The Effect of Heat Stress on Ovarian Function of Laying Hens

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ABSTRACT Reproductive failure associated with heat stress is a well-known phenomenon. The mechanism involved in this failure is not clearly understood. In order to test a possible direct effect of heat stress on ovarian function, 36 White Leghorn laying hens were housed in individual cages in 2 temperature- and light-controlled rooms (n = 18). At 31 wk of age, one group was exposed daily for 12 h to high temperature (42 ± 3°C), and the second group was maintained under thermoneutral conditions (24 to 26°C) and served as control. Body temperature, feed intake, egg production, and egg weight were recorded daily; heparinized blood samples were drawn every 3 d for plasma hormonal level of luteinizing hormone, follicular stimulating hormone, progesterone, 17β-estradiol, and testosterone. Six days after exposure half of the birds in each group were killed, and the ovary and oviduct were weighed and preovulatory follicles removed and extracted for mRNA of Cytochrome P 450 aromatase, 17-α hydroxylase. The same procedure was repeated 9 d later with the rest of the birds. Short and long heat exposure caused significant hyperthermia and reduction of egg production, egg weight, ovarian weight, and the number of large follicles. In addition, a significant reduction in plasma progesterone and testosterone was detected 2 d after exposing the birds to heat stress, and plasma 17β-estradiol was significantly reduced 14 d after initiation of heat stress. Short exposure to heat stress caused significant reduction in mRNA expression of cytochrome P450 17-α hydroxylase, exposing the birds to long-term heat stress caused significant reduction in expression of mRNA of both steroidogenic enzymes. No significant change was found in plasma luteinizing hormone and follicular stimulating hormone levels during the entire experimental period. We suggest a possible direct effect of heat stress on ovarian function.

Key words: heat stress, reproduction, egg production, steroid, gonadotropin

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INTRODUCTION

Heat stress is a major concern in modern poultry farming. Its debilitating effect on egg production is well recognized, but the mechanism involved is not clearly understood. Reduced feed consumption may account for part of the impairment in reproduction; however, the effect of high environmental temperatures on the rate of egg production appears largely unrelated to feed intake (Smith and Oliver, 1972).

The regulatory mechanisms for the reduced reproductive efficiency in the heat-stressed hen might be modulated at the level of the hypothalamus and pituitary (El Halawani et al., 1973; Saarela et al., 1977; El Halawani and Waibel, 1978; Braganza and Wilson, 1978a,b; Jeronen et al., 1978) or at the level of the ovary as found in mammalian species. Heat stress decreased ovarian function in cattle (Wolfenson et al., 1997), suggesting a differential inhibitory effect of heat stress on the functions of granulosa and theca cells by concurrent and delayed effects on the steroidogenic capacity of ovarian follicles.

Changes in reproductive hormone secretion represent the final sequence in the neuroendocrine pathway leading to the diminished reproductive performance associated with stress. Previous studies demonstrated that stress, in a number of forms and in a number of species, increased and decreased circulating prolactin (**PRL**) and gonadotropins (luteinizing hormone, **LH**; follicular stimulating hormone, **FSH**), respectively, in rats (Neill, 1970; Krulich et al., 1974), cows and goats (Johke, 1970), turkey poults (Opel and Proudman, 1982), laying hens (Johnson, 1981), and turkey hens (El Halawani et al., 1985; Rozenboim et al., 2004). The aim of the present study was to determine the role of the ovary in reproductive failure associated with heat stress in birds.

MATERIALS AND METHODS

Thirty-six 18-wk-old White Leghorn laying hens were housed in individual cages under ambient temperatures and were provided 16 h of light. Birds were fed ad libi-

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tum with commercial layer feed (17.5% protein). At 30 wk of age, birds were divided to 2 groups (n = 18) and transferred to 2 environmentally controlled rooms. Both rooms provided similar thermoneutral environmental conditions (24 to 26°C) and 16 h of light. Birds were housed under those conditions for 10 d prior to the beginning of the experiment.

