ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

# POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 3 (9): 588-592, 2004


# The Optimum Semen Dilution for the Sperm Quality Index that is Most Predictive of Broiler Breeder Fertility[1,2]

H.M. Parker and C.D. McDaniel
Department of Poultry Science, Mississippi State University, Mississippi State, MS, USA

**Abstract:** The sperm quality index (SQI) can accurately predict overall semen quality and fertility when broiler breeder semen is diluted, at most, 10-fold prior to analysis. The objective of the present study was to determine if a lower semen dilution rate yields an SQI that is an even better predictor of semen quality and fertility when hens are inseminated with a constant volume of semen. Individual ejaculates from 28 males were analyzed for sperm concentration, viability, and the SQI prior to insemination into 15 hens/male. Semen was diluted 2-, 4-, 8-, 10-, and 25-fold prior to analysis for the SQI. The SQI from 25-, 10-, and 8-fold dilutions produced the strongest correlations with total sperm concentration (r = 0.85, 0.82, and 0.80, respectively). Correlation coefficients for live sperm concentration were highest for the SQI from 25-, 10-, 8-, and 4-fold dilutions (r = 0.86, 0.86, 0.83, and 0.73, respectively). Correlation coefficients were similar for the SQI with fertility from the 4-, 8-, and 10-fold dilutions (r = 0.75, 0.72, and 0.72, respectively). It appears that an 8- to 10-fold SQI dilution is the most consistent at predicting fertility and semen quality.

**Key words:** sperm quality index, broiler breeder, fertility, semen dilution

## Introduction

The ability to predict semen quality and fertilizing potential of the male is of utmost importance in animal agriculture (Donoghue, 1999). In poultry, the Sperm Quality Index (SQI) is correlated with semen characteristics such as sperm concentration, viability, and motility (McDaniel *et al*., 1998; Neuman *et al*., 2002; Parker and McDaniel, 2003). The SQI has also been correlated to fertility as well as hatchability in broiler breeders (Parker *et al*., 2000; 2002; Parker and McDaniel, 2002; 2003). The SQI is a single number that provides an overall estimate of semen quality by measuring the deflections in a light path created by sperm movement. In poultry, due to high sperm concentrations, semen must be diluted prior to SQI analysis to allow for the free movement of sperm in the SQI capillary tubes (McDaniel *et al*., 1998).

Earlier avian research suggested diluting semen 10-fold prior to SQI analysis to accurately predict semen quality (McDaniel *et al*., 1998). The SQI from chicken semen diluted 10-fold has been used to predict fertility in laboratory research and to select males for house placement in a field trial. Using the SQI from semen diluted 10-fold prior to analysis to select males revealed improvements in fertility as well as hatchability (Parker *et al*., 2000; 2002; Parker and McDaniel, 2002). However, even though McDaniel *et al*. (1998) suggested using the SQI from a 10-fold semen dilution to obtain the SQI for semen quality, they did not correlate the SQI from the 10-fold semen dilution with fertility.

It has never been established at what semen dilution rate the SQI is most predictive of semen characteristics and fertility. Parker and McDaniel (2003) determined that diluting semen greater than 10-fold prior to SQI analysis did not enhance the ability of the SQI to predict semen quality and fertility. In fact, they found that the ability of the SQI to predict semen quality and fertility declined as semen dilution increased. Research has not been conducted to determine if semen diluted less than 10-fold prior to SQI analysis would yield an SQI that is an even better predictor of semen quality and fertility. McDaniel *et al*. (1998) found that diluting semen samples 3- to 5-fold yielded the highest SQI values. However, in that study, the SQI values from the 3- to 5-fold diluted semen samples were not correlated with semen quality and fertility. Therefore, the objective of this experiment was to determine if a semen dilution rate less than 10-fold would yield an SQI that is more predictive of semen quality and fertility.

