The Male Contribution to Broiler Breeder Heat-Induced Infertility as Determined by Sperm-Egg Penetration and Sperm Storage Within the Hen's Oviduct[1]

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ABSTRACT The purpose of the present study was to define the role of the male broiler breeder in heat-induced infertility. Seventy-two Arbor Acres roosters were individually caged at 21 wk of age and divided equally among three heated (H) and three control (C) temperature chambers. Control temperature chambers were held at 21 C. After an 8-wk pretreatment period (20 C), an 8-wk treatment period was conducted in which the temperature in all three of the H chambers was varied from week to week according to the following schedule: Week 1, 27 C; Week 2 through Week 4, 32 C; and Week 5 through Week 8, 21 C. On a weekly basis, semen was pooled by room and inseminated into 12 groups of 10 hens each (2 groups per room). During the 1st wk when males were maintained at 27 C for 12 h, *in vivo* sperm-egg penetration was reduced by 48% as compared to data obtained when males were maintained at 21 C. Fertility, *in vivo* sperm-egg penetration, and uterovaginal sperm storage were decreased when semen from males exposed to 32 C was used to inseminate hens as compared to insemination with semen from C males. However, during this same period, the ability of sperm to bind to and penetrate the egg, as determined by *in vitro* sperm-egg penetration, was similar between sperm from C and H males. After lowering the temperature in the H chambers back to 21 C, *in vivo* sperm-egg penetration as a result of insemination with semen from H males was analogous to results obtained when C males were used for insemination. Immediately after decreasing the temperature in the H chambers, fertilization of eggs by sperm from H males increased to a level similar to that obtained when eggs were fertilized by sperm from C males but then declined again during the later weeks.

(*Key words*: sperm-egg penetration, oviductal sperm storage, fertility, heat stress, broiler breeder)

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INTRODUCTION

The reproductive performance of the rooster can be greatly depressed during environmental stress. Whereas some information has been obtained on heat-stress infertility of exotic and small breeds of chickens, very little research has been conducted on the fertility of modern day broiler breeders that have been exposed to elevated ambient temperatures (for review see Edens, 1983). Recently, McDaniel *et al.* (1995) subjected sexually mature birds to high temperatures and determined that the male broiler breeder contributes more to heat-induced infertility than the female. When the male broiler breeder was exposed to 32 C, fertility declined 42% and *in vivo* sperm-egg penetration declined 52% as compared to results obtained from males maintained at 21 C. These differences, found when the male bird was maintained at high temperatures, were not evident when the female bird alone was exposed to elevated ambient temperatures. Also, significant effects of ambient temperature on male fertility were evident within 12 h of challenge at a typical summer temperature of 29 C. Semen characteristics such as semen volume, sperm concentration, and percentage of dead sperm were unaffected by heat treatment.

Although a reduction in *in vivo* sperm-egg penetration and fertility had been demonstrated when the male broiler breeder was subjected to high temperatures in this previous study, the mechanisms responsible for these reductions were not determined. The reduction in *in vivo* sperm-egg penetration may have been due to a decrease in the number of sperm being stored in the hen's oviduct when the male bird is exposed to elevated environmental temperatures. Sperm are stored within the uterovaginal junction (UVJ) and infundibulum sections of the hen's oviduct (for review see Bakst *et al.*, 1994). A decrease in oviductal sperm storage would result in fewer sperm being available to bind to,

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penetrate, and fertilize the egg. On the other hand, it was just as likely that sperm from heat-exposed (H) males were being stored properly in the hens oviduct but were unable to bind to and penetrate the ovum. In addition, questions still remain as to whether fertility of the H male broiler breeder would recover following removal of heat and whether or not sperm-egg penetration by sperm from male broiler breeders would be affected at an ambient temperature as low as 27 C.

These aforementioned questions instigated the four major objectives of the present study, which are as follows: 1) to determine whether a temperature as low as 27 C for 12 h would reduce *in vivo* sperm-egg penetration by sperm from broiler breeder males, 2) to determine whether fertility of H male broiler breeders improves following removal of heat, 3) to determine the effect of exposing broiler breeder males to high environmental temperatures on *in vitro* sperm-egg penetration, thereby removing any effect due to oviductal sperm storage, and 4) to determine the effect of exposing broiler breeder males to elevated ambient temperatures on subsequent storage of their sperm within the hen's oviduct.

MATERIALS AND METHODS

Housing and Environment

At 21 wk of age, 72 Arbor Acres roosters were obtained from a local breeder. The roosters were caged individually in six temperature-controlled rooms at a density of 12 males per room. Also at 21 wk of age, 120 Arbor Acres hens were obtained from a local breeder and housed in a closed-sided house with conventional environmental controls in which the ambient temperature ranged from 16 to 24 C. The hens were individually caged in 12 groups of 10 hens each. The roosters were placed on a restricted diet of 350 kcal ME per bird per d. The hens were fed the University of Georgia Breeder diet (2,915 kcal ME/kg, 15% CP, and 3% Ca) to maintain the recommended weight. All birds were exposed to 16 h of light/d (lights on at 0500 h; lights off at 2100 h).

The birds were given 8 wk to sexually mature and adjust to caging. The minimum and maximum temperature in each room was recorded daily with high-low thermometers. During the 8-wk pretreatment period, the temperature in all six of the controlled temperature rooms was maintained at 20 C (maximum = 20 ± 1 C; minimum = 18 ± 1 C). After the pretreatment period, an 8-wk treatment period was conducted. Three of the temperature rooms served as control (C) replicates in which the ambient temperature was maintained at 21 C (maximum = 21 ± 0.6 C; minimum = 18 ± 1 C) for the entire study. The other three rooms served as H replicates in which the ambient temperature varied from week to week according to the following schedule: Week 1, 27 C (maximum = 28 ± 1 C; minimum = 26 ± 1 C); Weeks 2 through 4, 32 C (maximum = 33 ± 1 C; minimum = 30 ± 1 C); and Weeks 5 through 8, 21 C (maximum = 22 ± 2 C; minimum = 19 ± 1 C). Room temperature changes were achieved at approximately 0100 h on the 1st d of each temperature treatment period. Relative humidity was measured daily with a VWR digital hygrometer model 1022486.[5] Relative humidity averaged 39 ± 11% across the 8-wk study.

Physiological and Individual Male Semen Characteristics Measured

As a general measure of the bird's response to elevated environmental temperatures, rectal body temperature was determined during the 1st d of each week with a Cole-Parmer thermistor thermometer Model 8402 and a YSI thermistor probe 403 inserted 6 cm into the rectum.[6] Body temperature measurements were made at 0800 h before feeding. Mortality and feed consumption were determined at the end of each week. Because pooled semen characteristics may differ from those of an individual male, semen characteristics for each individual bird were determined from 0800 to 1200 h during the 3rd d of each week. The semen characteristics measured included the following: semen volume, sperm concentration, and sperm viability. Sperm concentration was determined by the packed cell volume (PCV) method of Maeza and Buss (1976). Viability of spermatozoa was analyzed using the fluorometric method of Bilgili and Renden (1984).

