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The Effect of Semen Storage Temperature and Diluent Type on the Sperm Quality Index of Broiler Breeder Semen[1,2]

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Abstract: The sperm quality index (SQI) is predictive of fresh semen quality. Our objective was to examine if semen storage affects the SQI obtained from undiluted semen, or semen diluted with either Beltsville Poultry Semen Extender (BPSE) or Minimum Essential Medium (MEM) and held for 8 h at 4, 21, or 41°C. Dead sperm percentage was higher and SQI was lower from undiluted versus diluted semen. Dead sperm percentage was higher and SQI was lower for semen stored at 41°C than at lower temperatures. Overall, there was a linear increase in dead sperm percentage and linear decrease in SQI over storage length. Regardless of diluent, there was a linear increase in dead sperm percentage over time for semen stored at 4 and 21°C. For semen held at 41°C and diluted with BPSE or MEM there were respective quartic and linear increases in dead sperm percentage over time; a drastic linear increase existed for undiluted semen. There was a linear decrease in SQI from undiluted semen and semen diluted with MEM over time at 4°C; however, for semen diluted with BPSE, there was a linear increase. The SQI from undiluted semen stored at 41°C decreased linearly over time. At 41°C, a cubic relationship existed for SQI over time for semen diluted with BPSE, and a linear decline was detected for semen diluted with MEM. In conclusion, the SQI is indicative of changes induced by diluent type, storage temperature, and length of semen storage.

Key words: Sperm quality index, dead sperm, beltsville poultry semen extender, minimum essential medium

Introduction

The increasing use of artificial insemination (AI) in the poultry industry emphasizes the need for the distribution of good quality sperm. In order for the poultry industry to take advantage of modern AI techniques, proper storage of poultry semen is necessary. As chicken semen is highly concentrated and is of low volume, the extension of neat semen with a proper diluent is required prior to AI and storage. Several factors play a role in maintaining the quality of semen over storage. For example, the diluents used for semen extension and storage conditions such as time, aeration, and holding temperature play a major role.

It is known that sperm motility and the fertilizing ability of undiluted neat fowl semen stored *in vitro* usually decreases within 1 h of collection (Carter *et al*., 1957). Therefore, to store fowl semen, the type of diluent and storage temperature are very important to avoid a reduction in sperm quality. For example, Clarke *et al*. (1984) found that dilution of chicken semen resulted in a significant decrease in the percentage of dead sperm. They also revealed that increasing the incubation time to 6 h, raising the storage temperature to 41°C, or both, resulted in an increase in the percentage of dead sperm in chicken and turkey spermatozoa. For stored semen, Clarke *et al*. (1982) reported that sperm motility from both undiluted and diluted chicken semen is lowest when stored at 41°C, which is near the body temperature of the hen. This is in contrast to semen stored at 25, 15, or 5°C. They also revealed that sperm motility is not affected by dilution at storage temperatures of 15 and 5°C.

Although semen quality and therefore fertilizing ability of cock spermatozoa can be retained *in vitro* for 24 to 48 h at 5°C (Sexton, 1979; 1980), this ability is most often lost within a few hours when incubated *in vitro* at 41°C (Schindler *et al*., 1955). However, Ashizawa *et al*. (1976) reported that cock sperm stored in the presence of tissue cultures cells and minimum essential medium (MEM) for 2-4 days at 41°C had higher levels of fertility (89.9%) when compared to fresh semen. For semen diluted with MEM and stored 6 h at 41°C, Howarth (1983) reported significantly higher fertility when compared to semen stored at 41°C in either Beltsville poultry semen extender (BPSE) or Lake's Diluent A. In fact, Howarth (1983) found that semen stored at 41°C in MEM for 6 h had improved fertilizing capacity as compared to fresh semen. At much lower temperatures (2-5°C), semen diluted with BPSE stored up to 24 h yielded no loss in fertilizing capacity (Van Wambeke, 1970; 1972; Sexton, 1978). Furthermore, aeration of semen samples has allowed the storage of chicken spermatozoa for 48 hr. This resulted in the fertility of hens exceeding 90% of that of freshly diluted semen (Wishart, 1981).