## Materials and Methods

**Housing and environment:** Twenty-eight Cobb broiler breeder males, 49 wk of age, were obtained from a local integrator. Males were housed in individual cages, and maintained using conventional environmental controls. Males were fed the Mississippi State University male breeder diet (1.55 MJ/d per bird) to maintain primary breeder recommended weights. All males received 16 h of light daily throughout the experiment. Four hundred-twenty Hyline 98 Leghorn hens, 57 wk of age, were also housed in individual cages. Hens consumed the Mississippi State University layer diet (11.97 MJ/kg, 145 g/kg crude protein, and 40 g/kg calcium) *ad libitum* and were exposed to light from 0500 to 2100h daily throughout the experiment. The hens were caged in a house with conventional environmental controls.

**Semen, insemination, and fertilization evaluation:** Ejaculates were collected weekly from individual males for three weeks using the method of Burrows and Quinn

(1937). Each male=s SQI was determined in duplicate, simultaneously, using two Sperm Quality Analyzers (MEDICAL ELECTRONIC SYSTEMS LTD, Migdal Haemek, Israel). The CV between the two analyzers was 3.6%. Semen was diluted with 0.85% saline. These individual semen samples were diluted 2-, 4-, 8-, 10-, and 25-fold prior to SQI analysis. An aliquot of undiluted semen was used to determine the SQI for undiluted semen samples. To determine sperm concentration and sperm viability, two fluorometric measurements from each males undiluted semen sample was obtained (Bilgili and Renden, 1984). Each semen sample was analyzed within 2-3 minutes of collection.

After semen collection and analysis, 15 hens per male were inseminated with 20 ul of a constant volume of 4-fold diluted semen, 1 part semen in 3 parts minimum essential media (Howarth, 1981). Eggs were collected and labeled daily, stored at 18.3 C, and incubated weekly. On Day 6 of incubation, eggs were broken to determine the occurrence of embryonic development.

**Statistical analyses:** Shapiro and Wilk (1965) coefficient (W) was used to test for normality of the SQI and semen characteristic populations. The distribution curves were considered normal as W approached 1 at P>0.05. For each SQI dilution rate, Pearson's correlation coefficients were obtained for the relationship of the SQI with semen characteristics and fertility. Correlation analyses were also conducted to examine the relationship of semen characteristics, other than the SQI, with fertility (Steel and Torrie, 1980). Correlation coefficients were separated (P< 0.05) using the method of Steiger (1980). For fertility data, semen treatments were applied to groups of hens and groups of hens were the experimental units.

## Results

The population distribution for each semen characteristic and the SQI at each semen dilution is presented in Table 1. The population distributions for semen characteristics revealed that total and live sperm concentration were normally distributed. However, the population distribution for the percentage of viable sperm was skewed to the right. The population distribution for the SQI from semen diluted 2- and 25-fold was normal. The SQI obtained from undiluted semen revealed a population distribution that was skewed to the left, but the population distribution for the SQI from semen diluted 4-, 8-, and 10-fold was skewed to the right.

The correlation of the SQI at each semen dilution rate with total, percentage of viable, and live sperm concentration is presented in Table 2. There was a negative relationship for the SQI from undiluted semen with total and live sperm concentration. No relationship existed for the SQI from a 2-fold dilution with either total or live sperm concentration. The correlation coefficients for the SQI from undiluted and 4-fold diluted semen with total sperm concentration were significantly lower than the coefficients obtained from the 8-, 10-, and 25-fold semen dilutions. No relationship was detected between the SQI from undiluted semen and the percentage of viable sperm. Numerically, the SQI obtained from the 25-fold semen dilution had the weakest relationship with the percentage of viable sperm. The correlation of the SQI from the 4-, 8-, 10-, and 25-fold semen dilutions with live sperm concentration were similar and greater than the correlation from undiluted semen. The relationships of the SQI from undiluted, 2-, and 10-fold diluted semen with live sperm concentration are also graphically presented in Fig. 1. Even though the overall correlation of the SQI from the 2-fold dilution with live sperm concentration was non significant, a linear increase in the SQI was noted as live sperm concentration increased to 5 x $10^9$ live sperm/ml.