Fertility and In Vivo Sperm-Egg Penetration During and After Heat Exposure

Pooled Semen Characteristics and Artificial Insemination. At the beginning of each week, semen was pooled by room and sperm concentration, viability, and motility were quantified. Sperm motility was determined subjectively using the swirl method and scored from 5 (most motile) to 1 (least motile) (Cherms, 1968; Graham *et al.*, 1982). Semen was diluted to a concentration of 50 million sperm per 50 μL with minimum essential medium (MEM, Howarth, 1981) and used to inseminate hens. The 12 groups of broiler breeder hens were inseminated with 50 million sperm at 1400 h on a weekly basis such that two groups of hens received semen from the pool collected from one room of males. Only 50 million sperm were inseminated into hens so as to not overshadow any physiological effects with excess sperm.

Fertility Characteristics Measured. *In vivo* sperm-egg penetration was determined in each egg laid postinsemination during Week 1 by using the method of Bramwell *et al.* (1995). The perivitelline layer from oviposited eggs was removed, fixed with 20% formalin, and stained with Schiff's reagent. The number of holes created by sperm penetration were counted in a 1.35 mm^2 area surrounding the germinal disc.

[5]VWR Scientific, Marietta, GA 30066.
[6]Cole-Parmer, Niles, IL 60714-9930.

During Weeks 2 through 8, *in vivo* sperm-egg penetration was analyzed for three groups of hens inseminated with semen from each of the three H replicates and for three groups of hens inseminated with semen from each of the three C replicates. Eggs from the remaining groups of hens were used for determining candling fertility at 10 d of incubation and hatchability of fertile eggs for each day postinsemination. All unhatched eggs were opened to determine true fertility. Eggs were stored in a cooler at 13.3 C prior to incubation and were set on a weekly basis. Hen-day egg production averaged 77% over the 8 wk of the experiment. Therefore, approximately eight eggs were analyzed for *in vivo* sperm-egg penetration or fertility for each replicate during each day postinsemination.

In Vitro *Sperm-Egg Penetration*

During the 6th d of Weeks 1 and 3 of the experiment, 12 recently ovulated ova were collected from 32-wk-old White Leghorn hens that were individually caged in a closed-sided house at approximately 21 C. Only ova that were free in the body cavity and not engulfed by the infundibulum were used in the *in vitro* assay. The technique for determining *in vitro* sperm-egg penetration was similar to that of Bramwell and Howarth (1992). The perivitelline layer was removed from each ovum in deionized water and rinsed to remove adhering yolk material. The perivitelline layer was then placed in MEM and divided into six individual 0.5 cm^2 pieces that did not include the germinal disc area. Also during the 6th d of Weeks 1 and 3 of the experiment, semen was collected from each male and pooled by room. Semen was diluted 1 to 1 with MEM and centrifuged at 600 × *g* for 10 min. The supernatant was removed and the pellet was reconstituted with MEM. Sperm concentration of the suspension was determined using the packed cell volume method. The semen samples were diluted to obtain a sperm concentration of 1 million sperm per 100 μL. All semen samples and perivitelline layer sections were maintained at 39 C. To decrease egg to egg variation, the six pieces of each perivitelline layer were exposed for 10 min to 1 million sperm from each of the six temperature rooms. After incubation, the number of sperm penetration holes in three individual 0.27 mm^2 areas of each section were determined with a light microscope.

Oviductal Sperm Storage

To examine oviductal sperm storage, 33-wk-old White Leghorn hens, which were individually caged in a closed-sided house at approximately 21 C, were inseminated with semen from H and C males. During the 6th d of Weeks 2 and 4 of the experiment, 42 of these hens were inseminated with 100 million sperm at 1400 h from each pool of semen collected from each of the six rooms of males (7 hens per room per week). The technique for quantifying the number of sperm stored in the hens oviduct was that of McDaniel *et al.* (1996). Semen from each room was incubated with the nuclear fluorescent dye, *bis*-benzimide, before insemination. Twenty-four hours following insemination, hens were killed by cervical dislocation and a section of the oviduct from the uterus to the vagina was removed. The section of the UVJ containing the sperm storage tubules (SST) was excised and homogenized in 0.5 mL of a 0.85% NaCl solution. A 20-μL aliquot of each homogenate was placed on a microscope slide and air dried. The number of sperm per square millimeter was determined with the use of a fluorescence microscope. The total number of sperm in the UVJ was calculated with the following formula: (sperm per square millimeter) (total area of drop/20 μL) (total volume of the homogenate).

Statistical Analysis

An ANOVA with a completely randomized design and a split-plot in time was used to analyze all data except *in vivo* sperm-egg penetration, fertility, and hatchability. These fertilization data were analyzed using an ANOVA with a completely randomized design and a split-split plot in time. The two whole plots were C and H temperature treatments. Split-plots were the weeks of treatment, whereas split-split plots were the 7 d postinsemination during each week. The base experimental units were the rooms of males. Linear and curvilinear regression were also used to show relationships among means over weeks of the experiment and days postinsemination. Student-Newman-Keul's sequential range test was used to separate interaction means (Steel and Torrie, 1980).

Linear correlation was used to reveal the relationships of UVJ sperm storage with *in vivo* sperm-egg penetration, rectal body temperature, and sperm motility. For rectal body temperature and sperm motility correlations with UVJ sperm storage, C and H means for Weeks 2 and 4 were used as separate points in the analyses. As explained by McDaniel *et al.* (1996) and because hens were inseminated at 1400 h, the sperm stored in the UVJ 24 h postinsemination would most likely penetrate the ovum laid 3 d postinsemination. Therefore, *in vivo* sperm-egg penetration means over Weeks 2 and 4 and Day 3 postinsemination for each room were correlated with UVJ sperm storage means over Weeks 2 and 4 for each room of males.

