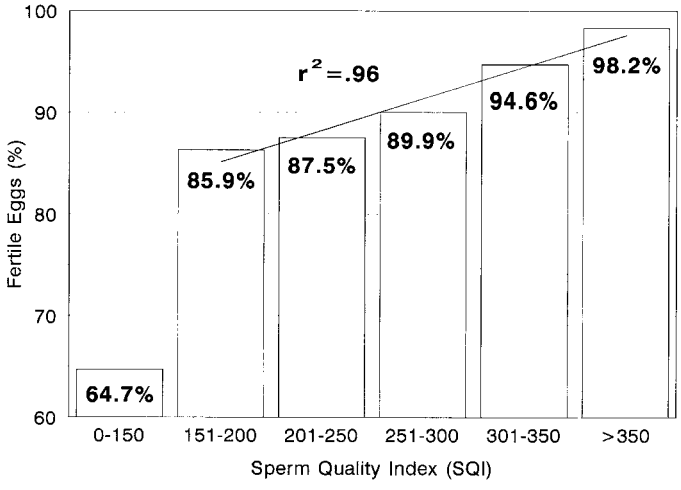


**FIGURE 3.** Relationship of sperm quality index (SQI) selection with fertility in Experiment 2. Each bar represents the SQI group mean over 8 wk of Days 2 to 8 postinsemination. There was a linear relationship between SQI and fertility. Fertility increased as the SQI increased (y = 3.18x + 78.54, $r^2 = 0.96$, $P < 0.0028$). Forty hens per SQI group were artificially inseminated with 50 $\mu$L of undiluted semen per hen (30 eggs laid per hen group per day).

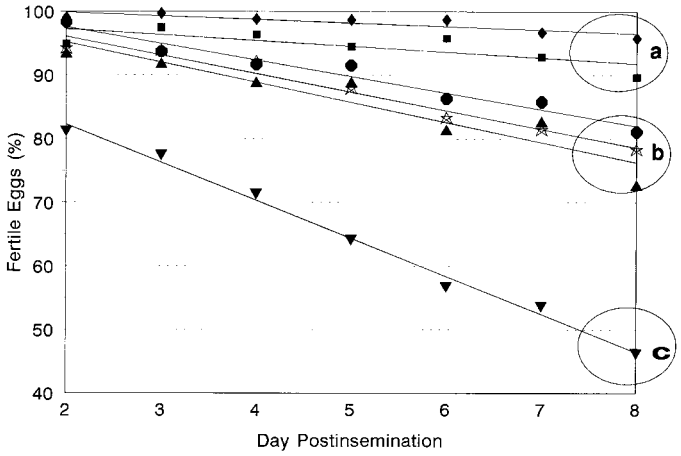


**FIGURE 4.** Relationship of sperm quality index (SQI) selection to fertility each day postinsemination in Experiment 2. Each point represents the SQI group mean for 8 wk. There is a linear decline in fertility each day postinsemination for all SQI groups. Lines labeled with the same superscript letter have similar slopes ($P < 0.05$). The linear slopes for the >350 (◆) and 301 to 350 (■) SQI groups are the same (y = –0.56x + 99.9, $r^2 = 0.75$, $P < 0.01$, and y = –.91x + 97.2, $r^2 = 0.58$, $P < 0.05$, respectively). The rate of decline in fertility for SQI groups 251 to 300 (●), 201 to 250 (☆), and 151 to 200 (▲) is significantly different from the other SQI groups (y = –2.6x + 97.6, $r^2 = 0.96$, $P < 0.0001$; y = –2.91x + 96.03, $r^2 = 0.96$, $P < 0.0001$; and y = –3.15x + 95.14, $r^2 = 0.88$, $P < 0.0018$, respectively). The rate of decline in fertility for the lowest SQI group, 0 to 150 (▼), was significantly different from all other SQI groups (y = –5.98x + 82.28, $r^2 = 0.99$, $P < 0.0001$). Forty hens per SQI group were artificially inseminated with 50 $\mu$L of undiluted semen per hen (30 eggs laid per hen group per day). Pooled SEM is 1.77.