To avoid losses in fertility, proper evaluation of semen prior to AI or storage is very important (Reddy and Sadjadi, 1990; Hammerstedt, 1992). Because semen evaluation is extremely important for semen storage and AI, a method of semen evaluation which is rapid, economical, objective and strongly predictive of fertility

would be beneficial to the poultry industry. One such method that would satisfy all of the aforementioned requirements is the sperm quality index (SQI), which is generated from a sperm quality analyzer. Research has shown that the SQI from freshly ejaculated semen is an objective predictor of chicken and turkey sperm motility, concentration, and viability (McDaniel *et al.*, 1998; Parker *et al.*, 2000; Neuman *et al.*, 2002a; Dumpala, 2006). Also, the SQI from freshly ejaculated semen has been positively correlated with broiler breeder fertility and hatchability (Parker *et al.*, 2000, 2002; Parker and McDaniel, 2002, 2003, 2004). Research conducted in turkeys revealed that the SQI accurately reflects declining semen quality with prolonged storage (Neuman *et al.*, 2002b). Recently, it has been determined that the SQI from freshly ejaculated chicken semen is a good predictor of stored semen quality when semen is stored up to 16 h (Dumpala, 2006).

Because chicken semen is highly concentrated, it must be diluted prior to SQI analysis (McDaniel *et al.*, 1998). It is also known that diluent type immediately affects sperm motility and, therefore, the SQI (Parker and McDaniel, 2006). For example, semen diluted excessively (more than 10-fold) yields higher SQI readings when diluted with MEM as compared to 0.85% saline or seminal plasma (Parker and McDaniel, 2006). However, these results were obtained immediately after diluting freshly ejaculated semen held at room temperature. To date, no research has been conducted which investigates if the storage of chicken semen in different diluents at various temperatures impacts the SQI. Therefore, the objective of the present study was to examine if changes occur in the SQI when semen is diluted with either BPSE or MEM and held at storage temperatures of 4, 21, or 41°C for 8 h. Such observations may contribute to our understanding of the ability of the SQI to measure changes in semen quality during storage and also to further elucidate the optimal conditions for proper storage of chicken semen.

Materials and Methods

Housing and environment: Twenty-five Ross broiler breeder males, 54 wk of age, were obtained from a local integrator. Roosters were randomly divided into two groups and 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. All males 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). Treatments were arranged in a 3 x 3 factorial (3 diluent types and 3 storage temperatures) using 2 semen pools from 2 groups of males. The semen treatments used for this study were undiluted neat semen, which served as the control, and semen diluted with either BPSE II (CONTINENTAL PLASTIC CORP., Delavan, WI) or MEM (Howarth, 1981). For BPSE and MEM, semen was diluted 1:1 immediately after semen collection. After the initial semen dilution, each semen group was further divided into 3 subpools and assigned a storage temperature of either 4, 21, or 41°C. All treatments were aerated during 8 h of storage using a rotary shaker (Clay Adams, Division of Becton, Dickinson and Co., Parsippany, NY). The SQI was obtained using the Sperm Quality Analyzer® (Medical Electronic Systems Ltd, Migdal, Haemek, Israel). To obtain the SQI, previously 1:1 diluted semen was further diluted 5-fold with 0.85% saline at room temperature immediately prior to analysis (McDaniel *et al.*, 1998). Undiluted neat semen was diluted 10-fold with saline prior to SQI analysis. 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 Bilgili and Renden (1984). Measurements were taken for each treatment at 0, 1, 2, 3, 4, 5, 6, 7, and 8 h of storage. All treatments within a group were tested randomly at each time period. After the completion of 0 h testing on semen from group 1 males, semen from group 2 was collected, pooled and divided as previously described and randomly tested at each time period.

