ABSTRACT  The present research was undertaken to determine the role of seminal plasma in heat stress (HS) infertility. Males were exposed to HS at 32 C or maintained at 21 C as controls. Centrifugation and reconstitution of semen samples created four final treatments: control sperm + control plasma (C\(_{Cp}\)), control sperm + HS plasma (C\(_{Hp}\)), HS sperm + HS plasma (H\(_{Hp}\)), and HS sperm + control plasma (H\(_{Cp}\)). Semen samples with HS males’ seminal plasma had lower sperm quality index values than those containing plasma from control males. Seminal plasma from HS males diminished fertility of control sperm, and control seminal plasma did not improve fertility of HS sperm. Therefore, regardless of seminal plasma source, HS sperm had a lower fertilization rate than control sperm. Also, seminal plasma from semen samples with HS sperm (H\(_{Hp}\) and H\(_{Cp}\)) contained lower Ca, Na\(^+\), and Cl\(^-\) concentrations than seminal plasma from semen samples with control sperm (C\(_{Cp}\) and C\(_{Hp}\)). When HS seminal plasma was mixed with control sperm, plasma ion concentrations increased, indicating an efflux of ions from the control sperm to the HS seminal plasma. The other hand, when control seminal plasma was mixed with HS sperm, plasma ion concentrations decreased, indicating an influx of ions from the control seminal plasma to the HS sperm. Therefore, control sperm appear to have higher intracellular ion concentrations than the sperm from HS males. In conclusion, high temperatures might decrease male fertility by decreasing seminal plasma and intracellular ion concentrations.

(Key words: seminal plasma, sperm quality index, semen, fertility, broiler breeder)

INTRODUCTION

An important concern for a broiler breeder manager is to obtain a maximal number of chicks per flock. Because many broiler breeder farms are located in tropical and subtropical regions of the world, elevated temperatures along with high humidity and insufficient ventilation decrease broiler breeder reproductive performance and, therefore, cause significant financial losses in these regions. In hot summer months, financial losses may be more serious when broiler breeder flocks are young (Singh, 1999).

There is a marked alteration in sperm function of broiler breeder males exposed to high temperatures, which results in poor sperm-egg penetration and fertility (McDaniel et al., 1995). Hood (1999) reported that heat exposure caused an increase in the percentage of dead sperm (29.1%) and a decrease in the sperm quality index (SQI) (10.2%). McDaniel et al. (1995) found that when hens were inseminated with semen from heat stressed (HS) males, sperm-egg penetration and fertilized egg production were decreased as compared to hens inseminated with semen from control males (5.4 vs. 14.9 sperm holes per germinal disc, 45.5 vs. 73.8% fertility). Also, Hood (1999) found a 54% decrease in sperm-egg penetration when hens were inseminated with semen from HS males as compared to control males.

Limited research exists concerning biochemical changes within spermatooza or seminal plasma when males are exposed to high environmental temperatures. Suboptimal fertility of HS broiler breeder males may be attributed to changes in sperm or seminal plasma ions, proteins, or pH. Ions, such as Ca\(^++\), Na\(^+\), K\(^+\), and Mg\(^++\), are involved in cellular metabolism and influence biochemical activities of spermatooza (Cummings and Huston, 1976; Alberts et al., 1994). For example, Ca\(^++\) is a key regulator of sperm physiology including motility, membrane function, and metabolism (Barna et al., 1998). The cell plasma membrane maintains an almost constant intracellular Ca\(^++\) concentration via Ca\(^++\) ion channels and Ca\(^++\) pumps (Alberts et al., 1994). In addition, when fowl sperm are exposed to warm temperatures in vitro, they release Ca\(^++\), and motility decreases. Once the incubation temperature is lowered, the sperm

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SEMINAL PLASMA AND HEAT STRESS INFERTILITY

then resequester this released Ca\textsuperscript{++} and regain motility (Ashizawa and Wishart, 1987; Thomson and Wishart, 1989, 1991; Ashizawa et al., 1992; Ashizawa et al., 1994; Abramowitz and Suki, 1996; Ashizawa et al., 1999). Also, when males were transferred from an ambient temperature of 19 C to a hot temperature of 30 C, there were increases in whole semen Na\textsuperscript{+} and K\textsuperscript{+} concentrations but not seminal plasma K\textsuperscript{+} concentration (Cummings and Huston, 1976). Lack of information on changes in seminal plasma and seminal ion concentrations in HS males led to the objective of the present trial, which was to determine the role of seminal plasma in HS infertility.

