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The Sperm Quality Index from Fresh Semen Predicts Chicken Semen Quality after Storage[1,2]

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Abstract: The Sperm Quality Index (SQI) is correlated with fresh broiler breeder semen quality. Our objective was to determine if the SQI from semen prior to storage is predictive of semen quality after storage. Prior to semen dilution, sperm concentration, viability, and SQI were determined for each male's neat semen sample. Each ejaculate was then diluted 1:1 with Beltsville Poultry Semen Extender and maintained at 4 C on a rotary shaker for 16 h. After semen dilution, sperm concentration, viability, and SQI were obtained at 0, 2, 4, 6, 8, 10, 12 and 16 h. The SQI increased from 0 to 4 h of storage then decreased in a quartic fashion as storage time further increased (r^2=0.83). There was a linear decrease in sperm viability as storage time increased (r^2=0.87). There was a negative relationship for the SQI from fresh semen with percentage of dead sperm over storage period yielding correlation coefficients ranging from r= -0.88 to -0.55. Over storage, positive correlation coefficients for the SQI from fresh semen with live sperm concentration ranged from 0.47 to 0.61. There were also strong positive correlations for percentage of dead sperm and live sperm concentration from fresh semen with their respective semen characteristic at each storage period (r=0.81 to 0.97 and r=0.80 to 0.96, respectively). There was a strong positive relationship for SQI from fresh semen with the SQI over storage (r=0.88 to 0.94). In conclusion, the SQI from semen prior to storage is predictive of chicken semen quality through 16 h of storage.

Key words: Sperm Quality Index, semen storage, broiler breeder

Introduction

Today's selection for heavy, broad-breasted broiler breeder strains has led to a continuous decline in fertility using natural mating (McDaniel, 1978). Similar to the turkey industry, if roosters become so large that they cannot mate hens successfully, artificial insemination (AI) will have to be applied to the broiler industry in the future. In the broiler industry, to utilize AI successfully, evaluation of semen quality before and after storage as well as prior to insemination is very important (Reddy, 1995). There are several reasons why semen quality should be examined prior to AI. For example, losses in fertility would be avoided by inseminating hens with semen that is of good quality. Therefore, an improvement in long-term storage procedures for poultry semen could have a significant impact on the poultry industry. As a result, the evaluation of stored semen is crucial to ensure a successful AI program.

Traditional methods used to determine fresh semen quality include parameters such as semen volume, color, concentration, and sperm motility, viability, and morphology (Donoghue and Wishart, 2000) as well as metabolic activity (Chaudhuri *et al*., 1988; Wishart, 1989). However, all of these methods of semen evaluation are based on a single sperm quality parameter and do not consider other semen quality characteristics. One method of semen evaluation that does include several measures of semen quality in a single index number is the sperm quality index (SQI).

The SQI is a single number, obtained in 20 sec, that provides an overall estimate of chicken and turkey semen quality (McDaniel *et al*., 1998; Parker *et al*., 2000; Neuman *et al*., 2002a). For example, as sperm viability, concentration, and motility increase from rooster and tom semen, there is an increase in the SQI (McDaniel *et al*., 1998; Neuman *et al*., 2002a; Parker and McDaniel, 2003). The SQI is also positively correlated with broiler breeder fertility and hatchability (Parker *et al*., 2000, 2002; Parker and McDaniel, 2002, 2003, 2004). However, the previously mentioned research involving the SQI was conducted on freshly ejaculated broiler breeder semen only, not stored semen (Parker and McDaniel, 2002, 2003, 2004). Dumpala *et al*. (2006) stored broiler breeder semen for 8 h in different diluents at various temperatures and found that the SQI declined as storage temperature and time increased. In turkeys, the SQI accurately reflected declining semen quality with prolonged storage (Neuman *et al*., 2002b). Also, research conducted on bovine spermatozoa revealed that SQI values were highly correlated with sperm motility of frozen-thawed semen (Zavos *et al*., 1996).