RESULTS

Physiological and Individual Male Semen Characteristics Measured

As shown in Figure 1, body temperature of the C males decreased slightly with each week of the study. Body temperature of the H birds was not significantly higher than the C males during the 1st wk of the study when the H males were maintained at 27 C. However, during the 2nd and 3rd wk of the experiment, when the H birds were maintained at 32 C, body temperature of the H males

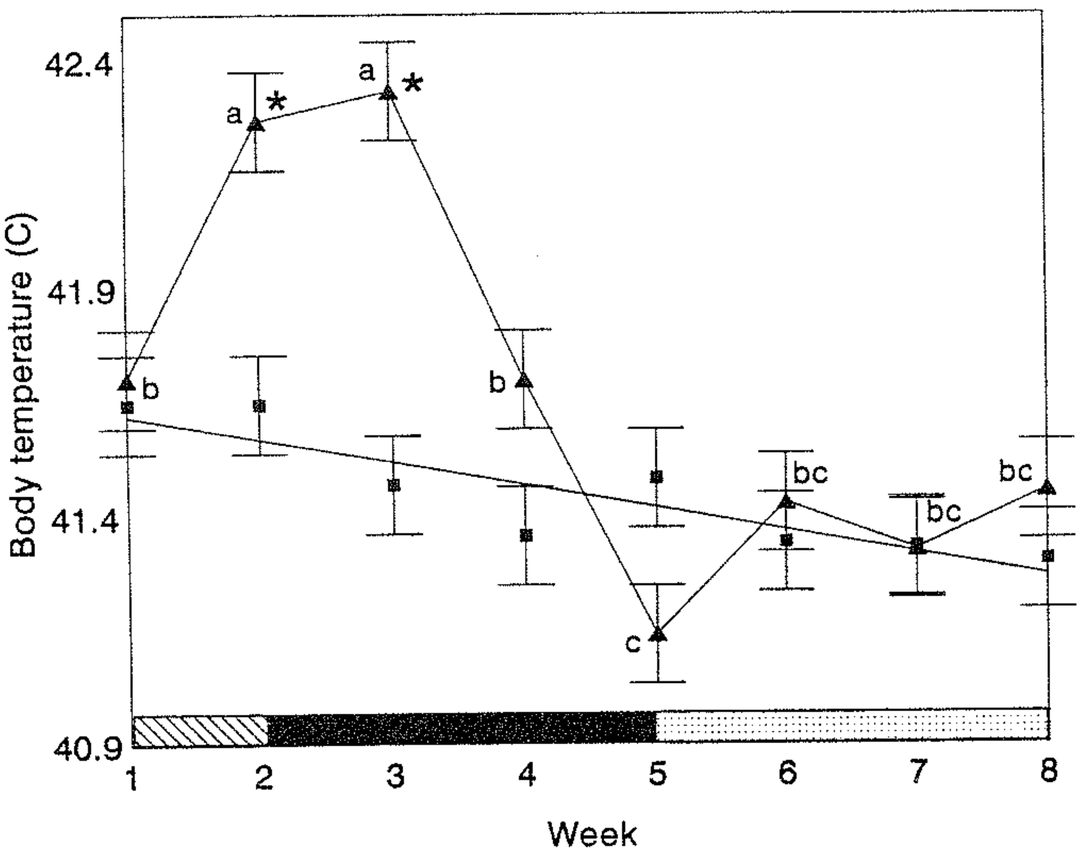
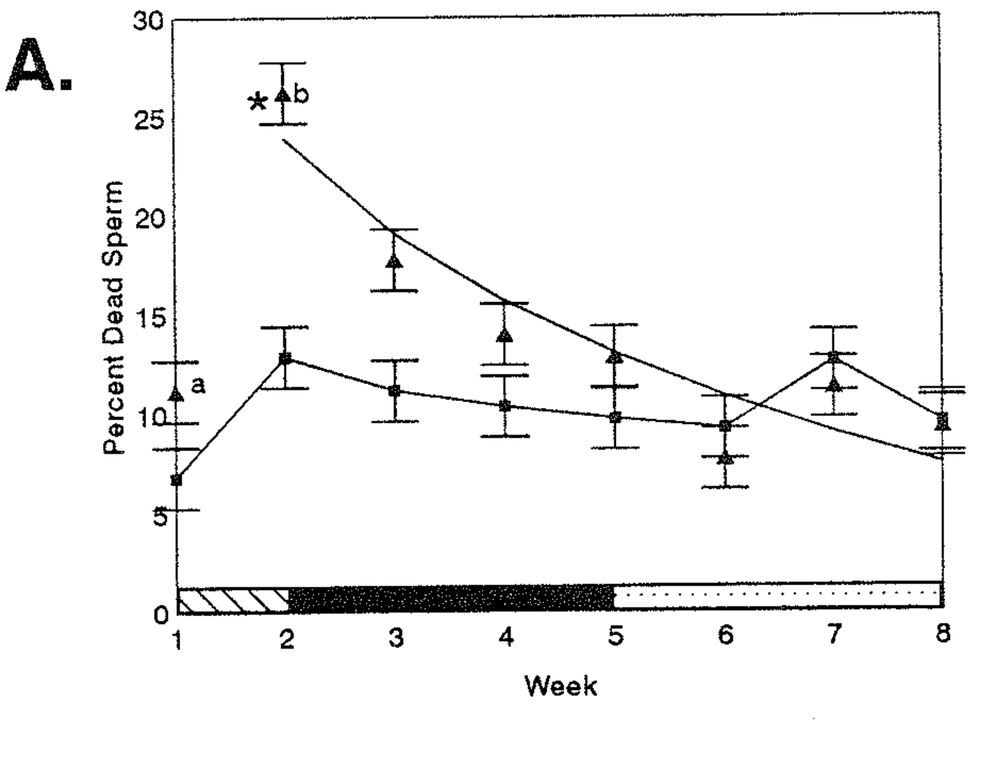


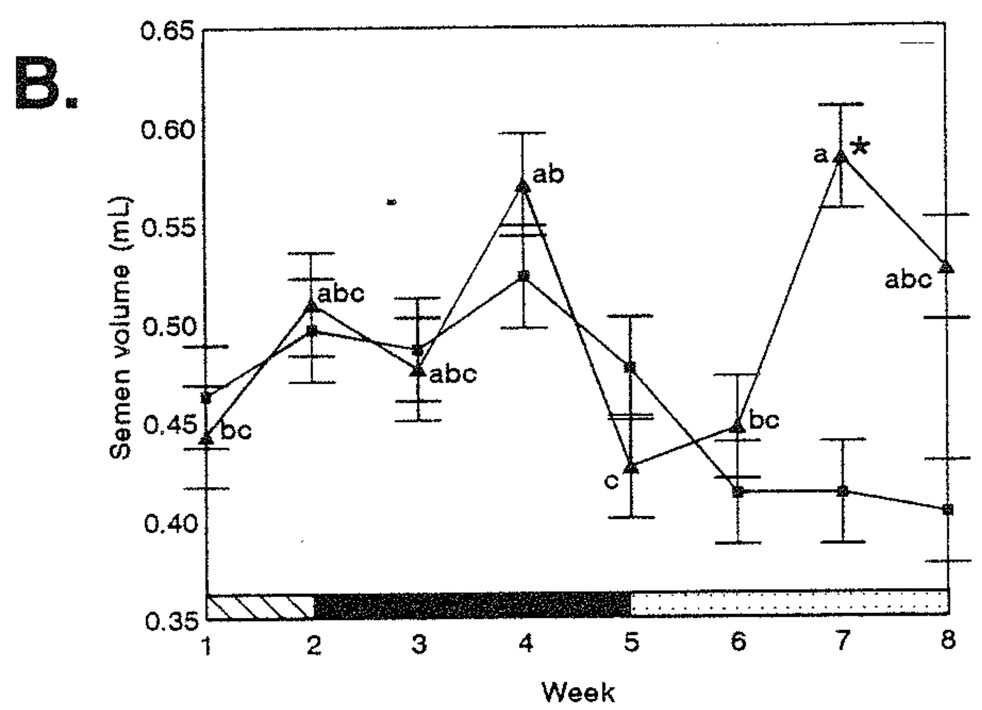
FIGURE 1. Rectal body temperature of control (■) and heat-exposed (▲) males during each week of the experiment (treatment by week interaction, $P < 0.0002$). The graphics bar along the x axis indicates the ambient temperature at which the heat-exposed birds were maintained during each week (hatched bar = 27 C, solid bar = 32 C, and stippled bar = 21 C). All control birds were maintained at 21 C throughout the experiment. A significant linear decline was detected for body temperature of the control males over each week of the study ($y = -0.05x + 41.67$, $r^2 = 0.79$; $P < 0.0031$). An asterisk indicates a significant difference between control and heat-exposed means for a specific week. Superscripts denote significant differences among heat-exposed means over weeks. Values represent the replicate means of three replicates with 12 birds each at initiation of the study (n = 3). The mean ± SEM is displayed for each week.