MATERIALS AND METHODS

Housing and Environment

One hundred and forty-four Cobb broiler breeder males, 19 wk of age, were obtained from a local integrator and individually caged in six temperature controlled rooms (21 C), with 24 males per room. The males were fed a restricted Mississippi State University breeder breeder diet of 370 kcal/d per bird to maintain the primary breeder’s recommended weight. At the integrator’s facilities, the males had previously been housed in a standard black-out house with 8 h of light/d. Throughout the present experiment the males were exposed to 16 h of light/d from 0500 to 2100 h.

Three hundred and sixty individually caged Babcock White Leghorn hens, 53 wk of age, were housed in a commercial type layer house for artificial insemination. The hens were fed the Mississippi State University layer ration (2,860 kcal of ME/kg, 14.5% crude protein, 4% Ca\textsuperscript{++}) ad libitum and exposed to 16 h of light/d from 0500 to 2100 h.

The males were maintained at 21 ± 1 C for a 7-wk acclimation period. During the last week of the acclimation period, semen was collected from each male and analyzed in duplicate for the SQI. The SQI was determined using a Sperm Quality Analyzer. Each semen sample was diluted 10-fold with 0.85% saline to obtain the SQI according to the method of Parker et al. (2000). In order to eliminate bias due to sperm quality, the males were equally divided among the six rooms so that the mean SQI value for each room would be similar.

After the 7-wk acclimation period, the ambient temperature of the three HS rooms was increased to 32 ± 1 C for 8 h (0800 to 1600 h) each day for 11 wk. The other three rooms served as control rooms and were maintained at 21 ± 1 C for the entire study. Room temperature and humidity were recorded daily.

Treatment Preparation

Semen from each male in a room was collected, and the entire ejaculate from every male in a room was pooled together. The semen pool from each room was placed into two centrifuge tubes so that each tube contained approximately 3.8 billion sperm as determined by sperm packed cell volume (Maaza and Buss, 1976). The balance of the semen pool from each room not utilized for treatment sperm preparation was placed into another centrifuge tube to obtain additional seminal plasma. All tubes were centrifuged at 600 \texttimes g for 10 min. After centrifugation, seminal plasma was removed from the sperm pellet, sperm in the extra tubes was discarded, and seminal plasma was retained. Each of the three control rooms was paired with n HS room so that the sperm pellets from each of the three control rooms could be mixed with the seminal plasma from each of the three HS rooms. Also, the sperm pellet from each HS room was mixed with seminal plasma from a corresponding control room. Seminal plasma was added to the sperm pellets to yield a total of 1.75 mL of reconstituted semen with 1.875 billion sperm/mL. One tube from each room served as a control and received seminal plasma from the same room as the sperm pellet. Therefore, the following four reconstituted semen treatment groups were created for each pair of rooms: control sperm + control plasma (CsCp), control sperm + HS plasma (CsHp), HS sperm + HS plasma (HsHp), and HS sperm + control plasma (HsCp). A schematic of semen reconstitution is depicted in Figure 1 showing the treatment preparation process for this experiment.

Physiological and Semen Characteristics

On a weekly basis, body temperature was measured rectally using a thermistor probe and digital thermometer.
Body weights were recorded every 2 wk. Mortality was recorded daily, and feed consumption was measured weekly.

Every Monday for 11 wk, semen was collected and pooled by room. The pooled semen sample from each room was divided for treatment preparation as previously explained. After centrifugation, pellet separation, and preparation of the treatment combinations, the reconstituted semen samples were mixed aerobically on an Adams7 nutator for 30 min. Sperm viability was measured in triplicate for each reconstituted semen sample using the fluorometric method of Bilgili and Renden (1984). Also, four SQI readings were obtained for each of the reconstituted semen samples. The SQI was measured in the reconstituted semen samples following a 10-fold dilution (0.85% saline). Immediately after these semen characteristics were measured, reconstituted semen samples were centrifuged again at 3000 × g for 40 min at 5 C. Seminal plasma was removed from the pellet at this time. Seminal plasma was then refrigerated at −20 C until the next day so that pH and ion concentration measurements could be obtained. Ionized Na+, K+, and total Ca concentrations were measured using a Kodak Ektachem DT System.8 Chloride concentration was measured by using an Orion6 chloride sensitive electrode. Total protein was analyzed using the Bradford assay (Bio-Rad DC Protein Assay).9

**Insemination and Fertilization Evaluation**

Each Wednesday for 11 wk, semen from each room was collected and pooled by room. The pooled semen was then divided for semen treatment preparation as previously explained. After preparing treatment combinations, reconstituted semen was aerobically mixed on a nutator for 30 min at room temperature. Immediately after semen preparation, hens were artificially inseminated. There were six replications (three rooms × two hen groups) per treatment resulting in a total of 24 groups of hens. Each hen group consisted of 15 hens. Each hen was inseminated with 40 µL (75 million sperm) of reconstituted semen.