**TABLE 3. Linear relationship of sperm quality index (SQI) selection to the duration of fertility in Experiment 2**

| SQI Category | Duration of fertility[1] |
|---|---|
| | (days) |
| 0–150 | 15.5 |
| 151–200 | 15.5 |
| 201–250 | 17.0 |
| 251–300 | 16.8 |
| 301–350 | 18.0 |
| >350 | 17.8 |
| $r^2$ | 0.85 |
| $P <$ | 0.009 |
| Linear equation | $y = 0.53 \times + 15$ |

[1]Forty hens per SQI group were each inseminated with 50 $\mu$L of undiluted semen (30 eggs laid per hen group per day). The duration of fertility was defined as the number of days of fertilized egg production until 2 consecutive d of infertile eggs.

201 to 250, and 251 to 300 had a sharper linear decline in fertility, and their percentage of fertilized eggs remained >75%. However, the linear decline in fertility expressed by these three SQI groups was significantly lower than that found for the 0 to 150 SQI category. The lowest SQI group, 0 to 150, fell approximately 30 percentage points to less than 50% fertility over the 8-d period.

Increased selection for the SQI also resulted in a linear increase in the total duration of fertility (Table 3). The duration of fertility was approximately 18 d for SQI groups 301 to 350 and >350, about 17 d for SQI groups 201 to 250 and 251 to 300, and 15.5 d for the lowest two SQI groups, 0 to 150 and 151 to 200.

## DISCUSSION

At the present, a majority of the poultry integrators choose males for house placement by judging physical characteristics for reproductive performance. Poultry breeders could benefit by selecting males for reproductive traits. Amman (1999) reported that a male should be able to produce and ejaculate sperm that is capable of fertilization. Presently, turkeys are culled if their semen has a watery appearance (Amman, 1999). Donoghue (1999) noted that management procedures should include sperm quality assessment. One possible way to achieve this objective is to develop a test that will quickly and correctly identify a fertile male. However, according to Donoghue (1999), most tests developed to assess sperm quality are time consuming, labor intensive, and do not always predict fertility. The SQI can be obtained quickly (40 s) and easily, and it provides valuable information for male fertility selection (McDaniel et al., 1998a,b). According to Hammerstedt (1999), industry should focus on producing the most fertilized eggs possible from the breeder stock available.

Three important semen characteristics necessary for the fertilization of an egg are sperm concentration, viability, and motility. If there is a defect in any one of these characteristics, fertility is negatively affected. The ovum of the hen has to be penetrated several times by sperm to ensure a fertilized egg (Bramwell et al., 1995). Males with a higher SQI have more viable, motile sperm per ejaculate, which would be available for egg penetration. McDaniel and coworkers (1998b) have shown that the SQI gives an accurate overall view of avian sperm concentration, viability, and motility. By using bull semen, Zavos and colleagues (1996) found that the motility of sperm diluted in a hypotonic media was correlated with the SQI. Bartoov et al. (1991) and Johnston et al. (1995) observed very similar results in humans. In addition, Experiment 1 revealed that the SQI was directly correlated with sperm concentration, sperm viability, and fertility.

McDaniel and colleagues (1998b) demonstrated that the SQI declined as the percentage of dead sperm increased when total sperm concentration was held constant for all samples. In the present study, the weaker correlation of the percentage of dead sperm with the SQI is logical when one considers that the Sperm Quality Analyzer® measures the amount of sperm movement across a light path. For example, a semen sample with no dead sperm but very few total sperm would result in very little apparent movement across the light path and a low SQI reading. A similar SQI reading could also be obtained for a sample with a large percentage of dead sperm and an enormous sperm concentration. Therefore, two samples with the same concentration of motile, live sperm and not necessarily the same percentage of live sperm would produce the same SQI. Because only motile, live sperm cells are capable of fertilizing the egg, the SQI holds promise as a male fertility selection tool.

The correlation coefficient for the SQI-fertility relationship (r = 0.73) was greater than all other coefficients obtained for correlations of individual semen characteristics with fertility. These data indicate that the SQI provides more information about the fertilizing ability of rooster sperm than do any of these single semen characteristics. This larger coefficient obtained for the correlation of the SQI with fertility is most likely a result of sperm concentration, viability, and motility all contributing to the SQI, because all three of these sperm characteristics are necessary for fertilization of the hen ovum. Results for live sperm concentration produced the second largest correlation coefficient with fertility in this experiment (r = 0.61), most likely because both sperm concentration and viability are included within the variable live sperm concentration.

