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Correlation of the Sperm Quality Index with ATP Utilization, Gas Exchange and Ionic Balance of Broiler Breeder Semen[1]

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Abstract: The Sperm Quality Index (SQI) is capable of predicting semen quality when semen is diluted 10-fold prior to analysis; yet when semen is diluted, changes in motility, gas exchange, Adenosine Triphosphate (ATP) and ion content occur. Therefore, the objectives of this research were to determine if the SQI could be a predictor of ATP, gas and ion content of semen and to determine if O_2 and ATP content of semen could be used as predictors of seminal ion content when ejaculates are diluted 10-fold. Prior to semen analysis, ejaculates from 70 Ross males were diluted 10-fold using 0.85% saline and immediately analyzed to determine each ejaculate's SQI as well as ATP, pH, O_2, CO_2, Na^+, K^+, Ca^{2+} and Cl^- content. There was a positive correlation between the SQI and ATP present per sperm ($r = 0.70$, $p<0.0001$). However, the coefficients for the SQI with concentrations of O_2, Ca^{2+}, Na^+ and Cl^- present per sperm were negative ($r = -0.70$, -0.49, -0.51 and -0.53, respectively). The coefficient for the concentration of O_2 per sperm with ATP per sperm was negative ($r = -0.41$) yet the coefficients for CO_2, Ca^{2+}, Na^+, K^+ and Cl^- were positive ($r = 0.30$, 0.74, 0.77, 0.50 and 0.77, respectively). In conclusion, the SQI and O_2 present per sperm can predict ATP and ion content in broiler breeder semen samples diluted 10-fold.

Key words: Sperm quality index, broiler breeder, ion, adenosine triphosphate, gas exchange

Introduction

The Sperm Quality Index (SQI) is a semen testing tool which is correlated with poultry sperm concentration, viability and motility (McDaniel *et al*., 1998; Neuman *et al*., 2002; Parker and McDaniel, 2003). The SQI, from semen diluted no greater than 10-fold, is also correlated with broiler breeder fertility and has been used to select males for house placement to improve hatchability (Parker *et al*., 2000, 2002; Parker and McDaniel 2002, 2003 and 2004). The SQI is determined by the Sperm Quality Analyzer (SQA; MEDICAL ELECTRONIC SYSTEMS LTD., Migdal Haemek, Israel) and is a single number that is generated when sperm movement disrupts an internal light path. Because broiler breeder sperm concentrations are high, sperm are unable to move within the SQA capillary unless semen is diluted prior to SQI analysis (McDaniel *et al*., 1998).

However, when semen is diluted excessively, a dilution effect occurs and sperm motility is altered (Mann, 1964). Factors such as Adenosine Triphosphate (ATP), O_2 and seminal ion concentrations are associated with sperm motility (Wishart 1984; Nevo, 1965; Thomson and Wishart, 1991). Factors that are known to impact sperm motility, such as gas exchange, ATP and ion utilization, are also altered when semen is diluted (Parker and McDaniel, 2006).

Parker and McDaniel (2006) reported that freshly ejaculated neat semen has no free O_2, yet ample O_2 is available in the diluent. Because semen must be diluted prior to SQI analysis, it appears that this increase in diluent O_2 results in increased motility. Parker and McDaniel (2006) also reported an increase in motility when seminal ions were utilized by sperm. Therefore, diluent O_2 may also impact the utilization of seminal ions by sperm.

Because the SQI from 10-fold diluted semen is highly correlated with sperm motility and fertility (McDaniel *et al*., 1998; Parker and McDaniel, 2003, 2004), it is possible that a correlation exists for the SQI with factors that are associated with sperm motility. The SQI could possibly be an indicator of each factor's ability to influence sperm motility as well as fertility. Also, the utilization of seminal ions by sperm from diluted semen is altered as the concentration of O_2 increases. Therefore, it is possible that the O_2 available from the diluent could be an indicator of sperm motility as well as ATP and seminal ion utilization. Because ATP is correlated with sperm motility and is a known energy source for spermatozoa, it is possible that the relationship of ATP with O_2, Na^+, K^+, Ca^{2+} and Cl^- present in diluted semen could be used as an indicator of a diluted ejaculate's sperm motility. Therefore, the objective of this study was three-fold. The first objective was to determine if correlations exist for the SQI from 10-fold diluted semen with the concentrations of ATP, gas and seminal plasma ions per sperm in diluted semen samples. The second objective was to examine if relationships exist for the concentration of O_2 available per sperm with CO_2 and seminal plasma ions available per sperm from 10-fold diluted semen. The final objective was to determine if relationships exist for ATP with O_2, Na^+, K^+, Ca^{2+} and Cl^- present per sperm when broiler breeder semen is diluted 10-fold.