Temperature Treatments

The first group was exposed daily for 12 h to high temperature (42 ± 3°C), and the second control group was maintained under thermoneutral conditions (24 to 26°C) during the whole day.

During the experiment body temperature was measured 6 times/d by using a telethermometer (YSI Instruments, Yellow Springs, OH); egg production and egg weight were recorded daily, and heparinized blood samples were drawn every 3 d for plasma levels of LH, FSH, progesterone, 17β-estradiol, and testosterone.

Hormonal Analysis

Each hormonal determination was conducted in a single assay. Plasma progesterone, estrogen, and testosterone were measured by an ELISA (Nash et al., 2000). Plasma LH levels were measured by RIA according to Bacon and Long (1996). Plasma FSH levels were measured by RIA according to Krishnan et al. (1993).

Six days after exposure, half of the birds in each group were euthanized by an overdose of pentobarbital, the ovary and oviduct were quickly removed and weighed, and preovulatory follicles were removed and stored in liquid nitrogen. The same procedure was conducted 9 d later with the remainder of the birds in each group.

RNA Extraction

Total RNA was isolated from the largest ovarian follicle (F1-F3) using Tri Reagent (1 mL/100 mg of tissue) according to the manufacturer's protocol (MRC Molecular Research Center, Cincinnati, OH).

Isolation of cDNA Probes for Chicken Cytochromes P 450 Aromatase, 17-α Hydroxylase, and β-Actin

The following primers were used in a reverse transcription PCR (**RT-PCR**) to amplify a 692-bp sequence of the mRNA coding region of the aromatase gene (McPhaul et al., 1988): 5′-CACACGACCTCTACTACTAAC-3′ (sense primer corresponding to coding nucleotides 671-690) and 5′- CCCGAAACCACTTCTTCCCAG-3′ (complement primer corresponding to coding nucleotides 1078-1099).

The following primers were used in a RT-PCR to amplify a 529-bp sequence of the mRNA coding region of the 17-α hydroxylase gene (Ono et al., 1988): 5′-TGGAAGTCCGTACCGAAGTCG-3′ (sense primer corresponding to coding nucleotides 722-741) and 5′-CGTACTCTTCCTCACCCTATT-3′ (complement primer corresponding to coding nucleotides 925-943).