The use of the SQI offers new possibilities in selecting males for predicting future fertility. The average SQI for the population of males used in Experiment 2 was 286, which was in the 251 to 300 SQI selection category. This SQI group had an average fertility rate of 90%. Not only was the average male fertility 90%, the overall weighted average across all SQI groups was also 90% fertilized egg production. Therefore, if males in this study had not been selected for the SQI, the fertilization rate would have been 90%. In the present study, the upper 78% of the males, those with an SQI >250, produced, on average, 94% fertilized eggs each week of the study. The males with an SQI >300 represented 53% of the population, and their

percentage of fertilized eggs was 95%. On the other hand, males with a SQI <250 represent 22% of the population with a weighted average for fertility of 81%. Elimination of the 0 to 150 SQI group from the overall male population could increase fertility by 3 percentage points, from 90 to 93%. A 4% increase in fertility, 90 to 94%, could be realized if males were selected for a SQI value >250, the upper 78% of the population. By using artificial insemination, this study has shown that, by selecting a male for the SQI, his fertilizing capabilities can be predicted, and overall flock fertility can be improved.

Other methods have also been developed for selecting males for fertilizing potential. Froman and colleagues (1999) observed that the Accudenz® method of determining sperm mobility was very accurate at predicting male fertility, especially within the range of 0 to 75% fertility. Froman and Feltmann (1998) showed that by categorizing New Hampshire roosters as average or high mobility, fertility rates differed by 30%: 64 to 94%, respectively. Average males were within one standard deviation below and high males were one and one-half standard deviations above the sperm mobility mean. They also found a similar frequency distribution for sperm mobility as was found in the present study for the SQI. Donoghue and colleagues (1998) used a sperm-mobility assay to predict fertilizing potential of turkey toms. In their first trial, by selecting toms for low or high mobility, they obtained differences in fertility ranging from 90 to 96%, respectively. In their second trial, the fertility rate of low mobility males was 82% compared with 89% for the high mobility males. They also noted the importance of the sperm's ability to traverse the vagina and reach the sperm storage tubules for fertilization to be successful. Also with turkeys, Gill and colleagues (1999) have developed a sperm-binding assay to predict fertility. By using their method and categorizing toms as low or high by their sperm-binding ability, they found a difference in fertility from 78 to 84%, respectively, when using fresh semen. Therefore, Gill and coworkers (1999) illustrated that sperm motility is not the only sperm characteristic responsible for fertilization.

The SQI cannot evaluate male libido, but it can afford the portion of the poultry industry that uses natural mating an opportunity to place fertile males in their breeder houses. It is also possible that by selecting males for the SQI, industry could use a lower male to female ratio, allowing for a maximized fertilizing potential and profitability. With only fertile males in a breeder house, even if hens are mated less frequently, it is probable that they will produce a high percentage of fertilized eggs because the efficacy of each male's ejaculate would be excellent. If the lowest SQI males are also very aggressive, they could be responsible for a disproportionate number of matings in the hen house, causing an even greater decrease in fertility. Therefore, removal of these subfertile males could substantially improve fertility. However, if males with an SQI >300, which represents 53% of the population, are responsible for 100% of the matings, then removal of males with an SQI <300 from the flock would not improve fertility. Research using the SQI and natural mating should answer these questions.

The research in Experiment 2 indicates that when males were selected and grouped by SQI, there was a linear relationship of the SQI to live sperm concentration, live sperm insemination dose, and fertility. It is interesting to note that hens of the 251 to 300 and 301 to 350 SQI groups were inseminated with almost the same number of live sperm, 185 and 196 $\times 10^6$ (Table 2), yet fertility of these two groups differed by almost 5% (Figure 3). Also note that SQI groups 151 to 200, 201 to 250, 251 to 300, and 301 to 350 received total sperm insemination doses of 217, 218, 222, and 217 $\times 10^6$, respectively, yet there was a difference of nine percentage points, 86 to 95%, in fertility. These results indicate how the SQI can accurately separate males of differing fertilizing potentials that cannot be separated using individual semen characteristics such as sperm concentration and viability as fertility predictors.