The relationship of fertility with the SQI for each semen dilution is presented in Table 3. No relationship existed between the SQI from undiluted semen and fertility. The correlation coefficients for the SQI from semen diluted 2-, 4-, 8-, 10-, and 25-fold with fertility were similar. Numerically, the SQI from the 2- and 25-fold semen dilutions had the lowest correlation coefficients. There was a strong positive relationship of the SQI from 10-fold diluted semen with fertility as shown in Fig. 2 (r = 0.72).

The relationship of semen characteristics other than the SQI with fertility is presented in Table 4. There were no differences among the coefficients for the correlation of total sperm concentration, percentage of viable sperm, and live sperm concentration with fertility.

## Discussion

Previous research has shown that the population distribution is skewed to the right for the SQI obtained from semen diluted 10-fold (Parker *et al.*, 2000, 2002; Parker and McDaniel, 2002; 2003). In this study, the population distribution for the SQI from semen diluted 25-fold as well as the population distributions for total sperm concentration and the percentage of viable sperm were similar to what was reported by Parker and McDaniel (2003). As dilution increases the SQI population distribution shifts from the left, to the right, and then becomes normally distributed. However, at the high semen dilutions required for a normal SQI population distribution, the SQI is not very predictive of fertility or semen quality (Parker and McDaniel, 2003). Apparently, excessive semen dilution alters semen quality yielding an SQI that is no longer indicative of fertility (Parker and McDaniel, 2003).

As previously reported, the SQI is an index that is dependent upon sperm concentration, viability, and sperm motility collectively. Also, semen must be diluted prior to SQI analysis to enhance the ability of the SQI to predict semen quality (McDaniel *et al.*, 1998; Parker and

Table 1: Population distribution characteristics for total sperm concentration, percentage of viable sperm, live sperm concentration, and the sperm quality index (SQI) at each semen dilution rate[1]

| | Mean | Skewness | W | P value[2] | Distribution |
|---|---|---|---|---|---|
| Total Sperm Conc. (sperm/ml) | 5.82 x $10^9$ | -0.055 | 0.954 | 0.2642 | Normal |
| Viable Sperm (%) | 90% | -2.371 | 0.731 | 0.0001 | Right |
| Live Sperm Conc. (sperm/ml) | 5.24 x $10^9$ | -0.353 | 0.944 | 0.1371 | Normal |
| SQI from undiluted semen | 135 | 1.077 | 0.781 | 0.0001 | Left |
| SQI from 2-fold dilution | 400 | -0.328 | 0.961 | 0.3697 | Normal |
| SQI from 4-fold dilution | 435 | -1.571 | 0.813 | 0.0002 | Right |
| SQI from 8-fold dilution | 399 | -1.342 | 0.854 | 0.0011 | Right |
| SQI from 10-fold dilution | 387 | -1.172 | 0.873 | 0.0029 | Right |
| SQI from 25-fold dilution | 246 | -0.410 | 0.954 | 0.2484 | Normal |

[1]These data are from averages obtained from individual ejaculates of 28 males for each of the 3 wk of insemination.
[2]P-value for Shapiro and Wilk coefficient (W).

Table 2: Correlation coefficients for the sperm quality index (SQI) at each semen dilution rate with total sperm concentration, percentage of viable sperm, and live sperm concentration of undiluted semen[1]

| SQI at each Semen Dilution Rate | Sperm Characteristic | | |
|---|---|---|---|
| | Total sperm conc. | % Viable Sperm | Live sperm conc. |
| | (r) | | |
| Undiluted semen | -0.77[c] | NS | -0.73[b] |
| 2-fold | NS | 0.68[a] | NS |
| 4-fold | 0.68[b] | 0.65[a] | 0.73[a] |
| 8-fold | 0.80[a] | 0.63[a] | 0.83[a] |
| 10-fold | 0.82[a] | 0.65[a] | 0.86[a] |
| 25-fold | 0.84[a] | 0.49[a] | 0.86[a] |

[a-c]Correlation coefficients within a column with different superscripts are significantly different (P<0.05). NS indicates a nonsignificant correlation coefficient. [1]These data are from averages obtained from individual ejaculates of 28 males for each of the 3 wk of insemination