increased to levels significantly greater than that of the controls. Following this elevation, body temperature of the H males decreased to levels similar to that of the controls during Weeks 4 through 8.

Mortality and feed consumption (data not shown) were also different between the C and H males. When males were exposed to a temperature of 27 C during Week 1, no mortality was encountered. By Week 2, mortality increased to approximately 40% (14 birds) when the birds were exposed to 32 C, whereas no C birds died during Week 2 (treatment by week interaction, $P < 0.0009$). During the following weeks, mortality of the H males immediately returned to C levels. At termination of the study 18 H males and 1C male had died. Feed consumption was also unaffected by heat exposure at 27 C. However, when the ambient temperature in the H rooms was increased to 32 C during Week 2, feed consumption decreased approximately 10 g per bird per d. After Week 2, feed consumption of the H males began a logarithmic return to levels similar to that of the C birds (treatment by week interaction, $P < 0.0005$).

Weekly means for individual male semen characteristics, including percentage of dead sperm, semen volume, and sperm PCV, are reported in Figure 2. By the 2nd wk of the experiment, there was a significant elevation in the percentage of dead sperm produced by the H males. Immediately following Week 2, the percentage of dead sperm produced by the H males returned to a level similar





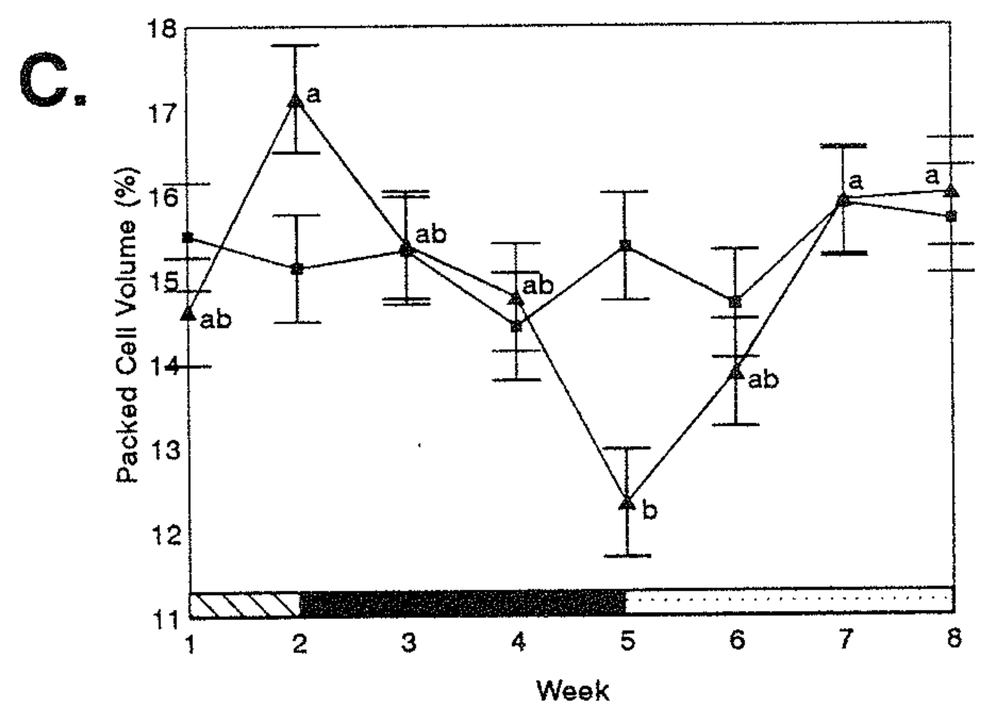


FIGURE 2. The percentage of dead sperm (A), semen volume (B), and packed cell volume (C) for control (■) and heat-exposed (▲) males during each week of the experiment (treatment by week interactions: $P < 0.0008$, $P < 0.0033$, $P < 0.037$, respectively). The graphics bar along the x axis indicates the ambient temperature at which the heat-exposed birds were maintained during each week (hatched bar = 27 C, solid bar = 32 C, and stippled = 21 C). All control birds were maintained at 21 C throughout the experiment. A significant logarithmic decrease was detected for the percentage of dead sperm produced by the heat-exposed males over Weeks 2 through 8 of the study ($y = -27.25 \log x + 32.09$, $r^2 = 0.88$; $P < 0.0019$). An asterisk indicates a significant difference between control and heat-exposed means for a specific week. Superscripts denote significant differences among heat-exposed means over weeks. Values represent the replicate means of three replicates with 12 birds each at initiation of the study (n = 3). The mean ± SEM is displayed for each week.

to the controls and decreased logarithmically throughout the rest of the study. Semen volume was extremely variable throughout the experiment, and the production of semen by the H males was significantly lower during Week 5 as compared to Weeks 4 and 7. It was only during Week 7, when all birds were maintained at 21 C, that semen volume of the previously H males was significantly different than that of the C males. Sperm PCV was also extremely variable for the H males throughout the experiment. At no time during the experiment was sperm PCV significantly different between the C and H males. On the other hand, sperm PCV of the H males was lower during Week 5 than during Weeks 2, 7, and 8.