Statistical analyses: Data were analyzed as a randomized complete block with each male group representing a block and treatments arranged in a 3 x 3 factorial (3 diluent types and 3 storage temperatures). For the SQI and the percentage of dead sperm, means were separated using Fisher's protected least significant difference at $\alpha < 0.05$. Regression analyses were used to determine the relationships of the SQI and percentage of dead sperm over storage time (Steel and Torrie, 1980).

Results

The main effect of diluent type on the percentage of dead sperm and the SQI is presented in Table 1. The percentage of dead sperm from undiluted neat semen was higher than semen diluted with BPSE and MEM ($P < 0.03$). The SQI from semen diluted with BPSE and MEM were significantly higher than the SQI from undiluted neat semen ($P < 0.0001$). When comparing the percentage of dead sperm or the SQI from semen diluted with BPSE to that of semen diluted with MEM, no differences were detected.

Table 1: The main effect of dilution type on the percentage of dead sperm and the sperm quality index (SQI)

	Diluent type[1]				
	BPSE[2]	MEM[3]	Undiluted Neat	SEM[4]	P-value[5]
Dead sperm (%)	10.9[b]	11.9[b]	17.5[a]	1.29	0.0292
SQI	438[a]	457[a]	370[b]	6.1	0.0001

[a-b]Means within a row with different superscripts are significantly different at P < 0.05. Averages obtained from the undiluted neat semen and semen diluted with respective diluent stored over 8 h. [2]Beltsville poultry semen extender. [3] Minimum essential medium. [4]Pooled standard error of the mean. [5]P < 0.05 considered statistically significant.

Table 2: The main effect of storage temperature on the percentage of dead sperm and the sperm quality index (SQI)

	Temperature[1]				
	4°C	21°C	41°C	SEM[2]	P-value[3]
Dead sperm (%)	10[b]	10.7[b]	20.1[a]	1.29	0.0008
SQI	427[a]	430[a]	404[b]	6.1	0.0413

[a-b] Means within a row with different superscripts are significantly different at P < 0.05. [1]Averages obtained from the semen held at respective temperatures over 8 h. [2]Pooled standard error of the mean. [3]P < 0.05 considered statistically significant.

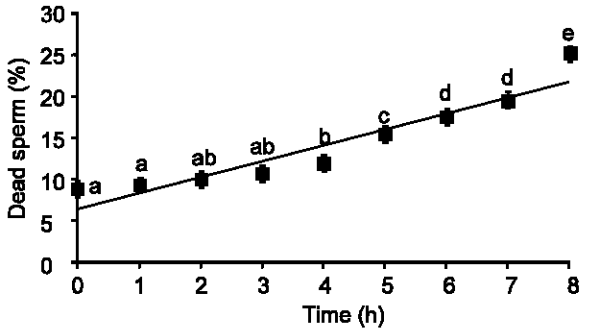

Fig. 1: The main effect of storage period on the percentage of dead sperm. Each point represents the mean of 2 replicates from 3 diluents at 3 temperatures for each time period. a-e Points with different superscripts are significantly different (P < 0.05). There was a linear increase in the percentage of dead sperm as storage time increased (y = 1.93x + 6.32, r^2 = 0.89, P <0.0001).

The main effect of storage temperature on the percentage of dead sperm and the SQI is presented in Table 2. The percentage of dead sperm from semen held at 41 C was significantly higher when compared to semen held at 4 or 21°C (P < 0.0008). The SQI from semen stored at 41°C was significantly lower than that of semen held at 4 or 21°C. No differences in percentage of dead sperm or SQI were seen when comparing storage temperatures of 4 and 21°C.

Averages over diluent type and temperature of storage revealed a linear increase in the percentage of dead sperm over storage time resulting in a significant increase in the percentage of dead sperm after 3 h of storage (Fig. 1, r^2 = 0.89). When averages over diluent type and storage temperature were examined, there was a linear decrease in the SQI over storage time (Fig. 2, r^2 = 0.82). After the first hour of storage, the SQI began to decline significantly.