Materials and Methods

Housing and environment: Seventy Ross broiler breeder males, 68 wk of age, were obtained from a local integrator. Males were housed in individual cages and maintained using conventional environmental controls. Males were fed a standard breeder diet (1.55 MJ day^{-1} per bird) and feed restricted according to primary breeder recommendations. All males received 16 h of light daily throughout the experiment and were treated in accordance with the Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching.

Semen preparation and evaluation: Ejaculates from each of the 70 males were collected using the method of Burrows and Quinn (1937). Immediately after semen collection, sperm concentration was determined for each male's undiluted ejaculate using two absorbance readings obtained from a MicroReader (IMV INTERNATIONAL, Maple Grove, MN). Each ejaculate was then diluted 10-fold. Two replications from each male's 10-fold diluted semen sample was used to determine the SQI, sperm viability, ATP, gas and ion concentrations. These measurements were taken on whole semen immediately (within 1 min) after an ejaculate was diluted. The method of McDaniel *et al.* (1998) was used to determine the SQI. For sperm viability, the fluorometric method of Bilgili and Renden (1984) was utilized. To determine ATP content, the technique of Gottlieb *et al.* (1987) was used on an Lmax luminometer (MOLECULAR DEVICES CORP., Sunnyvale, CA; Donoghue and Walker-Simmons, 1999). A gas and electrolyte analyzer, ABL77 (RADIOMETER, Copenhagen, Denmark) was used to determine pH, O_2, CO_2, Na^+, K^+, Ca^{2+} and Cl^- concentrations present in each diluted semen sample. This analyzer uses potentiometry to measure pH, CO_2, Na^+, K^+, Ca^{2+} and Cl^- ; and amperometry to determine O_2 concentrations (Wishart, 1984). For ATP, O_2, CO_2, Na^+, K^+, Ca^{2+} and Cl^-, each concentration present in the diluted sample was divided by each ejaculate's respective sperm concentration to correlate the SQI with ATP, O_2, CO_2, Na^+, K^+, Ca^{2+} and Cl^- present per sperm, O_2 present per sperm with CO_2, Na^+, K^+, Ca^{2+} and Cl^- present per sperm as well as ATP with Na^+, K^+, Ca^{2+} and Cl^- present per sperm.

Statistical analyses: Pearson's correlation coefficients were obtained for the SQI with pH, as well as ATP, O_2, CO_2, Na^+, K^+, Ca^{2+} and Cl^- content present per sperm from individual 10-fold diluted semen samples (Steel and Torrie, 1980). Pearson's correlation coefficients were also used to determine the relationship of O_2 present per sperm with ATP, CO_2, Na^+, K^+, Ca^{2+} and Cl^- present per sperm, as well as ATP with Na^+, K^+, Ca^{2+} and Cl^- present per sperm from diluted semen samples. Correlation coefficients were considered significant at $p< 0.05$.

Results

The concentration of ATP, O_2, CO_2, Na^+, K^+, Ca^{2+} and Cl^- as well as the pH of 0.85% saline is presented in Table 1. The pH of 0.85% saline prior to semen dilution was 7.43. The diluent contained no detectable ATP, CO_2, K^+, or Ca^{2+}. However, 178 nmol/mL of O_2, 149 µmol/mL of Na^+ and 126 µmol/mL of Cl^- were present in the diluent. The mean SQI for this trial was 362 units with the values ranging from 0 to 531 units (Table 2). The correlation coefficients for the SQI from 10-fold diluted semen with ATP, gas and ion content present per sperm are shown in Table 2. There was a positive correlation for the SQI with ATP content present per sperm (r = 0.70). However, the correlation coefficients for the SQI with O_2, Na^+, Ca^{2+} and Cl^- present per sperm were negative. For O_2 present per sperm, the correlation coefficient was r = -0.70. The correlation coefficients for the content of Na^+, Ca^{2+} and Cl^- present per sperm were r = -0.51, -0.49 and -0.53, respectively. There was no relationship for the SQI with CO_2 and K^+present per sperm or between the SQI and pH (Table 2).