These results indicate how semen samples of similar total or live sperm concentration can produce very different rates of fertilization. In general, fertility, as well as the number of sperm stored in the hen's oviduct, increases with increasing numbers of sperm inseminated (Bakst et al., 1994). However, because sperm and oviductal characteristics both regulate the ability of sperm to be stored in the oviduct, it is possible for even very large insemination doses to produce very low fertility rates. For example, sperm motility is essential for sperm to traverse the vagina and reach the sperm storage tubules (Allen and Grigg, 1957; Bakst et al., 1994). Semen samples with a low SQI do have poor sperm motility (McDaniel et al., 1998b), even if sperm concentration is large, resulting in low fertility. In addition, the oviduct may regulate sperm movement and select sperm by immune reactions within the oviductal fluid (Bakst et al., 1994; Steele and Wishart, 1992).

Brillard and McDaniel (1986) found that a single insemination dose of 125 $\times 10^6$ total sperm per hen resulted in maximum fertility. The research of McDaniel and colleagues (1997) revealed that insemination doses higher than 100 $\times 10^6$ total sperm did not result in an increase in the amount of sperm stored in the oviduct. All SQI groups in the present study produced a minimum insemination dose of 100 $\times 10^6$ live sperm per hen. The 0 to 150 SQI group had the lowest insemination dose of 103 $\times 10^6$ viable sperm, yet their fertility rate was only 65%. From previous research (Brillard and McDaniel, 1986; McDaniel et al., 1997) it would seem that the hen's storage capacity should have been maximized, even in the lowest SQI group, 0 to 150. However, this group's duration of fertility was only 15.5 d compared with the >300 SQI groups whose duration of fertility was approximately 18 d. It is possible that the sperm with low SQI values are unable to enter the sperm storage tubules, or they are able to enter the sperm storage tubules but are unable to remain viable once stored there, resulting in poor duration of fertility.

The SQI appears to give an accurate measurement of sperm motility (McDaniel et al., 1998b), which may be a

reason for the linear effect of SQI on fertility. The SQI >250 groups had fertility rates of 90% and greater. These higher fertility rates could be explained because the sperm from these groups were more motile and were capable of reaching the fertilization and storage sites. The 0 to 150 SQI group had the worst fertility, dropping to 46% over the 8 d postinsemination. Again, the low SQI sperm apparently is either unable to reach storage and fertilization sites or does not remain viable for long once in these sites.

In conclusion, the SQI shows promise as a useful tool that can offer the opportunity to select roosters for their reproductive performance and for predicting fertilizing ability. It quickly and easily evaluates sperm concentration, viability, and motility. This research suggests that using the SQI can improve flock fertility.

The aforementioned results indicate that selection of males for fertilizing potential by using the SQI is ready to test in natural mating flock conditions. Selection of males for the SQI at sexual maturity would eliminate birds with poor semen quality, allowing fertile males to do the mating. An increase in the percentage of fertilized eggs would result in an increase in hatchability. Even slight increases in hatchability can be very profitable. For example, Pollock (1999) noted that if an integrator produces 15 million eggs a week, increasing hatchability 1% would increase profits by $30,000 weekly.

## REFERENCES


Allen, T., and G. Grigg, 1957. Sperm transport in the fowl. Aust. J. Agric. Res. 8:788–799.

Amann, R. P., 1999. Lessons for the poultry industry gleaned from experiences with other commodity species. Poultry Sci. 78:419–427.

Bakst, M. R., G. Wishart, and J. P. Brillard, 1994. Oviducal sperm selection, transport, and storage in poultry. Poultry Sci. Rev. 5:117–143.

Bartoov, B., J. Ben-Barak, A. Mayevsky, M. Sneider, L. Yogev, A. Lightman, 1991. Sperm motility index: A new parameter for human sperm evaluation. Fertil. Steril. 56:108–112.

Bilgili, S. F., and J. A. Renden, 1984. Fluorometric determination of avian sperm viability and concentration. Poultry Sci. 63:2275–2277.