It would be beneficial to poultry breeders if a method was available that could use freshly ejaculated semen to predict overall semen quality of stored semen. If such a method existed, only males that responded favorably to semen storage could be selected for breeding purposes. Because the SQI is capable of predicting semen quality and fertility of freshly ejaculated chicken

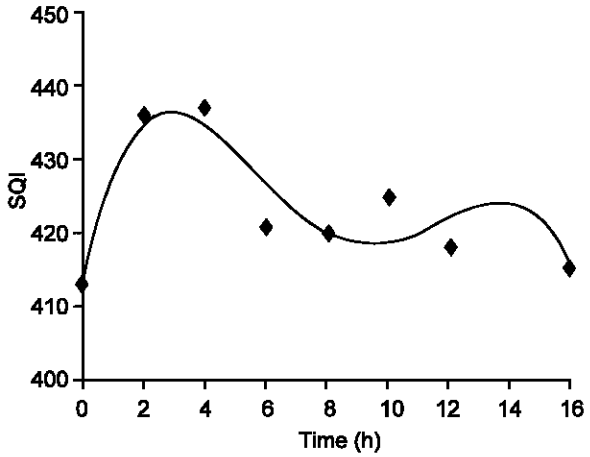

Fig. 1: The relationship of the sperm quality index (SQI) with storage period. Each point represents the mean of 20 individual males at each time period. There was a quadratic relationship for the SQI over storage ($y = -0.0128x^4 + 0.44x^3 - 5.0064x^2 + 18.97x + 414$, $r^2 = 0.83$, $P < 0.0001$).

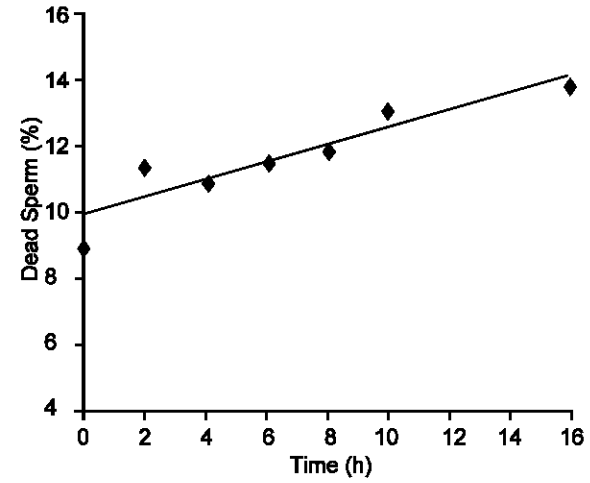

Fig. 2: The relationship of the percentage of dead sperm with storage period. Each point represents the mean of 20 individual males at each time period. There was a linear increase in the percentage of dead sperm as storage time increased ($y = 0.259x + 9.93$, $r^2 = 0.87$, $P < 0.0001$).

semen, it is possible that the SQI of freshly ejaculated semen can predict the semen quality of stored chicken semen. Therefore, the objective of this study was to determine if the SQI from freshly ejaculated semen prior to storage is predictive of broiler breeder semen quality when semen is stored up to 16 h.

Materials and Methods

Housing and environment: Twenty Ross broiler breeder males, 68 wk of age, were obtained from a local integrator. Roosters were housed in individual cages. Broiler breeder males were fed a standard breeder diet (1.55 MJ/d per bird) and feed-restricted according to the primary breeder's recommendations. All males received 16 h of light per day throughout the experiment and were treated in accordance with the Guide for Care and Use of Agricultural Animals in Agricultural Research and Training.