For the percentage of dead sperm, a significant interaction among diluent type, temperature, and storage period was noticed (P < 0.0001). The relationship for the percentage of dead sperm from undiluted neat semen and semen diluted with either BPSE or MEM stored at 4°C is presented in Fig. 3. There was a slight linear increase over storage time for undiluted neat semen and semen diluted with BPSE and MEM (r^2 = 0.93, r^2 = 0.74, and r^2 = 0.81, respectively). There was also a slight linear increase in the percentage of dead sperm over time for semen stored at 21°C and undiluted or diluted with BPSE and MEM (Fig. 4, r^2 = 0.60, r^2 = 0.83, and r^2 = 0.83, respectively). For semen diluted with BPSE and stored at 41°C, there was a slight quartic increase over time in the percentage of dead sperm (Fig. 5, r^2 = 0.99). However, there was a drastic linear increase in the percentage of dead sperm over time for undiluted neat semen held at 41°C (r^2 = 0.92), yet only a slight linear increase was noted for semen held at 41°C and diluted with MEM (r^2 = 0.83). Significant differences among diluent types were noted for percentage of dead sperm when semen was stored 4 h or more at 41°C. During this period, the percentage of dead sperm from undiluted neat semen was significantly higher than that of semen diluted with BPSE and MEM. When comparing semen diluted with BPSE to semen diluted with MEM, no differences in the percentage of dead sperm occurred over time at 41°C.

For the SQI, a significant interaction between diluent, storage temperature, and time was detected (P< 0.0001). The relationship of the SQI from undiluted neat semen and semen diluted with either BPSE or MEM stored at 4°C over time is presented in Fig. 6. There was a linear decrease in the SQI over storage for both undiluted neat semen and semen diluted with MEM (r^2 = 0.85, r^2 = 0.55, respectively). However, when compared to undiluted neat semen, this decline in the SQI was slower for semen diluted with MEM. For semen diluted

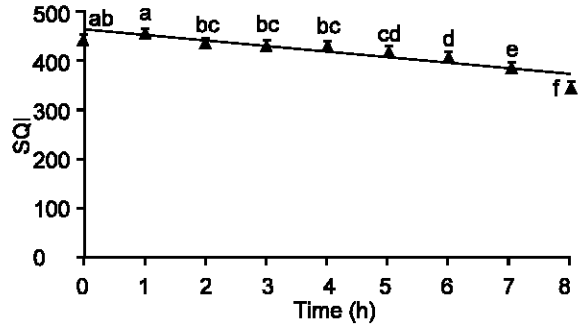

Fig. 2. The main effect of storage period on the sperm quality index (SQI). Each point represents the mean of 2 replicates from 3 diluents at 3 temperatures for each time period. a-f Points with different superscripts are significantly different ($P < 0.05$). There was a linear decrease in the SQI as the storage period increased ($y = -11.06x - 462$, $r^2 = 0.82$, $P < 0.0001$).

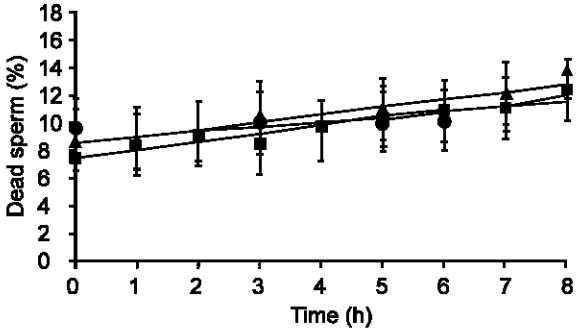

Fig. 3: The relationship of the percentage of dead sperm from undiluted neat semen (■) and semen diluted with either Beltsville poultry semen extender (BPSE, ●) or minimum essential medium (MEM,▲) stored at 4°C over time. Each point represents the mean of 2 replicates at each time period. There was a linear increase in the percentage of dead sperm for undiluted neat semen and semen diluted with BPSE and MEM over time ($y = 0.55x + 7.46$, $r^2 = 0.93$, $P < 0.0001$, $y = 0.39x + 8.45$, $r^2 = 0.74$, $P < 0.0001$, and $y = 0.55x + 8.33$, $r^2 = 0.81$, $P < 0.0001$, respectively).