The correlation coefficient was negative between O_2 present per sperm and ATP present per sperm (r = -0.41), yet the coefficients were positive for O_2 present per sperm with CO_2 per sperm as well as the seminal ions present per sperm when semen was diluted 10-fold immediately prior to analysis (Table 3). The coefficient for O_2 present per sperm with CO_2 per sperm was r=0.30. The correlation for the concentration of O_2 present per sperm with Na^+ and Cl^- present per sperm were the numerically highest coefficients (r = 0.77 and 0.77, respectively). The correlation coefficient for O_2 present per sperm with K^+ was r = 0.50 and r = 0.74 for Ca^{2+} present per sperm. No significant correlation was reported for ATP present per sperm with Na^+, K^+, Ca^{2+} and Cl^- present per sperm (data not shown).

Discussion

There are several factors such as ATP (Wishart and Palmer, 1986), O_2 (Nevo, 1965) and seminal ions, i.e., Ca^{2+} (Thomson and Wishart, 1991), that are associated with avian sperm motility. Because semen must be diluted prior to SQI analysis, sperm motility and factors affecting motility such as ATP, gas exchange and ionic balance are also altered due to dilution rate as well as diluent composition (Parker and McDaniel, 2006).

Another factor known to affect sperm motility is pH. Holm and Wishart (1998) reported an increase in both sperm velocity and the percentage of motile sperm when the pH increased from 7.0 to 8.0. Even though it has been reported that pH has an impact on sperm motility and the SQI is predictive of sperm motility (McDaniel *et al.*, 1998), there was no relationship for the SQI with pH in this trial. In this trial, the diluent pH was 7.43 with pH values from diluted semen samples ranging from 6.66

Table 1: Adenosine Triphosphate (ATP), pH, gas and ion content of 0.85% saline prior to dilution[1]

ATP (µmol/mL)	0.00
pH	7.43
O_2 (nmol/mL)	178.00
CO_2 (µmol/mL)	0.00
Na^+ (µmol/mL)	149.00
K^+ (µmol/mL)	0.00
Ca^{2+} (µmol/mL)	0.00
Cl^- (µmol/mL)	126.00

[1]These averages were obtained from 2 replications.

to 7.57, yielding an overall mean pH of 7.24 (Table 2), which is at the lower range of pH reported by Holm and Wishart (1998). Because the overall mean pH from diluted semen samples was 7.24 and the range in pH was so narrow, this most likely explains why there was no correlation for the SQI (motility) with pH.

Unlike the correlation between the SQI with pH, there was a positive correlation for the SQI with ATP present per sperm. Research has shown that both the SQI (McDaniel *et al.*, 1998; Neuman *et al.*, 2002) and concentration of ATP (Wishart and Palmer, 1986) are highly correlated with avian sperm motility and that rooster sperm are capable of efficiently producing ATP under aerobic as well as anaerobic conditions (Sexton, 1974). Because the correlation coefficient was positive between the SQI and ATP present per sperm, it is apparent that as the SQI increases, sperm are capable of producing ATP to maintain increased sperm motility and that this production of ATP is aerobic as there was ample O_2 found in the diluent to allow sperm to produce ATP aerobically (Sexton, 1974). This aerobic production of ATP to maintain sperm motility is further evidenced by the negative correlations of the SQI with O_2 present per sperm as well as for the concentration of O_2 present per sperm with ATP present per sperm. For example, it is evident that as the SQI (motility) increases, sperm utilize O_2 and produce ATP to maintain increased sperm motility. These results are in agreement with the results reported by Parker and McDaniel (2006). They reported that when semen is diluted 10-fold with saline, sperm are producing ATP, while sperm motility and O_2 utilization is increasing rapidly.

Similar to the relationship between the SQI and pH in this trial, there was no relationship detected for the SQI with CO_2 present per sperm. Using pooled semen diluted with saline, Parker and McDaniel (2006) found that as sperm motility changes, O_2 utilization is more variable than CO_2 generation. In fact in the present study, the range in O_2 per sperm was also much greater when compared to the range for CO_2 per sperm. This greater range in O_2 utilization may explain why there was a negative relationship for the SQI with O_2 present per sperm and why there was no relationship detected between the SQI and CO_2 present per sperm.