Semen evaluation: Prior to the study, semen was collected from each rooster every other day for a total of 3 collections to evacuate residual sperm from the bird's body. Semen was collected using the method of Burrows and Quinn (1937). Each male's ejaculate was diluted 1:1 with Beltsville Poultry Semen Extender II (BPSE; CONTINENTAL PLASTIC CORP., Delavan, WI). The diluted ejaculates were then maintained aerobically at 4 C on a rotary shaker (Clay Adams, Division of Becton, Dickinson and CO., Parsippany, NY.) for 16 h. Sperm concentration, viability, and the SQI were obtained at 0, 2, 4, 6, 8, 10, 12, and 16 h from the 1:1 diluted semen samples. Sperm concentration was measured using an IMV micro reader (IMV INTERNATIONAL, Maple Grove, MN) at 540 nm (King and Donoghue, 2000). Sperm viability was determined using the fluorometric method of Biligili and Renden (1984). A Sperm Quality Analyzer® (Medical Electronic Systems Ltd, Migdal, Haemek, Israel) was used to obtain the SQI from 1:1 diluted semen samples that were further diluted 5-fold with 0.85% saline at 21°C.

Statistical analyses: Regression analyses were used to examine the relationships of the SQI and sperm viability over storage time. Pearson's correlation coefficients were used to explore the relationship of the SQI from fresh semen with semen quality characteristics from each storage period. Correlation coefficients were also obtained for the relationships of percentage of dead sperm and live sperm concentration from fresh semen with the same semen characteristics after storage (Steele and Torrie, 1980).

Results

There was a quartic relationship for the SQI over 16 h of storage (Fig. 1). The SQI increased from 0 to 4 h of storage then decreased and stabilized after 4 h of storage. The relationship of the percentage dead sperm over 16 h of storage is presented in Fig. 2. There was a slight linear increase in the percentage of dead sperm as the length of storage period increased. No linear or curvilinear relationship existed for live sperm concentration over storage as live sperm concentration remained virtually constant over time (Fig. 3).

Table 1: Correlation coefficients for the sperm quality index (SQI) from freshly ejaculated semen with the percentage of dead sperm at each storage period

Storage period (h)	Fresh SQI vs % of Dead sperm
	Correlation coefficient
Fresh	-0.79
2	-0.81
4	-0.54
6	-0.59
8	-0.44
10	-0.71
12	-0.59
16	-0.51

Table 2: Correlation coefficients for the sperm quality index (SQI) from freshly ejaculated semen with live sperm concentration at each storage period

Storage period (h)	Fresh SQI vs live sperm concentration
	Correlation Coefficient
Fresh	0.50
2	0.57
4	0.49
6	0.58
8	0.52
10	0.51
12	0.51
16	0.51

The correlation of the SQI from freshly ejaculated semen with the percentage of dead sperm at each storage period is shown in Table 1. The correlation coefficients were negative for the SQI obtained from freshly ejaculated semen with the percentage of dead sperm at each storage period ($P < 0.03$). There was a strong negative correlation at 2 h ($r = -0.81$). However, after 2 h of storage the correlation coefficients became weaker but remained better than $r = -0.44$.

The correlation of the SQI obtained from freshly ejaculated semen with live sperm concentration at each storage period is also shown in Table 2. The correlation coefficients were positive for the SQI from freshly ejaculated semen with live sperm concentration at each storage period ($P < 0.01$). The correlation coefficients at each storage period remained at approximately $r = 0.50$ throughout storage.

Very strong positive correlations existed for the percentage of dead sperm, live sperm concentration, and the SQI obtained from freshly ejaculated semen with their respective sperm quality characteristics obtained from stored semen at each storage period (Table 3; $P < 0.0001$). The correlation coefficients were highest for the percentage of dead sperm from freshly ejaculated semen with the percentage of dead sperm of semen stored for 2 and 10 h ($r = 0.97$). The correlation coefficients were greater than $r = 0.81$ at each storage period. For live sperm concentration of freshly ejaculated semen with live sperm concentration of stored semen, high correlation coefficients of $r = 0.91$ to 0.96 were obtained except for semen stored for 4 h ($r = 0.80$). The correlation coefficient was highest for the SQI obtained from freshly ejaculated semen with the SQI from semen stored 8 h ($r = 0.94$). However, correlation coefficients for the SQI obtained from freshly ejaculated semen with the SQI obtained from semen stored were always $r = 0.88$ or greater. Correlation coefficients for percentage of fresh dead sperm versus stored dead sperm, and correlation coefficients for fresh SQI versus stored dead sperm over storage period are graphically presented in Fig. 4. Directional changes over storage period in the correlation coefficients for both the relationships of dead sperm and the SQI from fresh semen with stored dead sperm paralleled one another.