Individual eggs were collected and labeled daily at 1600 h and stored at 18 C for 1 wk. The eggs were then incubated in a Petersime incubator10 at a wet-bulb temperature of 29 C and a dry-bulb temperature of 37.5 C. On the seventh day of incubation, eggs were broken out to determine fertility by observing the presence of embryonic development.

**Statistical Analyses**

Each of the three HS rooms was paired with one of the three control rooms for seminal plasma transfer creating a randomized complete block design. Statistical analyses were made in SAS programs using the mixed model procedure (Littell et al., 1996). Blocks were the room pairs and weeks and days within a week were the repeated measurements in the mixed model for semen quality and fertility. Means were separated using Fisher’s protected LSD at alpha < 0.05. Orthogonal contrasts were used to compare sperm source, control sperm (CsHp + CsHp) vs. HS sperm (HsHp + HsHp); seminal plasma source, control plasma (CsHp + CsHp) vs. HS plasma (HsHp + HsHp), and sperm-seminal plasma mixing, not mixed (CsHp + HsHp) vs. mixed (CsHp + CsHp) (Steel and Torrie, 1980).

**RESULTS**

**Physiological Characteristics**

The body temperatures at 21 and 32 C were 41.7 and 42.1 C, respectively (SEM = 0.13 C, P < 0.1). However, heat treatment did not alter BW (4,164 g vs. 4,251 g, SEM = 0.40). Feed consumption and mortality were also not affected by high ambient temperature (120 g vs. 117 g, SEM = 1.1 g, P > 0.19, and 19% vs. 13%, SEM = 5.2%, P > 0.39, respectively).

**Semen Characteristics**

Seminal plasma pH was not different among the four reconstituted semen treatment groups (Table 1). Orthogonal contrast tests revealed that there were no plasma pH differences due to sperm source, seminal plasma source, or sperm-seminal plasma treatment mixing (data not presented). The percentage of dead sperm was also not different among reconstituted treatments (Table 1). Furthermore, no differences in sperm viability were observed for sperm source, seminal plasma source, or sperm-seminal plasma treatment mixing.

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7Clay Adams, Division of Becton, Dickinson and Co., Parsippany, NY.
8Johnson & Johnson Ortho-Clinical Diagnostics, Rochester, NY.
9Bio-Rad Laboratories, Hercules, CA.
TABLE 1. Seminal plasma pH, the percentage of dead sperm, sperm quality index (SQI), and fertility in reconstituted semen samples

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Seminal plasma pH</th>
<th>Dead sperm (%)</th>
<th>SQI (%)</th>
<th>Fertility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CₛCₚ</td>
<td>7.52</td>
<td>11.6</td>
<td>126ᵃ</td>
<td>67.7ᵃ</td>
</tr>
<tr>
<td>CₛHₛ</td>
<td>7.58</td>
<td>12.6</td>
<td>100ᵇ</td>
<td>61.0ᵇ</td>
</tr>
<tr>
<td>HₛHₛ</td>
<td>7.59</td>
<td>13.0</td>
<td>106ᵇ</td>
<td>64.9ᵇ</td>
</tr>
<tr>
<td>HₛCₛ</td>
<td>7.61</td>
<td>11.8</td>
<td>111ᵇ</td>
<td>57.4ᵇ</td>
</tr>
<tr>
<td>SEM</td>
<td>0.041</td>
<td>0.81</td>
<td>11.1</td>
<td>2.7</td>
</tr>
<tr>
<td>P</td>
<td>0.49</td>
<td>0.61</td>
<td>0.05</td>
<td>0.001</td>
</tr>
<tr>
<td>n</td>
<td>33</td>
<td>33</td>
<td>33</td>
<td>226</td>
</tr>
</tbody>
</table>

ᵃ⁻ᵇMeans within a column with different superscripts are significantly different at α < 0.05.


However, when control sperm were reconstituted with HS male seminal plasma, SQI values were lower (P < 0.05) as compared to reconstitution of control sperm with control plasma (Table 1). Furthermore, orthogonal contrast test indicated a trend toward higher SQI values when HS or control sperm was reconstituted with control male seminal plasma, as compared to reconstitution with HS male seminal plasma (P < 0.09, Figure 2). There were no sperm source and treatment mixing effects for SQI.