As with O_2 present per sperm, the SQI was negatively correlated with the seminal plasma ions, Na^+, Ca^{2+} and Cl^-, present per sperm in this study. When using pooled semen, it has been revealed that sperm externalize Na^+ and Cl^- and internalize Ca^{2+} as motility initially increases due to dilution with saline (Parker and McDaniel, 2006). The diluent used in this study contained concentrations of Na^+ and Cl^- which would be available for sperm to use for motility if necessary. Interestingly, the correlation coefficients were slightly more negative for the SQI with Na^+ and Cl^- present per sperm when compared to Ca^{2+} present per sperm. It is possible that the correlation coefficients were more negative for Na^+ and Cl^- present per sperm because Na^+ and Cl^- were present in the diluent as well as in each ejaculate's seminal plasma immediately after dilution. Because these ions were present in the diluent and the correlation coefficients were negative for the SQI with Na^+ and Cl^- present per sperm, it is apparent that semen from individual males with good sperm motility contain less free Na^+ and Cl^- per sperm.

Unlike Na^+ and Cl^-, there was no free Ca^{2+} available in the diluent. It has been reported that seminal plasma Ca^{2+} levels are lower in males with good sperm motility versus males with poor sperm motility when males were grouped according to their SQI (Karaca *et al.*, 2002). In the present study, the SQI ranged from 0 to 531 units. Apparently as the SQI increases, sperm are utilizing more extruded Ca^{2+} found in the seminal plasma to maintain motility resulting in a negative relationship for the SQI with Ca^{2+} present per sperm.

The correlations for the SQI with the seminal plasma ions present per sperm were negative, yet the correlation coefficients were positive for the content of O_2 present per sperm with CO_2, Na^+, K^+, Ca^{2+} and Cl^- present per sperm. Using pooled semen diluted 10-fold, it has been reported that as sperm motility increases the utilization of O_2 and the generation of CO_2 increase but not at the same rate (Parker and McDaniel, 2006). Because sperm motility is dependent on O_2 (Nevo, 1965) and CO_2 is a by-product of sperm movement, this would explain the positive correlation coefficient for the content of O_2 present per sperm with CO_2 present per sperm.

The correlation coefficients for the amount of O_2 present per sperm with the seminal ions were greater when compared to O_2 present per sperm with CO_2 present per sperm. It has been reported that intracellular Na^+ and Cl^- are externalized when pooled semen is diluted 10-fold with saline (Parker and McDaniel, 2006). In this trial, it is apparent that concentrations of intracellular Na^+ and Cl^- extruded into the seminal plasma are dependent on the amount of O_2 available per sperm.

Even though concentrations of Na^+ and Cl^- were found in the diluent, their correlation coefficients were similar to the coefficients for O_2 present per sperm with Ca^{2+} present per sperm; yet the coefficient was lower for O_2 present per sperm with K^+ present per sperm. Research

Table 2: Correlation coefficients from 10-fold diluted semen for the sperm quality index (SQI) with adenosine triphosphate (ATP), gas and ion content present per sperm[1]

	Mean	Std. Dev	Min.	Max.	Correlation coefficient (r)	P value[2]
SQI	362.00	141.00	0	531	-------	------
pH	7.24	0.16	6.66	7.57	NS	0.7345
ATP/sperm (fmole)	0.02	0.02	0.002	0.10	0.70	0.0001
O_2/sperm (fmole)	0.41	0.93	0.013	6.60	-0.70	0.0001
CO_2/sperm (fmole)	0.14	0.11	0.028	0.67	NS	0.8514
Na^+/sperm (fmole)	437.00	818.00	130.400	6120.00	-0.51	0.0001
K^+/sperm (fmole)	4.24	8.97	0.702	69.80	NS	0.0713
Ca^{2+}/sperm (fmole)	1.56	4.46	0.238	35.80	-0.49	0.0001
Cl^-/sperm (fmole)	369.00	704.00	11.600	5291.00	-0.53	0.0001

[1]These data are from averages obtained from individual ejaculates of 70 males from 2 replications.
[2]P< 0.05 considered statistically significant. NS indicates a nonsignificant correlation coefficient.

Table 3: Correlation coefficients from 10-fold diluted semen for the concentration of oxygen present per sperm with adenosine triphosphate (ATP), CO_2 and seminal ions present per sperm[1]

	Correlation coefficient	P value[2]
ATP per sperm	-0.41	0.0005
CO_2 per sperm	0.30	0.0136
Na^+ per sperm	0.77	0.0001
K^+ per sperm	0.50	0.0001
Ca^{2+} per sperm	0.74	0.0001
Cl^- per sperm	0.77	0.0001

[1]These data are from averages obtained from individual ejaculates of 70 males from 2 replications.
[2]P< 0.05 considered statistically significant.

has revealed that K^+ has an influence on O_2 uptake by sperm (Lake, 1966). Because K^+ impacts the utilization of O_2 by sperm, this would explain why O $_2$present per sperm is correlated with K^+ present per sperm. Interestingly, even though K^+ impacts O_2 uptake by sperm and the SQI was correlated with O_2 present per sperm, there was no relationship for the SQI with K^+ present per sperm in this study.