Discussion

Efficient storage and evaluation of semen quality prior to storage and AI are important in order to take advantage of modern AI techniques in the poultry industry (Donoghue and Wishart, 2000). One method of semen evaluation that is capable of providing a rapid and precise assessment of overall fresh semen quality is the SQI, because it is highly correlated with live sperm concentration as well as sperm viability and motility (McDaniel *et al.*, 1998; Parker *et al.*, 2000, 2002; Parker and McDaniel, 2003, 2004).

In the present study, the percentage of dead sperm increased only 5 % over 16 h of storage and was negatively correlated with the SQI from freshly ejaculated sperm. Negative correlation for the SQI with the percentage of dead sperm is most likely due to the immotility of dead sperm. Because dead sperm are immotile they cannot interact with the internal light path of the analyzer and therefore are unable to increase SQI readings. For example, if a semen sample contains 40 % dead sperm, the SQI will be lower when compared to the SQI from a sample with only 20 % dead sperm (Dumpala *et al.*, 2006). Even though the percentage of dead sperm increased slightly from 9 to 14% as the storage period increased in this study, the SQI from semen stored for 16 h was similar to that of fresh semen. Dumpala *et al.* (2006) reported that there were no differences in the SQI from semen samples containing approximately 80 to 100% viable sperm, and in the present study, the percentage of viable sperm only ranged from 86 to 90%.

A strong negative correlation existed for the SQI obtained from freshly ejaculated semen with the percentage of dead sperm in the first 2 h of storage ($r = -0.81$), and the correlation coefficients became weaker as the storage period increased, but remained better than $r = -0.44$. Apparently, the SQI obtained from freshly ejaculated semen is more predictive of changes in sperm viability during the first 2 h of storage as opposed to the end of storage. Also in this trial, there was a slight linear

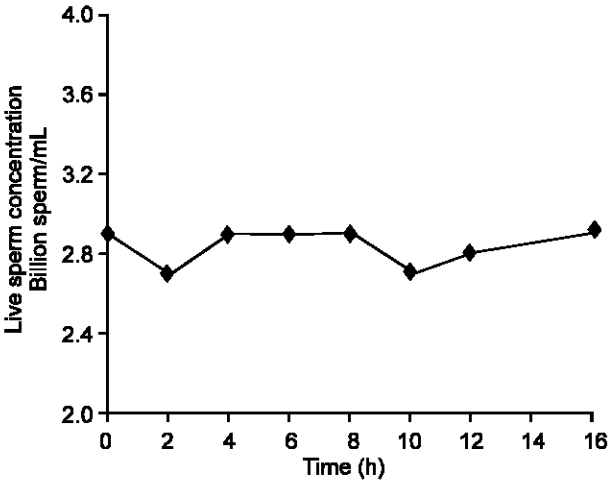

Fig. 3: The relationship of live sperm concentration with storage period. Each point represents the mean of 20 individual males at each time period. Each individual males sperm concentration was obtained from semen diluted 1:1 with BPSE. No relationship existed for sperm concentration over storage.