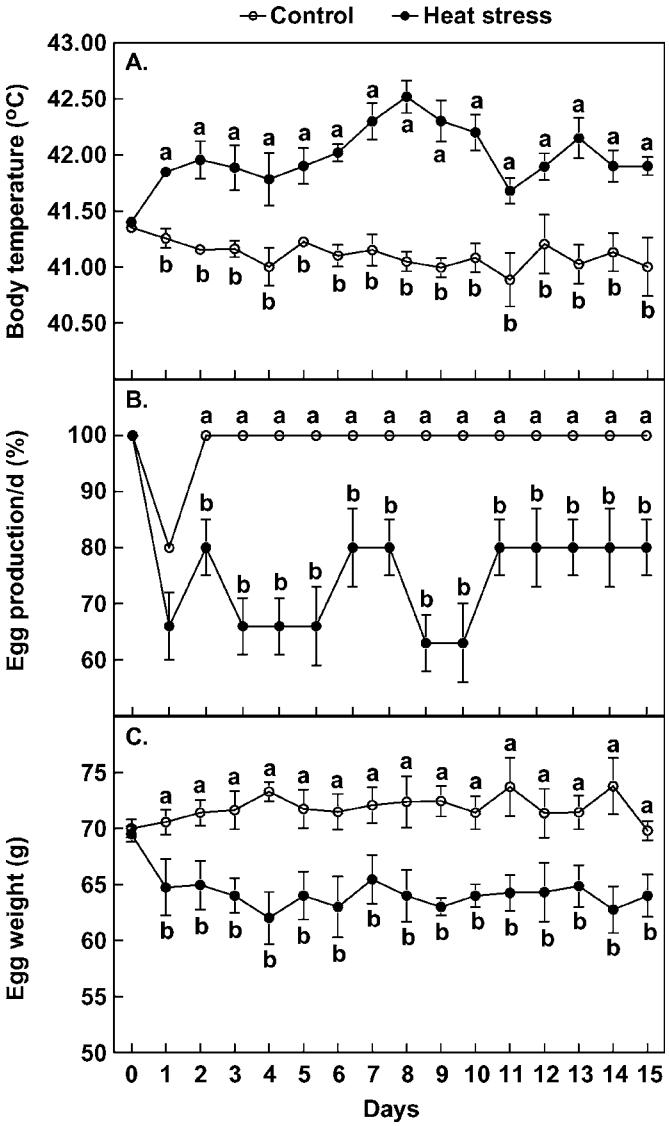

Figure 1. Body temperatures (°C; A), egg production (%; B) and egg weight (g; C) of White Leghorn layers that were exposed to 12 h/d for 15 d to 42 ± 3°C (heat stress) or that remained under thermoneutral conditions (24 to 26°C; control). Data represent mean ± SE. [a,b]Values marked with different letters are significantly different ($P < 0.05$).

The following primers were used in a RT-PCR to amplify a 241-bp sequence of the mRNA coding region of the β-actin gene: 5′-AACCCTAAGGCCAACCGTGAAAAG-3′ (sense primer corresponding to coding nucleotides 331-354) and 5′-TCATGAGGTAGTCTGTCAGGT-3′ (complement primer corresponding to coding nucleotides 551-571).

The RT-PCR products were visualized on a 1.5% agarose gel, stained with ethidium bromide, excised from the gel, and purified with DNA isolation system (DNA Isolation Kit, Biological Industries, Kibbutz Beit Haemek, Israel). To confirm that the fragments obtained correspond to the original sequence, fragments were sequenced by automated sequencing using an Applied

Biosystem 373A DNA sequencer (Applied Biosystem, Foster City, CA). Nucleic acid sequences were analyzed using the GCG suite programs (Devereux et al., 1984).

Northern Blot

For Northern blot analysis, 30 μg of total RNA was denatured and separated by electrophoresis on 1.5% agarose/1.1 mol/L of formaldehyde gel. After electrophoresis, RNA was transferred overnight by capillary transfer onto a nylon membrane, Hybond-N (Amersham Pharmacia Biotech, Amersham, UK), and then fixed on the membrane by ultraviolet at 340 nm for 2 min.

Hybridization

Three probes were used for hybridization: 1) the isolated 692-bp cDNA fragment of chicken aromatase, 2) the isolated 529-bp cDNA fragment of 17-α hydroxylase, and 3) the β-actin cDNA, used to normalize variations in the total RNA loading. The probes were labeled with ^{32}P-dCTP by random priming (Biological Industries, Kibbutz Beit Haemek, Israel). Prehybridization was done at 42°C for 4 h, hybridization was conducted at 42°C overnight, and a high-stringency wash (0.1× saline sodium citrate/0.1% SDS at 60°C) was conducted according to the procedures recommended by Amersham for Hybond N membranes (Amersham Pharmacia Biotech). Blots were exposed for 28 h at –80°C to Kodak XAR 5 film in the presence of an intensifying screen. The 1.9-, 4.0-, and 2.2-kb bands were visualized using 17-α hydroxylase, aromatase, and β-actin probes, respectively. All data were normalized for β-actin mRNA expression.