with BPSE, there was a linear increase in the SQI over time ($r^2 = 0.84$). At 8 h of storage, the SQI was significantly higher for semen diluted with BPSE and MEM as opposed to the SQI from undiluted neat semen. For semen stored at 21°C, regardless of the diluent type, no linear or curvilinear relationships were detected for the SQI over time (Fig. 7). At time 0, the SQI from semen diluted with BPSE and MEM was significantly higher that the SQI from the undiluted neat sample. However, after 8 h of storage, there was no difference in the SQI due to diluent type.

Over storage time, there was a steady linear decrease in the SQI from undiluted neat semen stored at 41°C yielding a zero SQI value at 8 h (Fig. 8, $r^2 = 0.86$). Also, after 1 h of storage the SQI from undiluted neat semen was significantly lower than semen diluted with either BPSE or MEM. For semen diluted with MEM, a slow linear decline ($r^2 = 0.65$) in the SQI was also noted. However, a cubic increase existed for the SQI over time for semen diluted with BPSE ($r^2 = 0.88$).

Discussion

In the present study, average percentage of dead sperm over storage was significantly greater for undiluted neat semen samples (17.5 %) in comparison to semen diluted with BPSE or MEM, and for semen samples stored at 41°C (20.1 %) in comparison to the other storage temperatures examined. Also, a steady increase in average percentage of dead sperm (7 to 25%) was noticed as the length of storage increased. This significant rise in percentage of dead sperm over storage was partially due to a drastic increase in the percentage of dead sperm for undiluted neat semen samples stored at 41°C. This increase in dead sperm at 41°C began after 3 h of storage and continued to increase yielding 75% dead sperm at 8h.

On the other hand, significantly lower average SQI readings over storage were obtained for undiluted neat semen samples (370) and for samples stored at 41°C (404). Also, there was a steady decline in the average SQI (445 to 346) as the length of storage increased to 8 h. This significant reduction in the average SQI readings for undiluted neat semen and for samples held at 41°C over storage was mostly due to the drastic reduction in the SQI from undiluted neat semen samples held at 41°C. This dramatic reduction in the average SQI from undiluted neat semen stored at 41°C began after 3 h of storage and continued to decline, yielding zero readings at 8 h.

It is known that as the percentage of dead sperm in a semen sample increases, the SQI declines (McDaniel *et al.*, 1998; Neuman *et al.*, 2002a; Dumpala, 2006). In the present study, as the percentage of dead sperm increased in undiluted semen, primarily at 41°C over storage, the number of live, motile sperm interacting with the light path decreased, yielding a reduction in SQI readings. McDaniel *et al.* (1998) demonstrated that the SQI declined as the percentage of dead sperm increased, when total sperm concentration remained constant. In the present study, sperm concentration remained constant for each treatment at each storage period, as all of the subpools were obtained from a single semen pool for each male group (data not shown). Similar to the present study, Clarke *et al.* (1984)

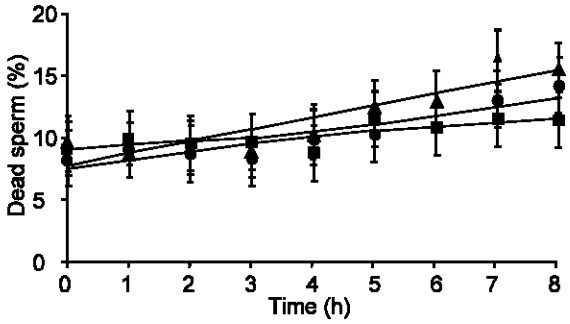

Fig. 4: The relationship of the percentage of dead sperm from undiluted neat semen (■) and semen diluted with either Beltsville poultry semen extender (BPSE, ●) or minimum essential medium (MEM, ▲) at 21 C over time. Each point represents the mean of 2 replicates at each time period. There was a linear increase in the percentage of dead sperm for undiluted neat semen and semen diluted with either BPSE or MEM over storage ($y = 0.31x + 9.11$, $r^2 = 0.60$, $P < 0.0001$, $y = 0.7x + 7.63$, $r^2 = 0.83$, $P < 0.0001$, and $y = 0.96x + 7.87$, $r^2 = 0.83$, $P < 0.0001$, respectively).