Fertility and In Vivo *Sperm-Egg Penetration During and After Heat Exposure*

None of the pooled semen characteristics, which were obtained on the day of insemination, were significantly affected by increasing the environmental temperature (Table 1). However, when semen collected from males within 12 h after initiation of heat exposure at 27 C was used for insemination, *in vivo* sperm-egg penetration was reduced by 48% as compared to results obtained when hens were inseminated with semen from C males (Figure 3a). In addition, *in vivo* sperm-egg penetration of sperm from the H males remained significantly lower than that of the C males until 1 wk after removal of heat. A linear decline over time was detected for *in vivo* sperm-egg penetration when semen from C males was used to inseminate hens. On the other hand, when semen from H males was used to inseminate hens, *in vivo* sperm-egg penetration responded in a quadratic manner over time. During heat treatment, *in vivo* sperm-egg penetration by sperm from H males declined until the last week of exposure to 32 C. Then, almost immediately following removal of heat, *in vivo* sperm-egg penetration by sperm from H males began to return to levels comparable to those of controls.

Figure 3b illustrates the treatment by day postinsemination interaction means for *in vivo* sperm-egg penetration. During the first 5 d postinsemination, *in vivo* sperm-egg penetration was significantly lower when hens were inseminated with semen from H males than when they were inseminated with semen from C males. The greatest difference between the two groups was during Day 2 postinsemination, but this difference decreased with increasing time postinsemination. A sharp linear decline was seen in *in vivo* sperm-egg penetration over days postinsemination when semen from C males was used to inseminate hens. However, a slower declining quadratic relationship was seen between *in vivo* sperm-egg penetration and day postinsemination if semen from H males was used to inseminate hens.

Unlike *in vivo* sperm-egg penetration, the percentage of eggs fertilized by the C and H male treatment groups was similar during the 2nd wk of the study when the H males were first exposed to 32 C (Figure 4a). By Weeks 3 and 4, however, fertility of the H males was only 70 and 60%, respectively, that of the C males. Within 12 h of heat removal, fertility of the H males increased to a value that was similar to that of the C males; yet during the last 3 wk of the study, fertility of the H males returned to levels significantly lower than that of the controls.

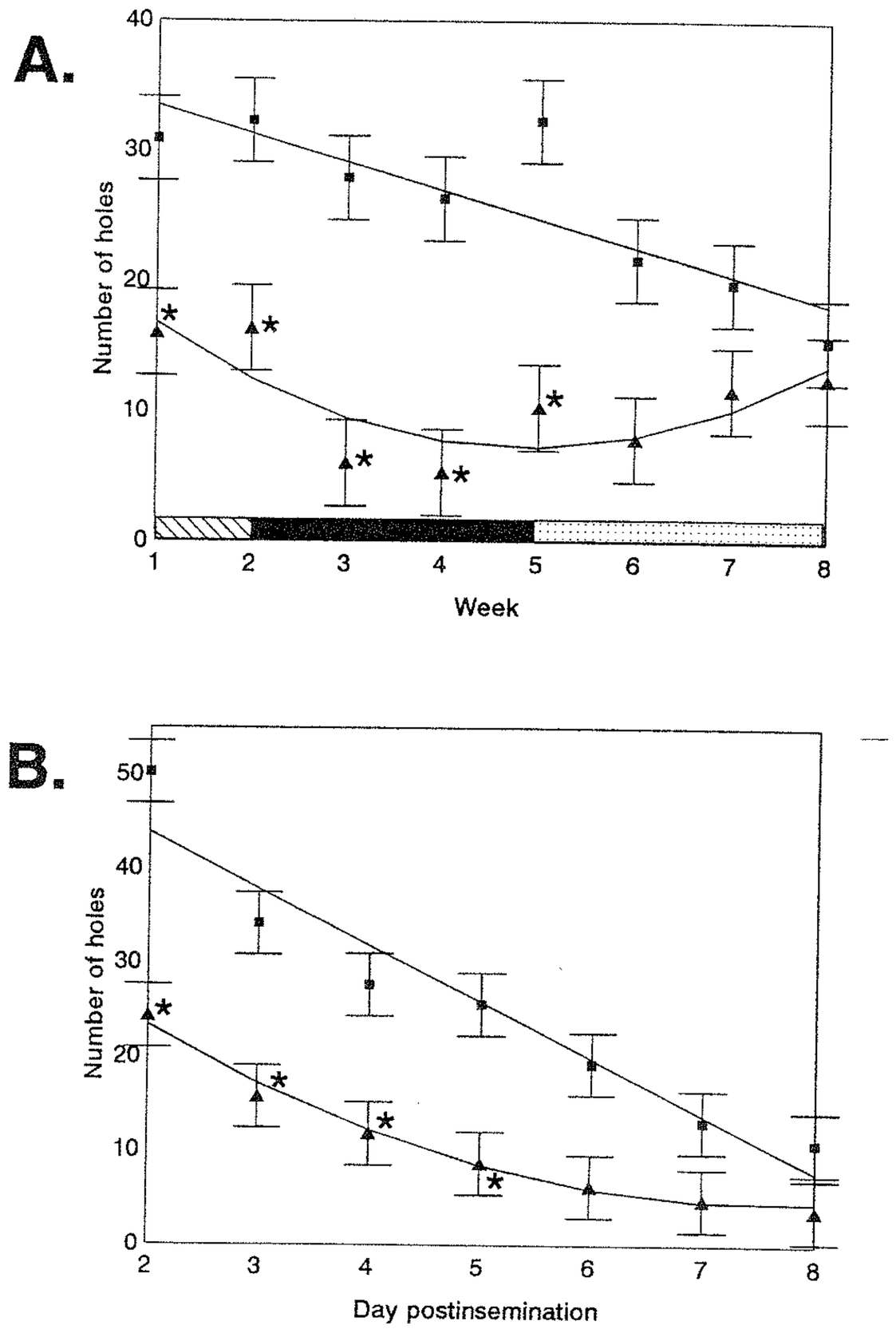


FIGURE 3. *In vivo* sperm-egg penetration during each week of the experiment (A, treatment by week interaction, $P < 0.05$) and each day postinsemination (B, treatment by day postinsemination interaction, $P < 0.13$). Hens were inseminated weekly with 50 million sperm from control (■) or heat-exposed (▲) males. The graphics bar along the x axis indicates the ambient temperature at which the heat-exposed birds were maintained during each week (hatched bar = 27 C, solid bar = 32 C, and stippled bar = 21 C). All control birds were maintained at 21 C throughout the experiment. A significant linear decline was detected for *in vivo* sperm-egg penetration of the control males over each week of the study ($y = -2.2 x + 35.7$, $r^2 = 0.73$, $P < 0.0072$) and each day postinsemination ($y = -6.02 x + 55.9$, $r^2 = 0.93$, $P < 0.0005$). When semen from heat-exposed males was used to inseminate hens, a quadratic relationship was seen in sperm-egg penetration over each week of the experiment ($y = -6.2 x + 0.64 x^2 + 22.4$, $r^2 = 0.63$, $P < 0.08$) and each day postinsemination ($y = -9.2 x + 0.61 x^2 + 39.4$, $r^2 = 0.99$, $P < 0.0001$). An asterisk indicates a significant difference between control and heat-exposed means for a specific week or day postinsemination. For weekly data, values represent the replicate means of the 2nd through the 8th d postinsemination of three replicates (n = 21). For daily data, values represent the replicate means of the 1st through the 8th wk of treatment of three replicates (n = 24). The mean ± SEM is displayed for each point.