It has been reported that the concentrations of intracellular Ca^{2+} regulate sperm respiration as well as motility (Ashizawa *et al.*, 1992) and that sperm internalize Ca^{2+} for motility (Parker and McDaniel, 2006). In this trial the correlation coefficient was positive for O_2 with Ca^{2+} present per sperm and for Ca^{2+} present per sperm with CO_2 present per sperm (data not shown). Perhaps as the amount of unutilized O_2 increases, immotile sperm must be extruding intracellular Ca^{2+} into the seminal plasma and ultimately the diluent.

Both ATP and K^+ are known to affect sperm motility (Wishart, 1984). Also it has been reported that sperm utilize ATP dependent Na^+ pumps to extrude intracellular Na^+ (Quinn *et al.*, 1965). Interestingly, there was no correlation for the concentration of ATP per sperm with the seminal ions per sperm in the present study.

In conclusion, because the SQI obtained from 10-fold diluted semen is correlated with sperm motility, ATP, O_2, Na^+, Ca^{2+} and Cl^- present per sperm, it is possible that the SQI could be used as an indicator of ATP, seminal gases and seminal ions present per sperm in 10-fold diluted semen samples. Also, the relationship for the concentration of O_2 present per sperm with the seminal ions present per sperm could be used to predict the response of seminal ions to semen dilution and sperm motility. Surprisingly, the concentration of ATP present per sperm is not an indicator of the concentrations of seminal plasma ions present per sperm.

References

Ashizawa, K., A. Hashiguchi, and Y. Tsuzuki, 1992. Intracellular free Ca^{++} concentration in fowl spermatozoa and its relationship to motility and respiration in spermatozoa. J. Reprod. Fert., 96: 395-405.

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. and M.K. Walker-Simmons, 1999. The influence of wheat dehydration-induced proteins on the function of turkey spermatozoa after twenty-four hour in vitro storage. Poult. Sci., 78: 235-241.

Gottlieb, C., K. Svanborg, P. Eneroth and M. Bygdeman, 1987. Adenosine triphosphate in human semen: A study on conditions for a bioluminescence assay. Fert. Ster., 47: 992-999.

Holm, L. and G.J. Wishart, 1998. The effect of pH on the motility of spermatozoa from chicken, turkey and quail. Anim. Reprod. Sci., 54: 45-54.

Karaca, A.G., H.M. Parker and C.D. McDaniel, 2002. Elevated body temperature directly contributes to heat stress infertility of broiler breeder males. Poult. Sci., 81: 1892-1897.

Lake, P.E., 1966. Physiology and biochemistry of poultry semen. Adv. Reprod. Physiol., 1: 93-123.

Mann, T., 1964. The Biochemistry of Semen and of the Male Reproductive Tract. John Wiley, New York.

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 s perm 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. Utilisation of a sperm quality analyzer to evaluate sperm quantity and quality of Turkey breeders. Br. Poult. Sci., 43: 457-464.

Nevo, A.C., 1965. Dependence of sperm motility and respiration on oxygen concentration. J. Reprod. Fert., 9: 103-107.

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 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: 239-245.

Parker, H.M. and C.D. McDaniel, 2002. Selection of young broiler breeders for semen quality improves hatchability in an industry field trial. J. Applied Poult. Sci., 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. Intl. J. Poult. Sci., 3: 588-592.

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

Quinn, P.J., I.G. White and B.R. Wirrick, 1965. Studies of the distribution of the major cations in semen and male accessory secretions. J. Reprod. Fert., 10: 379-388.

Sexton, T.J., 1974. Oxidative and glycolytic activity of chicken and turkey spermatozoa. Comp. Biohem. Physiol., 48: 59-65.

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

Thomson, M.F. and G.J. Wishart, 1991. Temperature-mediated regulation of calcium flux and motility in fowl spermatozoa. J. Reprod. Fert., 93: 385-391.

Wishart, G.J., 1984. The effect of anoxia on fowl spermatozoa: recovery of fertilizing ability, motility and cellular concentrations of potassium and ATP. Gamete. Res., 10: 83-89.

Wishart, G.J. and F.H. Palmer, 1986. Correlation of the fertilising ability of semen from individual male fowls with sperm motility and ATP content. Br. Poult. Sci., 27: 97-102.

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