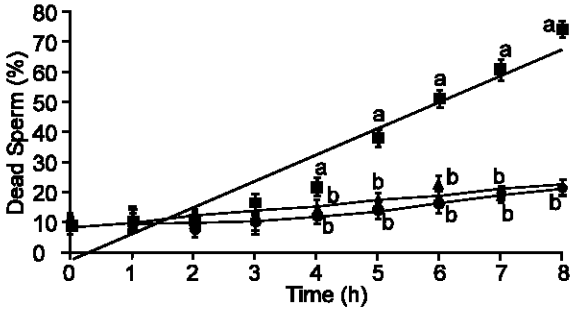

Fig. 5: The relationship of the percentage of dead sperm from undiluted neat semen (■) and semen diluted with either Beltsville poultry semen extender (BPSE, ●) or minimum essential medium (MEM, ▲) at 41 C over time. Each point represents the mean of 2 replicates at each time period. [a-b]Points with different superscripts are significantly different for the percentage of dead sperm at each time period ($P < 0.05$). There was a quartic increase in the percentage of dead sperm for semen diluted with BPSE ($y = -0.13x^4 + 0.21x^3 - 0.82x^2 + 1.66x + 8.07$, $r^2 = 0.99$, $P < 0.0001$). However, there was a linear increase over storage in the percentage of dead sperm for undiluted neat semen and semen diluted with MEM ($y = 8.65x - 2.47$, $r^2 = 0.92$, $P < 0.001$, and $y = 1.79x + 8.15$, $r^2 = 0.83$, $P < 0.0001$, respectively).

reported a relative increase in the percentage of dead sperm from samples stored for 6 h at 41°C. They also reported a higher incidence of morphological defects such as bent spermatozoa and sperm tail coiling as the length of storage period increases from 3 to 6 h. Hence, this deterioration in sperm quality due to storage temperature and the length of storage could explain why there was a significant reduction in SQI readings for semen samples held at 41°C over storage.

In the present study, the elevated number of dead sperm for undiluted neat semen stored at a high temperature (41°C) might be due to increased respiration rate of spermatozoa yielding substrate depletion, changes in the pH, or increased concentrations of metabolic by-products, which could directly or indirectly lead to sperm death (Clarke *et al.*, 1984). At higher temperatures, it is also known that the percentage of motile spermatozoa decreases as the length of storage increases. For example, holding semen at 41°C for 6 h, Clarke *et al.* (1982) reported a significant reduction in the percentage of progressively motile chicken and turkey spermatozoa when compared to fresh semen or semen samples held at lower temperatures such as 5, 15, or 25°C. This reduction in sperm motility could further explain why, in the present study, the SQI from semen at 41°C was significantly lower than that of the SQI from semen stored at 4 and 21°C.

However, these detrimental conditions at 41°C were at least partially alleviated by diluting semen with either BPSE or MEM. Similarly, in the present study, significantly higher SQI readings were obtained for semen samples diluted with either BPSE or MEM when compared to undiluted neat semen. These higher SQI readings for diluted semen samples indicate an increase in the total movement of spermatozoa. Clarke *et al.* (1982) reported that dilution of chicken semen increases sperm motility. This increase is possibly due to the increased availability of nutrients, such as fructose in BPSE (Kamar and Risik, 1972) and glucose in MEM (Parker and McDaniel, 2006). Diluting semen with various diluents has been shown to stimulate sperm metabolism (Sexton, 1976) and this increase in sperm metabolism can also be explained by an increase in activity of the enzymes involved in the tricarboxylic acid cycle (Smith *et al.*, 1957). Furthermore, Wilcox (1960) reported that the addition of fructose to diluted semen before storage at 10°C or insemination resulted in higher levels of fertility than that of samples containing no additional fructose. Hence, the higher SQI readings for semen diluted with either BPSE or MEM as compared to undiluted neat semen suggest that the SQI is capable of detecting the effects of diluent on sperm quality.