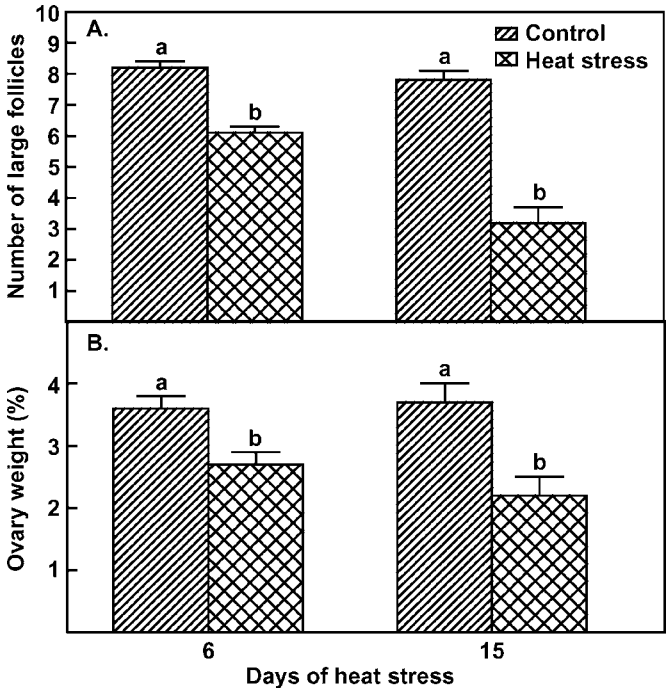

Figure 2. Number of large follicles (A) and percent ovary weight (B) of White Leghorn layers that were exposed to 12 h/d for 15 d to 42 ± 3°C (heat stress) or that remained under thermoneutral conditions (24 to 26°C; control). Data represent mean ± SE. [a,b]Values marked with different letters are significantly different ($P < 0.05$).

Statistical Analysis

Data were analyzed by 1-way ANOVA using JMP software (SAS Institute, Cary, NC).

RESULTS

Heat Exposure Effect on Reproductive Activities

Body Temperature. Exposure to high temperature caused a significant elevation in body temperature (Figure 1, panel A) during the 12 h of heat stress exposure.

Egg Production. A significant reduction in egg production was detected in heat-stressed hens (Figure 1, panel B). Egg production declined by an average of 20% after 2 d of heat exposure and increased on d 11 of heat stress remaining significantly lower then controls.

Egg Weight. Parallel to egg production, egg weight declined during exposure to heat stress (Figure 1, panel C) after 1 d of heat stress and remained low until the end of the experiment.

Effect of Heat Exposure on Ovary Weight and the Number of Large Follicles

A reduction in ovary weight and the number of large follicles was observed after 6 d of heat stress, and this was maintained until the end of the experiment at d 15 (Figure 2, panels A and B).

Effect of Heat Exposure Effect on Plasma LH and FSH and Ovarian Steroids

Little change was observed in plasma LH (Figure 3, panel A) and plasma FSH (Figure 3, panel B) due to heat stress. In contrast, a reduction in plasma progesterone and testosterone was detected 2 d after exposing the birds to high environmental temperatures (Figure 4, panels A and B). Plasma 17β-estradiol remained stable during most of the experimental period, decreasing in the last 3 d of heat exposure.

Effect of Heat Exposure Effect on Ovarian Steroidogenic Enzymes: Cytochrome P450 17α Hydroxylase and Cytochrome P450 Aromatase

Six days after exposure to heat stress, a significant reduction in the expression of mRNA for cytochrome P450 17α hydroxylase was observed; however, no effect on expression level of cytochrome P450 aromatase (Figure 5, panel A) was detected. After 15 d of exposure to