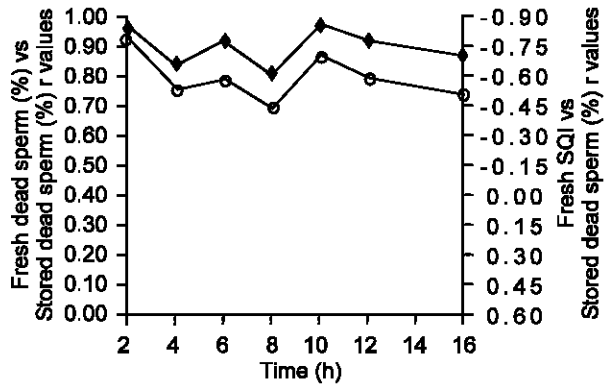

Fig. 4: Correlation coefficients for the percentage of dead sperm at time 0 with the percentage of dead sperm over storage (♦) and the sperm quality index (SQI) at time 0 with the percentage of dead sperm over storage (○). Each data point represents the r value obtained at each time period from 20 individual males.

increase in the percentage of dead sperm as the length of storage increased. Possibly due to this increase in the percentage of dead sperm, a few of the males during storage may have changed sperm viability rank position in the population, yielding weaker correlation coefficients over time for the SQI from fresh semen with the dead sperm percentage. In support of this reasoning, note that directional changes over storage period mirrored one another exactly in correlation coefficients for the relationships of the SQI and percentage of dead sperm from fresh semen with percentage of dead sperm from stored semen (Fig. 4). Therefore, as the ability of dead sperm from fresh semen to predict sperm viability over storage weakened, so did the ability of the SQI from fresh semen to predict sperm viability over storage and vice versa. For example, the lowest and highest correlation coefficients occurred at 8 h and 2 h of storage respectively, for both the relationship of the SQI and the relationship of the percentage of dead sperm from fresh semen with stored sperm viability. Apparently, even the slightest change in sperm viability during storage that alter a male's sperm viability rank in the population also impacts the ability of the SQI from the fresh semen to predict stored sperm viability. However, the SQI from fresh semen was always predictive of stored sperm viability with an average correlation coefficient of r = -0.60.

The lack of a dramatic reduction in sperm viability over 16 h of storage was most likely due to storage temperature (4°C). For example, sperm stored at 5°C showed a significant reduction in overall metabolic activity, by lowering oxygen consumption and energy production, as well as the metabolic by-products (Wishart, 1989). Because the overall aerobic metabolism of sperm is low at 4°C, there is less production of toxic end products such as 3-carbon glycolytic intermediates, which subsequently form toxic by-products (Riddle, 1968), oxygen-free radicals, and malonaldehydes (Wishart, 1989). As a result of the 4°C temperature used in this study, apparently toxic levels were not achieved, hence preserving sperm viability.

In the present study, the SQI from diluted semen increased 24 units in the first 4 h of storage and then stabilized, yielding similar SQI readings up to 16 h of storage. It is known that freshly ejaculated semen in its neat state contains no free O_2, however, ample O_2 is available from the diluent. As a result, sperm motility is increased due to the addition of O_2 (Parker and McDaniel, 2006). Wishart (1981) reported that aerobic metabolism of fowl spermatozoa can be encouraged by continuous introduction of air during storage. In this trial, semen samples were agitated continuously during storage using a rotary shaker. As a result of this agitation, sperm were provided with the necessary oxygen needed for aerobic metabolism to maintain sperm motility (Sexton, 1974; Wishart, 1981; Huyghebaert *et al*., 1984; Lake *et al*., 1984).

Another factor that could explain this increase in the SQI with storage time could be diluent composition. For example, diluents which contain an energy source such as fructose are known to stimulate motility in chicken sperm (Sexton and Fewlass, 1978). Therefore, this initial increase in the SQI is most likely due to the stimulation of sperm motility because of the availability of oxygen as well as fructose from the diluent. This increase in the SQI is similar to what was reported by Dumpala *et al.* (2006). He noted a 37 unit increase when comparing the

Table 3: Correlation coefficients for the percentage of dead sperm, live sperm concentration, and the sperm quality index (SQI) from freshly ejaculated semen with their respective semen characteristic at each storage period