TABLE 1. Effects of heat exposure on pooled semen characteristics[1]

Treatment	Dead sperm	Motility score	Packed sperm volume
	(%)		(%)
Control	9.8	4.5	15.4
Heat-exposed	11.3	4	15.9
SEM	0.52	0.24	0.53
P	0.11	0.17	0.50

[1]Values represent the means of the 1st through the 8th wk of the experiment of 3 rooms with 12 males per room (n = 24) at initiation of the study.

A significant treatment by day postinsemination interaction was also obtained for fertility (Figure 4b). When semen from H males was used to inseminate hens, fertility was reduced during Days 3 through 8 postinsemination as compared to insemination with semen from C males. During Day 2 postinsemination, fertility was similar between the two treatment groups. A quadratic decline in fertility over day postinsemination was seen for the C males. A much sharper linear decline in fertility over day postinsemination was noted for the H males.

The hatchability of fertile eggs appeared to be unaffected by exposing the male broiler breeder to elevated temperatures. Hatchability main effect means for the C and H males were 90.4 and 88.1%, respectively (SEM = 2.6, $P > 0.55$).

In Vitro *Sperm-Egg Penetration*

Sperm from H males penetrated the perivitelline layer *in vitro* at a rate that was similar to that obtained with sperm from C males (Table 2). In addition, there was no effect due to time ($P > 0.39$) or interaction with time ($P > 0.55$) with regard to *in vitro* sperm-egg penetration of sperm from the C and H males.

Oviductal Sperm Storage

When semen from H males was used to inseminate hens, the number of sperm stored in the SST of the UVJ was 60% less than when hens were inseminated with semen from C males (Table 3). During Week 4, more sperm were found in the UVJ than during Week 2. However, the treatment by week interaction for UVJ sperm storage was not statistically significant ($P > 0.22$). In addition, the number of sperm in the UVJ was positively correlated with *in vivo* sperm-egg penetration of eggs laid 3 d postinsemination (r = 0.85; $P < 0.034$). Oviductal sperm storage was also positively correlated with sperm motility (r = 0.97; $P < 0.032$) and negatively correlated with male rectal body temperature (r = –0.94; $P < 0.06$).

DISCUSSION

Results indicate that exposing male broiler breeders to 27 C does not significantly increase body temperature. McDaniel *et al.* (1995) found that an ambient temperature approximately 2 C higher does elevate rectal body temperature. Similar to data reported by McDaniel *et al.* (1995), body temperature of the male broiler breeder increased 0.62 C when birds were exposed to an ambient temperature of 32 C. In contrast to the linear increase in body temperature with time seen by McDaniel *et al.* (1995), body temperature of the H males in the current study decreased to a level similar to the controls by the 3rd wk of exposure to 32 C. Acclimation of the males to heat treatment may explain the conflicting results. On the other hand, McDaniel *et al.* (1995) subjected birds to 32 C for 8 wk, whereas in the present study birds were maintained at this temperature for 3 wk. It is possible that the body temperature of the birds in the present study could have increased linearly with time if the birds had been exposed to this high temperature for a longer amount of time. In addition, McDaniel *et al.* (1995) reported no increase in mortality or decrease in feed consumption as was seen in the present study when the male bird was exposed to 32 C.

TABLE 2. Effects of heat exposure on *in vitro* sperm-egg penetration[1]

Week	Control	Heat-exposed	Mean	SEM	*P*
	(holes)				
1	28	31	30	4.2	0.4
3	18	29	24		
Mean	23	30			
SEM	3.7				
P	0.3				

[1]Values represent the means of 3 replicates with 12 samples each (n = 3).