Fertility

When control sperm were reconstituted with seminal plasma from HS males, fertilizing ability of control sperm decreased (P < 0.0011) as compared to the fertility rate of the original CₛCₚ group (Table 1). In the reciprocal reconstitution, control male seminal plasma did not improve the fertility of HS male sperm; however, fertility of the HₛCₚ group was less than that of the original HₛHₛ group. Furthermore, sperm from HS males resulted in lower fertility (P < 0.009) than that of control males, regardless of seminal plasma source (Figure 3). Similarly, sperm reconstituted with their original seminal plasma exhibited higher fertility than that of sperm reconstituted with seminal plasma from the reciprocal treatment group (66.3% vs. 59.2%, P < 0.0003, Figure 3).

Seminal Plasma Ion Concentrations and Total Protein

Seminal plasma of the original HS group (HₛHₛ) had lower Ca, Na⁺, and Cl⁻ concentrations as compared to the original control group (CₛCₚ). Also, seminal plasma from HS and control males had less Ca, Na⁺, and Cl⁻ when reconstituted with sperm from HS males as compared to seminal plasma reconstituted with sperm from control males (Table 2). Orthogonal contrast results also revealed that reconstituted semen containing HS male sperm had lower seminal plasma Ca, Na⁺, and Cl⁻ concentrations as compared to semen containing control male sperm (Figure 4a,b,c; P < 0.02, 0.01, 0.04, respectively). Seminal plasma K⁺, K⁺/Na⁺ ratio, and total protein concentration did not differ among treatments (Table 2).

DISCUSSION

In the present study, exposing broiler breeder males to 32 C daily for 8 h resulted in a trend of increasing body temperature. These results are in agreement with past research (Harrison and Biellier, 1969; McDaniel et al., 1995, 1996; Jiang et al., 1999; Hood, 1999). However, exposing broiler breeder males to high ambient temperature in this study did not alter BW, feed consumption, or mortality. As reported in previous work, high ambient temperature caused a decrease in feed intake as well as increased mortality (Clark and Sarakoon, 1967; McDaniel et al., 1996; Hood, 1999). The lack of a HS effect in the present study on feed consumption and mortality may possibly be due to cyclic exposure to HS for only 8 h daily as compared to continuous heat exposure in these previous studies.

In this experiment, sperm concentration, sperm viability, and SQI were evaluated to determine the effects of HS on reconstituted semen characteristics obtained from broiler breeder males. Although Clark and Sarakoon (1967) and McDaniel et al. (1995, 1996) could not find any HS effects...
TABLE 2. Seminal plasma ion (mM/L) and total protein (g/L) concentrations of reconstituted semen samples

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ca</th>
<th>Na⁺</th>
<th>Cl⁻</th>
<th>K⁺</th>
<th>K⁺/Na⁺</th>
<th>Total protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>CₛCₚ</td>
<td>1.26a</td>
<td>123.8a</td>
<td>135.8a</td>
<td>13.6</td>
<td>0.19</td>
<td>5.7</td>
</tr>
<tr>
<td>CₛHₚ</td>
<td>1.24a</td>
<td>124.8a</td>
<td>137.2a</td>
<td>13.5</td>
<td>0.19</td>
<td>5.7</td>
</tr>
<tr>
<td>HₛCₚ</td>
<td>1.14b</td>
<td>121.9b</td>
<td>125.6b</td>
<td>13.3</td>
<td>0.19</td>
<td>5.6</td>
</tr>
<tr>
<td>HₛHₚ</td>
<td>1.14b</td>
<td>121.8b</td>
<td>128.0b</td>
<td>13.8</td>
<td>0.19</td>
<td>5.9</td>
</tr>
<tr>
<td>SEM</td>
<td>0.047</td>
<td>0.79</td>
<td>5.23</td>
<td>0.60</td>
<td>0.010</td>
<td>0.22</td>
</tr>
<tr>
<td>P</td>
<td>0.08</td>
<td>0.02</td>
<td>0.06</td>
<td>0.80</td>
<td>0.76</td>
<td>0.66</td>
</tr>
<tr>
<td>n</td>
<td>33</td>
<td>33</td>
<td>33</td>
<td>33</td>
<td>33</td>
<td>33</td>
</tr>
</tbody>
</table>

a,bMeans within a column with different superscripts are significantly different at α < 0.05.


on semen characteristics, Boone and Huston (1963) and Hood (1999) found decreases in sperm viability when males were HS. In the present study, when control sperm was reconstituted with HS males’ seminal plasma, SQI values were lower than that of the original reconstituted control group. In the reciprocal reconstitution, seminal plasma from control males did not improve the HS male’s SQI. Orthogonal contrast tests confirmed a seminal plasma effect. Reconstituted semen containing control seminal plasma had a higher SQI value when compared to reconstituted semen containing HS seminal plasma (Figure 2). This effect of seminal plasma source on SQI values is likely related to changes in seminal plasma ion concentration found in the present study when birds were HS.