Storage period (h)	Fresh % dead vs Stored % dead	Fresh live sperm concentration vs Stored live sperm concentration	Fresh SQI vs Stored SQI
		Correlation Coefficients	
2	0.97	0.91	0.89
4	0.84	0.80	0.88
6	0.92	0.95	0.93
8	0.81	0.95	0.94
10	0.97	0.96	0.88
12	0.93	0.96	0.92
16	0.88	0.95	0.89

SQI from time 0 h to the SQI at 4 h of storage after diluting semen with BPSE and storing it at 4°C. However, in that study the SQI continued to increase from 4 to 8 h of storage. In fact, when comparing the SQI from freshly ejaculated semen to the SQI from semen stored for 8 h in the present study, there was only a 7 unit increase in the SQI as opposed to a 49 unit increase previously reported (Dumpala *et al.*, 2006). This increase reported by Dumpala *et al.* (2006) as compared to the plateau in the SQI from 4 to 8 h of storage in the present study might be due to the use of younger males (54 vs 68 wks of age). Typically semen quality declines as the bird ages (Rosenstrauch *et al.*, 1994; Weil *et al.*, 1999).

Additionally, in the present study, similar SQI readings were obtained from 6 h to 16 h of storage. It is known that the SQI is highly correlated with live sperm concentration, sperm viability and motility (McDaniel *et al.*, 1998; Parker *et al.*, 2000, 2002; Parker and McDaniel, 2003, 2004). In the present trial, live sperm concentration remained constant throughout 16 h of storage and the correlation coefficients for the SQI obtained from freshly ejaculated semen with live sperm concentration at each storage period remained approximately 0.5. Also there was only a negligible increase in the percentage of dead sperm (11 to 14 %) after 4 h of storage. Furthermore, holding semen at lower temperatures such as 5 and 15 C, Clarke *et al.* (1982) reported no significant reduction in the percentage of progressively motile chicken and turkey spermatozoa after 6 h of storage when compared to fresh semen. These negligible changes in sperm viability, motility, and concentration over storage could explain why SQI values remained constant between 6 and 16 h of storage.

In the present study, strong positive correlations existed for dead sperm percentage as well as live sperm concentration of freshly ejaculated semen with their respective sperm quality characteristics in stored semen. These data indicate that an individual male's rank in the population with regard to their semen characteristics changed very little over semen storage time. Very strong positive correlations also existed for the SQI obtained from freshly ejaculated semen with the SQI obtained at each storage period in this trial, suggesting that each male's rank in the population with regard to the rate of sperm movement was also maintained over storage. This reasoning is supported by the fact that the SQI, which is also impacted by sperm motility, and the other two semen characteristics that influence the SQI, sperm viability, and concentration, remained rather stable over storage in the present study (McDaniel *et al.*, 1998; Parker *et al.*, 2000). Therefore, if a male's rank in the sperm motility population changed over storage, one would expect lower correlation coefficients for fresh semen SQI versus stored semen SQI. Because the SQI obtained from freshly ejaculated semen was strongly correlated with the SQI from each storage period, it is apparent that the SQI from fresh semen can predict sperm quality up to 16 h of storage. Therefore, the SQI from freshly ejaculated semen could be used to determine if a rooster will maintain superior semen quality even after 16 h of storage or if his semen quality will be poor following storage.

In conclusion, the results of this study reveal that the SQI obtained from chicken semen prior to storage predicts semen quality after storage. Therefore, roosters with a high SQI prior to semen storage should have the best semen quality after storage, and the converse is also true for the rooster with a low SQI. As a result, the SQI may be a useful tool for selecting avian males with the best semen storage capability.

References

Biligili, 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.

Chaudhuri D.G., G.J. Wishart, P.E. Lake and O. Ravie, 1988. Predicting the fertilizing ability of avian semen: a comparison of a simple colorimetric test with other methods for predicting the fertilizing ability of fowl semen. Br. Poult. Sci., 29: 847-51.

Donoghue, A.M. and G.J. Wishart, 2000. Storage of poultry semen. Anim. Reprod. Sci., 62: 213-32.