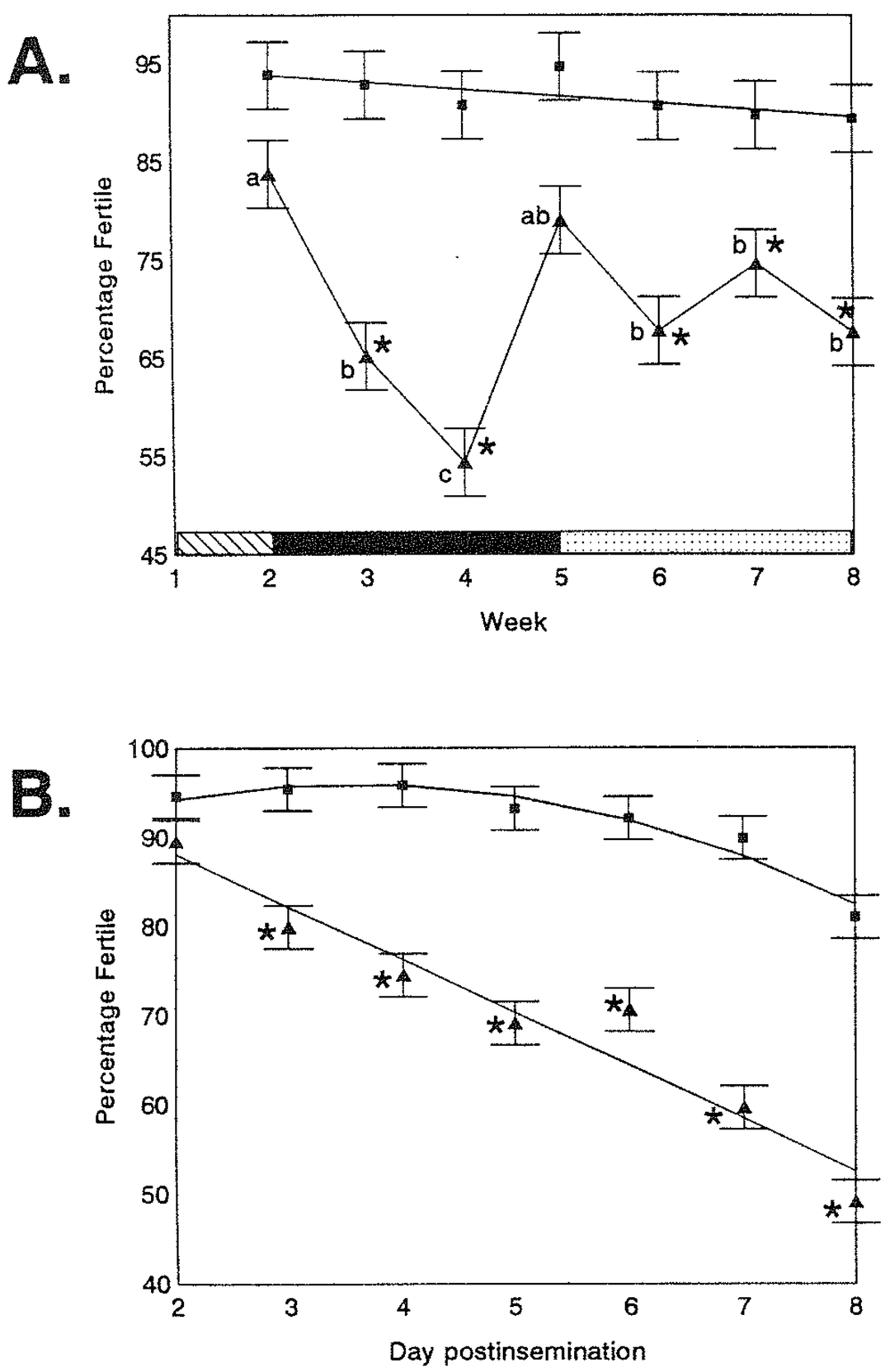


FIGURE 4. The percentage of fertile eggs produced during each week of the experiment (A, treatment by week interaction, $P < 0.017$) or each day postinsemination (B, treatment by day postinsemination interaction, $P < 0.0002$). Hens were inseminated weekly with 50 million sperm from control (■) or heat-exposed (▲) males. The graphics bar along the x axis indicates the ambient temperature at which the heat-exposed birds were maintained during each week (hatched bar = 27 C, solid bar = 32 C, and stippled bar = 21 C). All control birds were maintained at 21 C throughout the experiment. A significant linear decline was detected for fertility of the control males over each week of the study ($y = -0.69\ x + 94.5$, $r^2 = 0.53$, $P < 0.065$). When semen from control males was used to inseminate hens, a quadratic relationship was seen in fertility over each day postinsemination ($y = 4.9\ x - 0.69\ x^2 + 87.1$, $r^2 = 0.95$, $P < 0.0026$). A significant linear decline was detected for fertility of the heat exposed males over each day postinsemination ($y = -5.91\ x + 99.9$, $r^2 = 0.94$, $P < 0.0003$). An asterisk indicates a significant difference between control and heat-exposed means for a specific week or day postinsemination. Letter superscripts denote significant differences among heat-exposed means over weeks. For weekly data, values represent the replicate means of the 2nd through the 8th d postinsemination of three replicates (n = 21). For daily data, values represent the replicate means of the 1st through the 8th wk of treatment of three replicates (n = 24). The mean ± SEM is displayed for each point.

All of the aforementioned differences between the results of McDaniel *et al.* (1995) and the present study may relate to the differences in heat treatment temperatures used during the 1st wk of exposure in the two studies. Because the heat treatment temperature used during the 1st wk of the present study was only 27 C, the birds may not have adjusted to heat exposure as they did in the experiment of McDaniel *et al.* (1995), in which the birds were maintained at a higher temperature of 29 C during the 1st wk of heat treatment. If the birds in the present study did not completely acclimate to heat treatment during the 1st wk of the experiment, one would expect a more drastic increase in mortality and decrease in feed consumption when the birds were subjected to an even higher temperature of 32 C.

The percentage of dead sperm produced by individual H males suggested that sperm viability is decreased in comparison to C males during the 1st wk of exposure to 32 C. When semen was pooled by room, sperm viability was similar between the C and H males, indicating that those males producing the most sperm were also producing the most viable sperm. However, semen was collected on different days of the week for individual male and pooled measurements, so it is possible that time of semen collection may complicate this interpretation.

Although pooled semen characteristics were unaffected by heat treatment of the male, when pooled semen from H birds was used to inseminate hens, *in vivo* sperm-egg penetration was reduced as compared to insemination with semen from C males. Apparently exposure of males to high temperatures quickly diminishes the ability of sperm to penetrate the ovum, because semen was collected and used to inseminate hens approximately only 12 h after the initiation of the 27 C heat treatment. Interestingly, the immediate reduction in *in vivo* sperm-egg penetration after exposure of the males to only 27 C for 12 h is very similar to that obtained when McDaniel *et al.* (1995) heat-stressed males at a higher temperature of 29 C for 12 h. These results indicate that male heat-induced infertility may occur immediately following exposure to even mildly warm temperatures. This rapid response of the male to elevated ambient temperatures must not involve altering sperm formation which requires 10 to 12 d to reach completion and then 1 to 5 d for sperm to traverse the excurrent ducts (Lake, 1984). However, results also indicate that the decrease in *in vivo* sperm-egg penetration of H males is quickly eliminated within 2 wk following removal of heat.

Fertility results were similar to those of *in vivo* sperm-egg penetration with respect to heat treatment. However, during the 1st wk of exposure to 32 C, fertility of H males was not significantly less than that of the controls. In addition, during the 1st wk following removal of heat, fertility of the H males returned to a value not significantly different from that of the controls. Similarly, Renden and McDaniel (1984) found that exposing 17- to 20-wk-old broiler breeders to a cycling temperature of 24 to 36 C for 3 wk did not depress fertility after the removal of the high temperature. Interestingly, in the present study fertility of the H males became lower than that of the C males during the later weeks of the postheat period. These results may

TABLE 3. Effects of heat exposure on uterovaginal junction sperm storage[1]

Week	Control	Heat-exposed	Mean	SEM	*P*
	(sperm × 10^3)				
2	275.5	85.5	180.5	18.4	0.003
4	474.9	211.3	343.1		
Mean	275.2	148.4			
SEM	49.9				
P	0.05				

[1]Values represent the means of three replicates with 7 hens each (n = 3).

indicated a possible alteration in spermatozoa DNA after males are exposed to heat. It is possible that in testes of males after exposure to heat, abnormal nuclear division occurs during spermatocytogenesis resulting in sperm that are physically able to penetrate the ovum but genetically unable to initiate syngamy and therefore fertilization.

The present study also revealed that fertility of H males was significantly lower than that of C males for eggs laid every day postinsemination except Day 2. These results, coupled with those obtained for *in vivo* sperm-egg penetration, indicate that on average 25 to 30 sperm are required to penetrate the egg near the germinal disc before fertilization is successful (Bramwell *et al.*, 1995). Because *in vivo* sperm-egg penetration for H males was less than 25 sperm every day postinsemination except Day 2, one would expect fertility to be poor for the H birds. Wishart (1987) has also shown that as more sperm are trapped on the perivitelline layer the probability of successful fertilization increases.