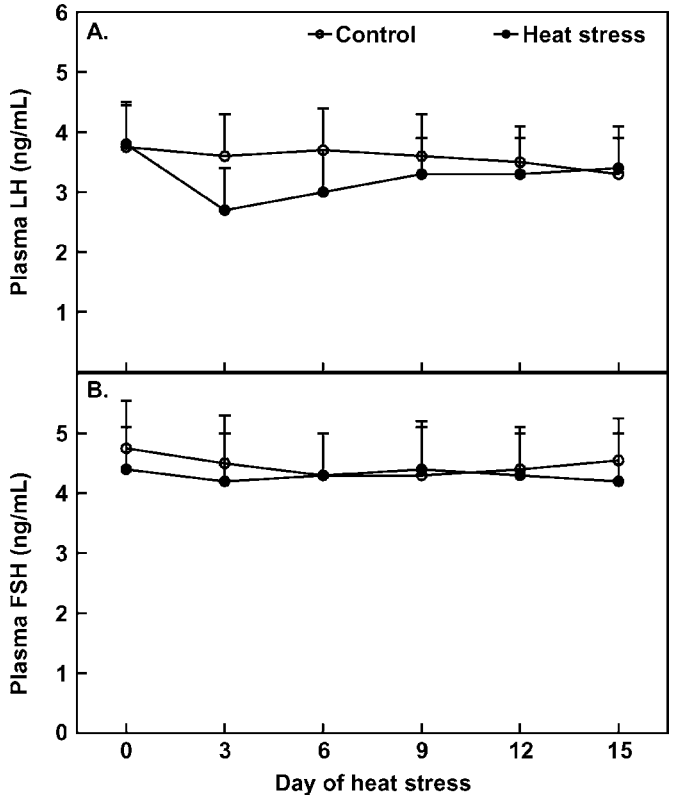

Figure 3. Plasma luteinizing hormone (LH; A) and follicle stimulating hormone (FSH; B) of White Leghorn layers that were exposed to 12 h/d for 15 d to 42 ± 3°C (heat stress) or that remained under thermoneutral conditions (24 to 26°C; control). Data represent mean ± SE.

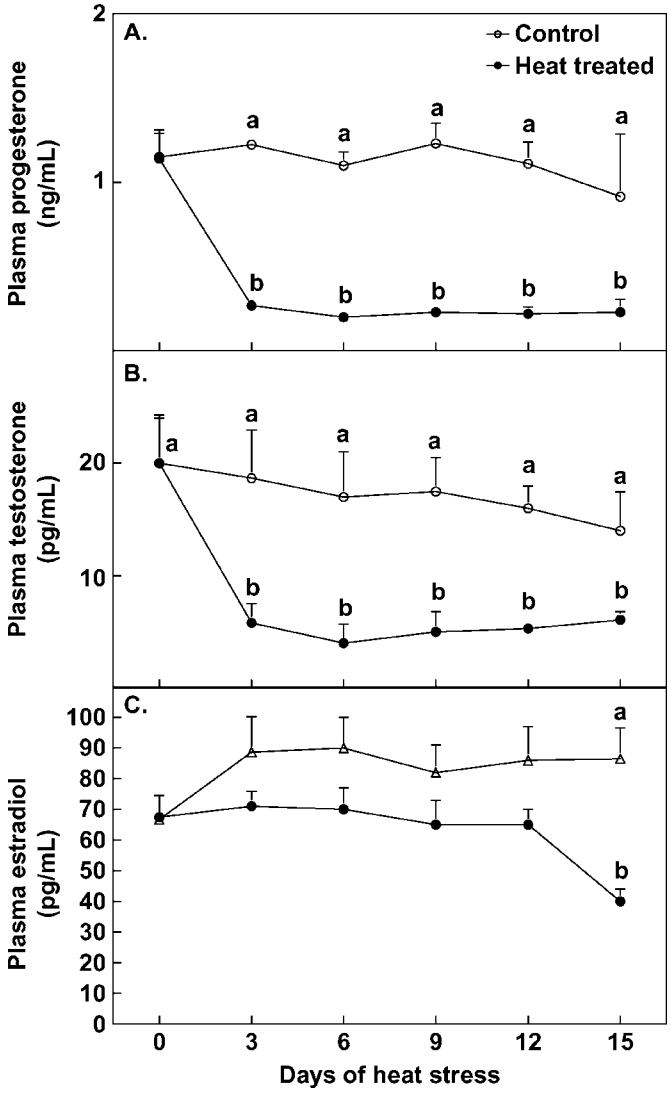

Figure 4. Plasma progesterone (ng/mL; A), testosterone (pg/mL; B), and estradiol (pg/mL; C), of White Leghorn layers that were exposed to 12 h/d for 15 d to 42 ± 3°C (heat stress) or that remained under thermoneutral conditions (24 to 26°C; control). Data represent mean ± SE. [a,b]Values marked with different letters are significantly different ($P < 0.05$).

high temperatures, expression of mRNA for both steroidogenic enzymes was reduced (Figure 5, panel B).