Dumpala, P.R., H.M. Parker and C.D. McDaniel, 2006. The effect of semen storage temperature and diluent type on the sperm quality index of broiler breeder semen. (Int. J. Poult Sci. submitted).

Huyghebaert, G., F. Van wambeke and G. De Groote, 1984. The effect of pH of diluent, no of spermatozoa and storage method on fertility and hatchability obtained with turkey semen stored for 6 hours and 24 hours. Arch. Geflugelkd., 48: 142-150.

King, L.M. and A.M. Donoghue, 2000. Adaptation of the sperm mobility test for identification of turkey toms with low fertilizing potential. J. Appl. Poult. Res., 9: 66-73.

Lake, P.E., F.L. Cherms and G.J. Wishart, 1984. Effect of aeration on the fertilizing ability of turkey semen stored for 48 h at 5 and 15 C; a study from the 33rd to the 47th week of age. Reprod. Nutr. Dev., 24: 147-153.

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.

McDaniel, G., 1978. Low fertility in broiler hatching eggs. Poult. Dig., (Sep.): 446-448.

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

Neuman, S.L., C.D. McDaniel, L. Frank, J. Radu and P.Y. Hester, 2002b. Use of a sperm quality analyser on semen of turkey breeders to monitor storage time effects and age-related changes during a reproductive cycle. Br. Poult. Sci., 43: 465-471.

Parker, H.M., J.B. Yeatman, C.D. Schultz, C.D. Zumwalt, and C.D. Mc Daniel, 2000. Use of a sperm quality analyzer for evaluating broiler breeder males. 2. Selection of young broiler breeder roosters 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 optibreed® sperm quality index when hens are inseminated with a constant number of sperm. Poult. Sci., 81: 239-45.

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.

Parker, H.M. and C.D. McDaniel, 2004. The optimum semen dilution for the sperm quality index that is most predictive of broiler breeder fertility. Int. J. Poult. Sci., 3: 588-592.

Parker, H.M. and C.D. McDaniel, 2006. The immediate effect of semen diluent and rate of dilution on the sperm quality index, ATP utilization, gas exchange, and ion balance of broiler breeder sperm. Poult. Sci., 85: 106-116.

Reddy, R.P., 1995. Artificial Insemination of broilers: Economic and management implications. In: Bakst M.R., Wishart G.J. (Eds.), Proc. 1st Int. Symp. on Artificial Insemination of Poultry. Poultry Science Association, Savoy, IL, pp: 73-89.

Riddle, V.V., 1968. Energy metabolism of fowl spermatozoa: evidence for mechanisms and artifacts in Ringer's phosphate buffer. PhD Dissertation, University of California at Davis.

Rosenstrauch, A., A.A. Degen and M. Friedlander, 1994. Spermatozoa retention by Sertoli cells during the decline in fertility in aging roosters. Biol. Reprod., 50: 129-136.

Sexton, T.J., 1974. Oxidative and glycolytic activity of chicken and turkey spermatozoa. Comp. Biochem. Physiol., 48B: 39-65.

Sexton, T.J. and T.A. Fewlass, 1978. A new poultry semen extender 2. Effect of the diluent components on the fertilizing capacity of chicken semen stored at 5 degrees C. Poult. Sci., 57: 277-84.

Steel, R.G.D. and J.H. Torrie, 1980. Principles and procedures of statistics. A biometrical approach. McGraw Hill Book Co. Inc., New York, NY.

Weil, S., I. Rozenboim, A.A. Degen, A. Dawson, M. Friedlander and A. Rosenstrauch, 1999. Fertility decline in aging roosters is related to increased testicular and plasma levels of estradiol. Gen. Comp. Endocrinol., 115: 23-28.

Wishart, G.J., 1981. The effect of continuous aeration on fertility of fowl and turkey semen stored above 0°C. Br. Poult. Sci., 22: 445-450.

Wishart, G.J., 1989. Physiological changes in fowl and turkey spermatozoa during *in vitro* storage. Br. Poult. Sci., 30: 443-54.

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

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