McDaniel, 2003). This study revealed a negative relationship for the SQI from undiluted semen with total and live sperm concentration. As evidenced in Fig. 1, the range in SQI values of undiluted semen was very small (79 units) when live sperm concentrations were above 5 x $10^9$ live sperm/ml. However, at live sperm concentrations below 5 x $10^9$ live sperm/ml the SQI values increased over 350 units with decreasing live sperm concentrations. McDaniel *et al.* (1998) observed sperm movement within the SQI capillary at different sperm concentrations and reported that sperm were unable to move freely within the capillary at high sperm concentrations. In this study, the negative relationship of the SQI from undiluted semen with total and live sperm concentration is mostly likely due to the restriction of sperm movement within the SQI capillary when live sperm concentration was greater than 5 x $10^9$ live sperm/ml.

Diluting semen two-fold revealed an overall increase in SQI values for each male as opposed to the SQI from undiluted semen of the same males (Fig. 1). Also, the range in SQI from males with a live sperm concentration greater than 5 x $10^9$ live sperm/ml was greater for semen diluted 2-fold when compared to the SQI values from undiluted semen (247 vs 79 units, respectively). The 2-fold SQI from males with live sperm concentrations below 5 x $10^9$ live sperm/ml covered a smaller range than the SQI obtained using undiluted semen from the same males (307 vs 356 units, respectively). Apparently, diluting semen 2-fold for males with live sperm concentrations below 5 x $10^9$ live sperm/ml allows for adequate movement within the SQI capillary as evidenced by a increase in the SQI values from these males. However, the SQI was still not maximized for males with a live sperm concentration greater than 6 x $10^9$ live sperm/ml, indicating that spermatozoa from these males were still unable to move freely within the SQI capillary when semen was diluted 2-fold.

In this study, there was a strong positive correlation of the SQI with live sperm concentration when semen was diluted 10-fold (r = 0.86). The reason for this high correlation coefficient was most likely because the sperm from all males were able to move freely in the SQI capillary regardless of live sperm concentration. The SQI from males with live sperm concentrations less than 5 x $10^9$ live sperm/ml decreased slightly when semen was diluted 10-fold as compared to 2-fold. However, the SQI from males with live sperm concentrations greater than 5 x $10^9$ live sperm/ml increased when semen was diluted 10-fold as compared to 2-fold.

Table 3: Correlation coefficients for the sperm quality index (SQI) from each semen dilution rate with fertility

| | Fertility[1] |
|---|---|
| SQI at each Semen Dilution Rate | Correlation coefficient |
| Undiluted semen | NS |
| 2-fold | 0.61 |
| 4-fold | 0.75 |
| 8-fold | 0.72 |
| 10-fold | 0.72 |
| 25-fold | 0.66 |

NS indicates a nonsignificant correlation coefficient.
[1]For each of 28 males, 15 hens were inseminated with 20µl of a 1:4 constant volume of diluted semen for each of the 3 wk of insemination (15 hens/male).

Table 4: Correlation coefficients for semen characteristics with fertility

| | Fertility[1] |
|---|---|
| Semen Characteristic | Correlation coefficient |
| Total Sperm Conc. | 0.56 |
| % Viable Sperm | 0.61 |
| Live Sperm Conc. | 0.62 |

[1]For each of 28 males, 15 hens were inseminated with 20µl of a 1:4 constant volume of diluted semen for each of the 3 wk of insemination (15 hens/male).

When semen was diluted only 4-fold, the relationship of the SQI with total sperm concentration was significantly weaker than that for semen diluted 8-, 10-, and 25-fold. Also, the SQI from semen diluted 4-fold had the lowest absolute numeric correlation coefficient with live sperm concentration. Again, this is mostly likely due to limited movement of spermatozoa within the SQI capillary. Apparently semen diluted either 8-, 10-, or 25-fold allows for freedom of sperm movement within the SQI capillary. With the exception of the SQI from undiluted semen, there were no differences due to dilution in the relationship of the SQI with the percentage of viable sperm. However, the correlation coefficient from semen diluted 25-fold was numerically lower than the other semen dilutions. Parker and McDaniel (2003) reported a non significant relationship for the SQI with the percent of viable sperm when semen was diluted 25-fold. In this study, the 25-fold semen dilution could be reaching the point of over dilution thus resulting in a lower correlation coefficient.