Bramwell, R. K., H. L. Marks, and B. Howarth, 1995. Quantitative determination of spermatozoa penetration of the perivitelline layer of the hen's ovum as assessed on oviposited eggs. Poultry Sci. 74:1875–1883.

Brillard, J. P., and G. R. McDaniel, 1986. Influence of spermatozoa numbers and insemination frequency on fertility in dwarf broiler breeder hens. Poultry Sci. 65:2330–2334.

Donoghue, A. M., 1999. Prospective approaches to avoid flock fertility problems: Predictive assessment of sperm function traits in poultry. Poultry Sci. 78:437–443.

Donoghue, A. M., D. R. Holsberger, D. P. Evenson, and D. P. Froman, 1998. Semen donor selection by *in vitro* sperm mobility increases fertility and semen storage in the turkey hen. J. Androl. 19:295–301.

Froman, D. P., and A. J. Feltmann, 1998. Sperm mobility: A quantitative trait of the domestic fowl (*Gallus domesticus*). Biol. Reprod. 58:379–384.

Froman D. P., A. J. Feltmann, M. L. Rhoads, and J. D. Kirby, 1999. Sperm mobility: A primary determinate of fertility in the domestic fowl. Biol. Reprod. 61:400–405.

Gill, S.P.S., A. M. Donoghue, D. R. Holsberger, R. P. Amann, and R. M. Hulet, 1999. Identifying potentially subfertile toms *via* a sperm-binding assay. Poultry Sci. 78:1208–1218.

Hammerstedt, R. H., 1999. Symposium summary and challenges for the future. Poultry Sci. 78:459–466.

Howarth, B., 1981. Preservation of the fertilizing capacity of cock semen incubated *in vitro* at 41 C. Poultry Sci. 60:1075–1078.

Johnston, R. C., G. N. Clarke, D. Y. Liu, H.W.G. Baker, 1995. Assessment of the Sperm Quality Analyzer. Fertil. Steril. 63:1071–1076.

McDaniel, C. D., R. K. Bramwell and B. Howarth, Jr., 1997. Development of a novel fluorescence technique for quantifying the total number of spermatozoa stored in the uterovaginal junction of hens. J. Reprod. Fertil. 109:173–179.

McDaniel, C. D., H. M. Parker, J. B. Yeatman, C. D. Schultz, and C. D. Zumwalt, 1998a. Selection of young broiler breeder roosters for the sperm motility index increases the percentage of fertilized eggs produced for weeks. Poultry Sci. 77(Suppl. 1):124. (Abstr.).

McDaniel, C. D., J. L. Hannah, H. M. Parker, T. W. Smith, C. D. Schultz, and C. D. Zumwalt, 1998b. Use of a sperm analyzer for evaluating broiler breeder males. 1. Effects of altering sperm quality and quantity on the sperm motility index. Poultry Sci. 77:888–893.

Pollock, D. L., 1999. Geneticist's perspective from within a broiler primary breeder company. Poultry Sci. 78:414–418.

Steel, R.G.D., and J. H. Torrie, 1980. Principles and Procedures of Statistics. A Biometrical Approach. 2nd ed. McGraw Hill Book Co., Inc., New York, NY.

Steele, M. G., and G. J. Wishart, 1992. Characterization of a mechanism impeding sperm transport through the vagina of the chicken. Proc. 12th Int. Cong. Anim. Reprod. 3:1593–1595.

Wilson, H. R., N. P. Piesco, E. R. Miller, and W. G. Nesbeth, 1979. Prediction of the fertility potential of broiler breeder males. World's Poult. Sci. J. 35:95–118.

Wishart, G. J., and Y. I. Wilson, 1997. Sperm motility and metabolism IV. Sperm motility analysis using the "Semen Quality Analyzer." Pages 54–55 *in*: Techniques for semen evaluation, semen storage, and fertility determination. M. R. Bakst and H. C. Cecil, ed. Poultry Science Association, Savoy, IL.

Zavos, P. M., J. R. Correa, and P. N. Zarmakoupis-Zavos, 1996. Measurement of the sperm motility index via the Sperm Quality Analyzer and its relationship to other qualitative sperm parameters. Theriogenology 46:421–427.