The original seminal plasma from HS males (HₛHₚ) contained less Ca, Na⁺ and Cl⁻ than that from the control males (CₛCₚ). In addition, regardless of the seminal plasma used for reconstitution, plasma Ca, Na⁺, and Cl⁻ concentrations were lower when sperm from HS males was present (HₛHₚ and HₛCₚ) as opposed to sperm from control males (CₛCₚ and CₛHₚ). However, there was no difference between HS and control male seminal plasma K⁺ concentrations. Cummings and Huston (1976) reported that there were increases in whole semen Na⁺ and K⁺ concentration when males were exposed to high ambient temperature, but they did not report on seminal plasma Na⁺ level. However, seminal plasma K⁺ concentrations in both studies were not affected by heat exposure. Seminal plasma Ca, Na⁺, K⁺, and Cl⁻ concentrations in this study were in the range found in previous works (Lake and El Jack, 1966; Graham et al., 1971; and Etches, 1996).

When HS seminal plasma was reconstituted with sperm from control birds, plasma Ca, Na⁺, and Cl⁻ ion concentrations increased as compared to the original HS plasma from the HₛHₚ group (Table 2). It appears that there was an efflux of ions from the sperm to the seminal plasma. On the other hand, when seminal plasma from control birds was reconstituted with sperm from HS birds, there were decreases in seminal plasma Ca and Na⁺ ion concentrations as compared to the original control plasma from the CₛCₚ group. This decrease in seminal plasma ion concentration could possibly be due to ion influx from seminal plasma to sperm. Perhaps sperm source affected seminal plasma ion concentrations due to lower intracellular ion concentrations in sperm from HS males. Recently Karaca et al. (2002) detected elevated seminal plasma Ca levels for males in the initial stages of HS. However, it is possible that this increase in Ca was due to extrusion of Ca from the reproductive tract and not from sperm.

These ionic differences between the sperm and seminal plasma of control and HS males may be partly responsible for the depressed fertility noted in the present study when sperm were reconstituted with the opposite treatment group’s seminal plasma. When control sperm was reconstituted with seminal plasma from HS males and vice versa, the fertilizing ability of the sperm decreased. Due to the

FIGURE 4. Orthogonal contrast test results for examining sperm source, seminal plasma source and treatment mixing effects on seminal plasma A. calcium (Ca); B. sodium (Na⁺); and C. chloride (Cl⁻) concentrations (mM/L). The black bar represents control males’ semen or semen not mixed, and the white bar represents heat stressed (HS) males’ semen or mixed semen samples. Bars with different letters are significantly different (P < 0.04, n = 60).
noted differences in sperm and seminal plasma ion concentrations for the two temperature treatments, reconstituting sperm with plasma from the reciprocal temperature treatment likely resulted in ionic flux and osmotic shock.

Also in the present study, HS sperm had a lower fertility rate than control sperm. As previously mentioned, results from this study indicated that intracellular sperm Ca concentration is less when males are HS. Similarly, Thomson and Wishart (1989, 1991) have shown that when chicken semen samples are exposed to elevated temperatures in vitro intracellular Ca\textsuperscript{++} concentrations decrease. Intracellular calcium plays a significant role in sperm motility and fertility. For example, sperm motility and the acrosome reaction are decreased at lower intracellular free Ca\textsuperscript{++} concentrations (Ashizawa et al., 1992, 1994, 1999; Thomson and Wishart, 1991; Mori et al., 1996). Therefore, it is likely that intracellular free Ca\textsuperscript{++} in HS male sperm is insufficient for the maintenance of sperm motility. In fact in the present study, the SQI, a measure of sperm motility, was depressed for semen samples that contained HS males’ seminal plasma and therefore low levels of extracellular and possibly intracellular Ca. Sperm must be motile to traverse the vagina and reach the sperm storage sites (Allen and Grigg, 1957). This lack of motility most likely results in an inadequate number of sperm reaching the oviductal sperm storage tubes and therefore a decrease in the fertility rate of HS males (McDaniel et al., 1996).

In conclusion, seminal plasma does play a major role in HS infertility, as evidenced by lower ion concentrations and sperm quality as compared to seminal plasma from males not HS. In addition, low seminal plasma and intracellular sperm Ca concentrations during HS may decrease sperm motility and subsequent fertility.

REFERENCES