Regardless of semen dilution, there was no statistical difference in the relationship of the SQI with fertility when hens were inseminated with a constant 4-fold volume of semen. However, the relationship of the SQI from 2-fold diluted semen with fertility was numerically weaker than that for the SQI from semen diluted 4-, 8-, and 10-fold, most likely because the SQI and fertility are both influenced by sperm concentration, and the SQI for 2-fold

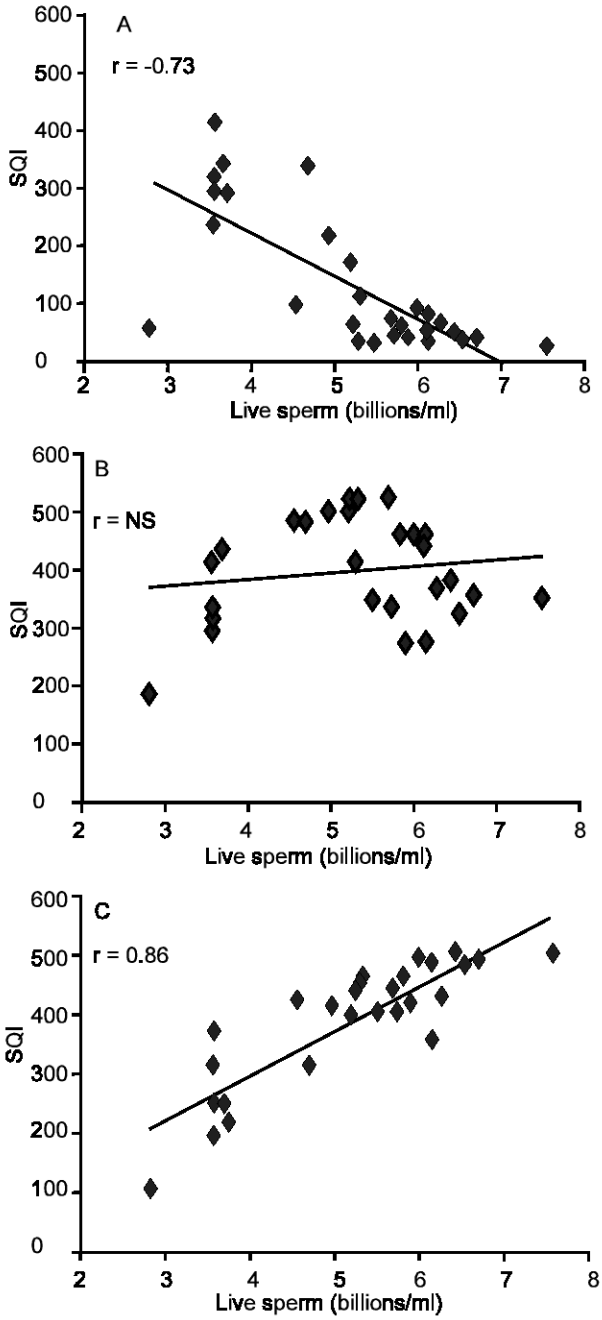


Fig. 1: The relationship of live sperm concentration in undiluted semen with the sperm quality index (SQI) from A. undiluted semen; B. 2-fold diluted semen; C. 10-fold diluted semen. These data are averages obtained from individual ejaculates of 28 males for each of the 3 wk of insemination.

diluted semen was not correlated with total and live sperm concentration. The correlation coefficient for the SQI from semen diluted 25-fold with fertility was also numerically lower than the coefficients obtained for the

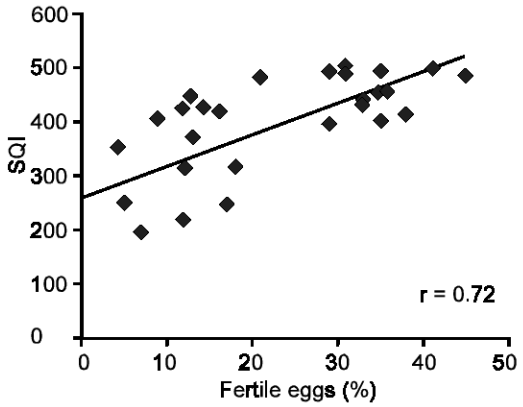


Fig. 2: The relationship of the sperm quality index (SQI) from the 10-fold semen dilution with fertility from hens inseminated with a constant 1:4 semen dilution for each of the 3 wk of insemination (15 hens/male). Each group of 15 hens laid on average 11 eggs/day.