DISCUSSION

Exposure of White Leghorn laying hens to high ambient temperatures caused reproductive failure manifested by a reduction in reproductive activities and egg quality. This is probably due to a decline in ovarian function indicated by the decline in plasma gonadal steroids and follicular expression of mRNA for steroidogenic enzymes. No effects on plasma LH and FSH levels were detected, suggesting a possible direct debilitating effect of high ambient temperature on ovarian function in domestic birds.

The reduction in reproductive performance associated with heat stress is a well-known phenomenon in domestic birds (Etches et al., 1995). Heat stress was found to reduce LH levels and hypothalamic gonadotropin-releasing hormone-I content (Donoghue et al., 1989), and in addition, a reduction of preovulatory surges of LH and progesterone was observed (Novero, et al., 1991). In our study, no significant reduction in plasma LH and FSH was found, suggesting a direct effect of heat stress on ovarian function. Wolfenson et al. (1997) reported a direct effect of heat stress on cattle ovarian functions. A possible mechanism for the reduction of ovarian function might be the reduction in blood flow to the ovary; differential ovarian blood flow pattern was found in hens exposed to high ambient temperature (Wolfenson et al., 1981).

The diminished reproductive performance in heat-stressed poultry may, in part, be related to increased PRL secretion (El Halawani et al., 1984; Donoghue et al., 1989). Convincing evidence implicating increased PRL secretion as a causative factor for reduced gonadotropins and ovarian regression has been presented (Youngren et al., 1991; Rozenboim et al., 1993; You et al., 1995). There are indications that elevated PRL levels can act through hypothalamic GnRH (Rozenboim et al., 1993) or directly on pituitary gonadotropes (You et al., 1995), causing the suppression of gonadotropin secretion. Together, the data presented in this study suggest that reproductive failure associated with high environmental

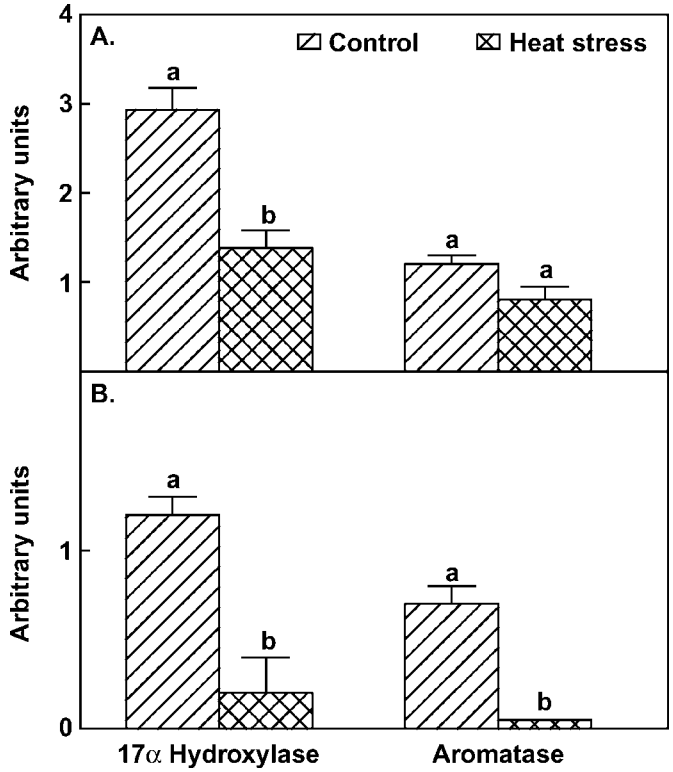

Figure 5. Cytochrome P450 17α hydroxylase and cytochrom P450 aromatase mRNA level of White Leghorn layers that were exposed to 12 h/d for 6 (A) and 15 d (B) to 42 ± 3°C (heat stress) or that remained under thermoneutral conditions (24 to 26°C; control). Data represent mean ± SE. [a,b]Values marked with different letters are significantly different ($P < 0.05$).

temperature might be caused directly by depressing ovarian functions.

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