In the present study we were successful in determining the relationship among *in vivo* and *in vitro* sperm-egg penetration and UVJ sperm storage when hens are inseminated with semen from both C and H males. Although *in vivo* sperm-egg penetration of H males was less than that of C males, *in vitro* sperm-egg penetration was not significantly different between the two treatment groups. This difference in *in vivo* and *in vitro* sperm-egg penetration may illustrate that sperm from H males are able to penetrate the ovum *in vivo*; however, there may be a smaller number of sperm from H males at the site of fertilization than there are from C males. This decrease in the number of H male's sperm at the site of fertilization may be a result of fewer sperm being stored in the UVJ region of the hen's oviduct, as was found in the present study. Because fewer H male's sperm are stored in the UVJ, fewer sperm would be released from the UVJ and become available at the site of fertilization as compared to C male's sperm.

McDaniel *et al.* (1995) found that, when semen from H males was used to inseminate hens, *in vivo* sperm-egg penetration was drastically less than controls during the 2nd d postinsemination, and this difference lessened with increasing time postinsemination. Similar results were obtained in the present study. McDaniel *et al.* (1995) reasoned that this declining difference in sperm-egg penetration over days postinsemination may be due to a decrease in oviductal storage of sperm from heat-stressed males. Indeed, results from the present study show that sperm from H males are not stored in the UVJ to the same degree as sperm from C males. In addition, our results indicate a strong relationship between *in vivo* sperm-egg penetration and UVJ sperm storage (r = 0.85). Brillard and Bakst (1990) have previously shown that the number of turkey sperm trapped on the perivitelline layer is positively correlated with the amount of sperm in the SST. It is also very interesting that in the present study the average reduction in UVJ sperm storage (60%) and *in vivo* sperm-egg penetration (63%), when the birds were exposed to high temperatures during Weeks 2 and 4, were strikingly similar. These data indicate that the entire decrease in *in vivo* sperm-egg penetration when the male bird is exposed to elevated ambient temperatures may be due to a lack of sperm being stored in the UVJ.

McDaniel *et al.* (1995) has indicated that, if there was a decrease in oviductal storage of sperm from H males, this decrease may be attributable to sperm motility. It was reasoned that because motility is essential for sperm to traverse the vagina (Allen and Grigg, 1957), a decrease in sperm motility would result in fewer sperm being stored in the SST. In the present study, we were unable to show that sperm motility was significantly reduced when the male bird was maintained at elevated temperatures. Interestingly, however, we did find a very strong correlation between motility and UVJ sperm storage (r = 0.97), which may indicate that the 11% decrease in motility of sperm from H males (Table 1), whereas not statistically significant, may be sufficient to decrease oviductal sperm storage. A less subjective technique, such as that of Wishart and Ross (1985), could provide more insight into sperm motility when the male is exposed to elevated environmental temperatures. Results from the present research also reveal a significant negative correlation between male rectal body temperature and UVJ sperm storage (r = –0.94).

In conclusion, sperm from males exposed to an ambient temperature as low as 27 C for 12 h were less capable of penetrating the perivitelline layer of the ovum. Although *in vivo* sperm-egg penetration and fertility were reduced in H males, they rebounded readily following removal of heat. However, fertility is

affected for a longer amount of time following heat removal than is *in vivo* sperm-egg penetration. In addition, male heat-induced infertility appears to be due to a decrease in oviductal sperm storage and not to a decrease in the ability of sperm to bind to and penetrate the ovum.

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Acute High Environmental Temperature and Calcium-Estrogen Relationships in the Hen[1]

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ABSTRACT Much is known about the effects of high environmental temperature (HT) on egg production, but very little is understood about the mechanisms that underlie them. Two experiments were conducted to examine the effects of acute heat stress on circulating estradiol, on calcium uptake by gut tissue, on bone resorption, and on the dynamic relationships between estradiol and calcium in the hen during one ovulatory cycle. In one study, hens were moved individually and randomly into a hot [HT: temperature (T) = 35 C, relative humidity (RH) = 50%; n = 18] or a control, thermoneutral (TN: T = 23 C, RH = 50%; n = 18) environment immediately after a mid-sequence oviposition and brachial vein cannulation. Blood samples (2 mL) were collected every 3 h for 21 h for ionized calcium (Ca^{2+}) and pH determinations and from which aliquots were frozen for 17β-estradiol (E_2), total calcium (TCa), and inorganic P analysis. Excreta and urine were assayed for TCa and hydroxyproline (OHPr), respectively. A second study was conducted to determine the effects of HT (T = 35 C, H = 50%, 12 h) vs TN (T = 23 C, RH = 50%, 12 h) on the ability of duodenal cells to take up calcium (CaT). Blood pH and calcium responded to HT as expected (pH increased, Ca^{2+} decreased, and TCa decreased) and the cyclic pattern of Ca^{2+} in blood was abolished. The ratio of Ca^{2+}:TCa decreased sharply at approximately the onset of shell calcification in control hens, but in HT hens there was no clear change in the ratio at any point in the cycle. The pattern of E_2 typical of hens under normal conditions was significantly depressed in plasma of HT hens. Calcium uptake by duodenal epithelial cells of HT hens was lower than in TN hens. There was a clear inverse correlation between blood Ca^{2+} and urine OHPr in TN hens (r^2 = –73, P = 0.0021) but not in HT hens (r^2 = –27, P = 0.32). In addition to alterations in acid-base balance and the status of Ca^{2+}, diminished ability of duodenal cells to transport calcium may be a critical factor in the detrimental effects of heat stress on egg production (numbers), eggshell characteristics, and skeletal integrity often documented in the laying hen.

(*Key words*: 17β-estradiol, ionized calcium, laying hen, heat stress, hydroxyproline)

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INTRODUCTION

High environmental temperature is one of the most serious factors affecting the production performance of laying hens in both tropical and temperate countries. In addition to effects on feed intake and any associated indirect effects on egg production performance, high temperature (HT) also adversely affects bone weight, ash, and strength, and shell thickness (Miller and Sunde, 1975; de Andrade *et al.*, 1976; Scott and Balnave, 1988), all of which implicate disruptions in calcium metabolism. Odom *et al.* (1986) reported an increase in blood organic acid levels, and documented increases in blood pH and decreases in blood ionized calcium (Ca^{2+}) during HT. Donoghue *et al.* (1989) attributed the reproductive decline in acutely HT hens to reduced luteinizing hormone (LH) releasing ability of the hypothalamus. Novero *et al.* (1991) noted that the preovulatory surges of both progesterone (P_4) and LH are depressed during the first ovulatory cycle after onset of high environmental temperatures. These latter studies suggest that heat stress disrupts neuroendocrine mechanisms controlling ovulation prior to declines in feed consumption, but do not suggest a causative mechanism. However, calcium and estrogen are required in the synthesis as well as the release of these hormones, and calcium and estrogen are also intricately associated with each other (Luck and Scanes, 1979a; Asem *et al.*, 1987; Onagbesan and Peddie, 1989).

The relationships between 17β-estradiol (E_2) and calcium are complex. Estrogen was shown to increase

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