4-, 8-, and 10-fold semen dilutions. This could be due to the low correlation coefficient for the SQI from the 25-fold semen dilution with the percentage of viable sperm. It is possible that the 25-fold semen dilution is approaching over dilution as reported by Parker and McDaniel (2003). The correlation coefficient for the SQI from semen diluted 10-fold is very similar to the coefficient that Parker and McDaniel (2003) reported for the SQI with fertility when hens were inseminated with a constant 4-fold volume of semen (0.72 vs 0.71).

In this study and as reported by Parker and McDaniel (2003), the individual correlation of total sperm concentration (r=0.56), percentage of viable sperm (r=0.61), and live sperm concentration (r=0.62) with fertility are weaker than the correlation of the SQI with fertility (r=0.72). This is most likely because the SQI is influenced by sperm concentration, viable sperm, and motility collectively and these semen characteristics are each very important in the fertilization process (Parker *et al.*, 2000).

In conclusion, it appears that diluting semen 8- or 10-fold yields an SQI that is most predictive of total sperm concentration, percentage of viable sperm, and live sperm concentration. Also, the SQI from semen diluted 8- or 10-fold is the best predictor of fertility when inseminating hens with a constant volume of semen.

## References

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

Burrows, W.H. and J.P. Quinn, 1937. The collection of spermatozoa from the domestic fowl and turkey. Poult. Sci., 16: 19-24.

Donoghue, A.M., 1999. Prospective approaches to avoid flock fertility: predictive assessment of sperm function traits in poultry. Poult. Sci., 78: 437-443.

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

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

Neuman, S.L., C.D. McDaniel, L. Frank, J. Radu, M.E. Einstein and P.Y. Hester, 2002. Utilization of a sperm quality analyzer to evaluate sperm quantity and quality of turkey breeders. Br. Poult. Sci., 43: 457-464.

Parker, H.M., J.B. Yeatman, C.D. Schultz, C.D. Zumwalt, and C.D. McDaniel, 2000. Use of a sperm analyzer for evaluating broiler breeder males. 2. Selection of young broiler breeder males for the sperm quality index increases fertile egg production. Poult. Sci., 79: 771-777.

Parker, H.M., A.G. Karaca, J.B. Yeatman, L.R. Frank and C.D. McDaniel, 2002. Fertility of broiler breeders following categorization by the Optibreed7 sperm quality index when hens are inseminated with a constant number of sperm. Poult. Sci., 81: 239-245.

Parker, H.M. and C.D. McDaniel, 2002. Selection of young broiler breeders for semen quality improves hatchability in an industry field trial. J. Appl. Poult. Res., 11: 250-259.

Parker, H.M. and C.D. McDaniel, 2003. Semen dilution prior to analysis influences the ability of the sperm quality analyzer to predict fertility whether inseminating with a constant number of sperm or a constant volume of semen. Poult. Sci., 82: 1808-1815.

Shapiro, S.S. and M.B. Wilk, 1965. Analysis of variance test for normality. Biometrika, 52:591-611.

Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics. A Biometrical Approach (New York McGraw-Hill).

Steiger, J.H., 1980. Tests for comparing elements of a correlation matrix. Psychological Bulletin, 87: 245-251.

[1]This is journal article number J10530 from the Mississippi Agricultural and Forestry Experiment Station supported by MIS-322140.

[2]Use of trade names in this publication does not imply endorsement by the Mississippi Agricultural and Forestry Experiment Station of the products mentioned or similar ones not mentioned.