For semen held at 4°C, there was a slight reduction in the SQI from undiluted neat semen and semen diluted with MEM as the length of storage period increased. A slight linear increase in the percentage of dead sperm

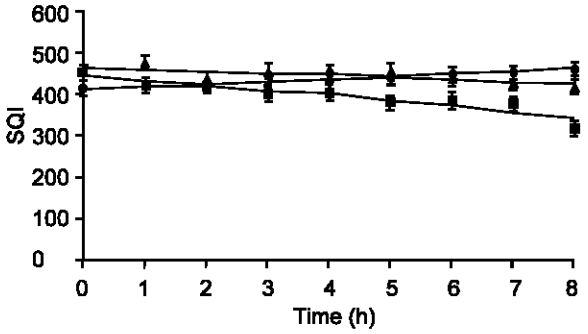

Fig. 6: The relationship of the sperm quality index (SQI) from undiluted neat semen (■) and semen diluted with either Beltsville poultry semen extender (BPSE, ●) or minimum essential medium (MEM, ▲) at 4°C over time. Each point represents the mean of 2 replicates for each time period. [a-b]Points with different superscripts are significantly different for the SQI at each time period ($P < 0.05$). A linear relationship existed for the SQI from undiluted neat semen and semen diluted with either BPSE or MEM. For semen diluted with BPSE there was an increase in the SQI as storage time increased ($y = 5.81x + 414$, $r^2 = 0.84$, $P < 0.0001$). However, for the undiluted neat semen and semen diluted with MEM the SQI declined over storage. ($y = -12.73x + 447$, $r^2 = 0.85$, $P < 0.001$, and $y = -4.83x + 464$, $r^2 = 0.55$, $P < 0.0001$, respectively).

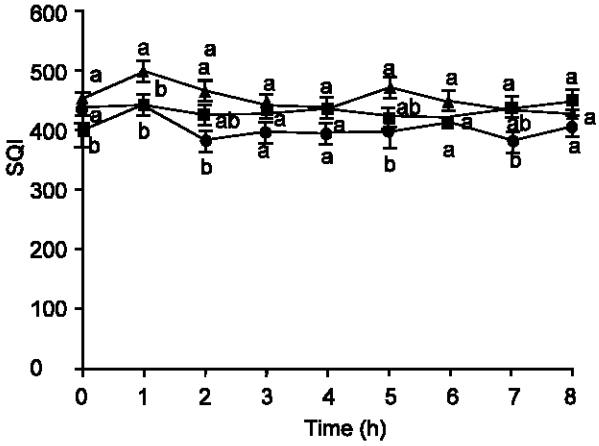

Fig. 7: The relationship of the sperm quality index (SQI) from undiluted neat semen (■) and semen diluted with either Beltsville poultry semen extender (BPSE,●) or minimum essential medium MEM,▲) at 21°C over time. Each point represents the mean of 2 replicates at each time period. a-b Points with different superscripts are significantly different for the SQI at each time period ($P < 0.05$). No linear or curvilinear relationships were detected for the SQI over storage for undiluted neat semen or semen diluted with either BPSE or MEM.

at 4°C was also noticed as the storage period increased (approximately 8-13%). This slight reduction in the SQI over 8 h of storage at 4°C might be due to the slight reduction in sperm viability (92 to 87 %). It is known that the overall aerobic metabolism of sperm is lower at 4°C (Wishart, 1989). As a result, 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). Apparently at this low temperature, these end products did not reach toxic levels in this trial, resulting in a very small increase in the percentage of dead sperm over storage.

The reduction over time in the SQI from undiluted neat semen stored at 4°C was greater than the reduction seen for semen diluted with MEM. However, for semen diluted with BPSE held at 4°C there was a slight increase in the SQI over time. It is well established that semen diluted with BPSE and held at 4°C maintains excellent semen quality over storage (Sexton and Fewlass, 1978; Giesen and Sexton, 1983). This difference in the SQI from undiluted versus MEM and BPSE diluted semen appears to be due to differences in sperm motility, because sperm viability and concentration were virtually identical among the three treatments over time at 4°C. Apparently, for sperm diluted with MEM and BPSE, this better motility is most likely due to the availability of nutrients such as glucose or fructose as well as oxygen (Parker and McDaniel, 2006). It is known that the extension of semen with a diluent leads to an improvement in the uptake of O_2 by spermatozoa (Rowell and Cooper, 1960). Previous research has shown that sperm motility is affected by the amount of O_2 available in diluted semen samples (Nevo, 1965). Furthermore, aeration of semen samples allows for the successful storage of chicken spermatozoa for 48 hr (Wishart, 1981). In the present study, all semen samples were aerated for the entire storage period. Hence the significantly higher SQI readings at 8 h of storage for semen diluted with MEM or BPSE at 4°C versus the SQI from undiluted neat semen might be due to improved sperm motility alone.

In the present study, for semen samples stored at 21°C, no major differences in the SQI were noted between undiluted semen and semen diluted with either BPSE or MEM over 8 h of storage. The percentage of dead sperm only increased from 9-15 % as the storage period increased. These findings are similar to those reported by Clarke *et al.* (1984). In that study, when storing semen at or below 25°C, they reported no significant changes in sperm viability due to storage. In another study, Clarke *et al.* (1982) reported no significant differences in

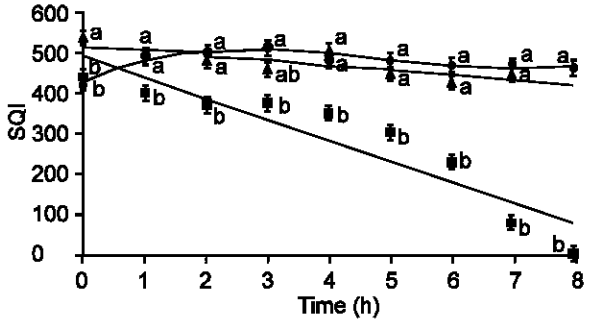

Fig. 8: The relationship of the sperm quality index (SQI) from neat undiluted semen (■) and semen diluted with either Beltsville poultry semen extender (BPSE, ●) or minimum essential medium (MEM, ▲) stored at 41°C over time. Each point represents the mean of 2 replicates at each time period. a-b Points with different superscripts are significantly different for the SQI at each time period ($P < 0.05$). A cubic relationship existed for the SQI from semen diluted with BPSE over storage ($y = 1.13x^3 - 16.49x^2 + 64.01x + 429$, $r^2 = 0.88$, $P < 0.0001$). However, there was a linear decrease in the SQI from undiluted neat semen and semen diluted with MEM ($y = -51.133x + 486$, $r^2 = 0.86$, $P < 0.001$, and $y = -11.75x + 514$, $r^2 = 0.65$, $P < 0.0001$, respectively).

percentage of progressively motile spermatozoa when semen was stored for 6 h at 15°C. The results of the present study reveal that at 21°C, storage conditions such as type of diluent does not affect sperm quality over 8 h of storage. These results also indicate that small changes in sperm viability and motility of sperm stored at 21 C do not affect the SQI.

In conclusion, the SQI reflected changes in chicken semen quality that occur due to dilution and prolonged storage, as well as storage temperature. To obtain maximum SQI values, semen should be diluted or stored at temperatures below 41°C. Because the SQI can be obtained easily and rapidly, it should be a very useful tool for developing better semen storage protocols.

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