Impact of acute short-term high thermal stress during early embryogenesis on hatchability, physiological body reaction, and ovarian follicles development of quails

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ABSTRACT This experiment aimed to evaluate the impact of continuous and intermittent thermal stress during early embryogenesis on hatchability, physiological body reaction, ovary weight, and follicle development of quails. A total of 540 eggs were divided into 3 equal groups (3 groups × 6 replicates × 30 eggs). In the first group (control), eggs were incubated at normal incubation conditions (37.5°C and 50–55% relative humidity) from day 0 till hatching. In the second group (continuous thermal stress [**CTS**]), eggs were daily exposed to 39.5°C and 50 to 55% during the early embryogenesis for 3 successive days (E4–E6) for 3 h (12:00–15:00). In the third group (intermittent thermal stress [**ITS**]), eggs were daily exposed to 39.5°C and 50 to 55% during the early embryogenesis for 90 min (12:00–13:30) then temperature was returned to 37.5°C for 60 min (13:30–14:30) after that the temperature was raised again for 39.5°C for 90 min (14:30–16:00) daily for 3 successive days (E4–E6). The findings showed that the highest relative water loss form egg (**RWL/%**) at 6 d of incubation was obtained in the CTS group ($P \leq 0.05$). The hatchability rate was significantly ($P \leq 0.05$) decreased in the thermal-treated groups compared with the control group. The body surface temperature and cloacal temperature in the CTS and ITS groups significantly ($P \leq 0.001$) increased compared with the control group. Chick weight (g) at 5 wk old, total weight gain, daily weight gain were significantly lower ($P \leq 0.05$) in the CTS group compared with the control group. Triiodothyronine (**T3**) hormone concentration and globulin level were significantly ($P \leq 0.05$) lower in the CTS and ITS groups compared with the control. The ovarian follicle weights (first, second, third, fourth, and fifth) and the diameter of the large follicle (fifth follicle) were significantly ($P \leq 0.01$) decreased by increasing incubation temperature. From these findings, it could be concluded that the hatchability and body weight at sexual maturity for quails produced from eggs exposed to CTS and IST were significantly decreased by 8 and 2.1% as well as 2.98 and 2.1%, respectively, compared with the control group.

Key words: quail, incubation temperatures, hatchability, embryonic development, reproductive performance

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INTRODUCTION

Quails (*Coturnix coturnix*) are farmed for various commercial purposes such as egg and meat production as well as the experimental purpose as a laboratory animal because its small body size makes its handling easy, so it is widely used as a tool in large-scale genetic studies (Nowaczewski et al., 2010; Alkan et al., 2013). Quails embryonic development, hatching, and survival can be influenced by several factors such as storage of the egg, turning of the egg, relative humidity, but it is to a large extent dependent on temperature (Wilson, 1990; Romao et al., 2009). The chicken embryo is poikilothermic and its development requires incubation temperature, which is defined as 37.5°C to 37.8°C (Wilson, 1990; Romao et al., 2009; Abuoghaba, 2017). The changes in the incubation temperature affect the size of the embryo, growth of the body organs, physiological process, metabolic performance, and hatchability (Yalcin and Siegel 2003). Optimum incubation temperature determination achieves maximum bird growth and hatchability, which linked with commercial production (Christensen et al., 2003; Boleli et al., 2016). Incubation temperature

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influences the birds' thermoregulation system after hatching phase; changes in the incubation temperature either low or high temperatures enhance the embryo capacity and improve the birds' thermotolerance after hatching against the cold or hot environments, respectively (Yalcin and Siegel 2003; Alkan et al., 2013). The thermoregulatory systems including peripheral and central nervous systems are affected with the incubation temperature manipulations (Tzschentke, 2008). Few studies from other researchers in the early days of incubation were used for the application of thermal stress. In bobwhite quails, the early and middle incubation periods are the critical periods in embryo body function development comparing with the late incubation period (Reyna, 2019).

The aim of the following study is to observe the effect of the short-term continuous and intermittent thermal stress during the early embryogenesis (E4–6 d) on embryonic development, hatchability, physiological body reaction, chick quality, and performance until slaughter age in quail chicks. Furthermore, the study observes if female white quails are sensitive to prenatal thermal manipulations.

MATERIALS AND METHODS

The experiment was conducted at the experimental Poultry Farm, Poultry Production Department, Agriculture Faculty, Sohag University, Sohag, Egypt.

Egg Incubation

A total of 540 freshly fertilized white quail eggs were collected from parent flock at 18 wk of age, which were reared under Sohag governorate, Sohag, Egypt. All eggs were divided into 3 groups (control, continuous, and intermittent thermal stress); each group (n = 180) consisted of 6 replicates of 30 eggs. In the first group (control), eggs were incubated under the normal incubation conditions (37.5°C and 50–55% RH) from day 0 till hatching. In the second group (continuous thermal stress [CTS]), eggs were daily exposed to 39.5°C and 50 to 55% during the early embryogenesis for 3 successive days (E4–E6) for 3 h (12:00–15:00). In the third group (intermittent thermal stress [ITS]), eggs were daily exposed to 39.5°C and 50 to 55% during the early embryogenesis for 90 min (12:00–13:30) in separate incubator at the same temperature and relative humidity, then temperature was returned to 37.5°C for 60 min (13:30–14:30) after that the temperature was raised again for 39.5°C for 90 min (14:30–16:00) daily for 3 successive days (E4–E6).

The eggs were turned automatically every 2 h. Embryonic day 0 (E0) was that day when the eggs were assigned in the incubator. The eggs were regularly observed to verify its hatching; the unhatched eggs were examined to detect the rate of embryonic mortality. All hatched chick weights were individually recorded at the hatch and 35 d old.

Studied Variables

Egg Weight Loss (Amount of Moisture Loss) The amount of water loss was determined as the difference in the weight of egg before assigning in the incubator and on the sixth day of incubation. The egg weight loss is represented as the initial egg weight percentage. Unfertile eggs and eggs with dead embryos were not included in the percentage egg weight loss calculation (Aygun and Sert, 2013).

Embryonic Mortality, Hatchability, and Duration of Incubation At the end of experiment, the unhatched eggs were broken to determine the embryonic mortality percentages during the early incubation (E0–E6), middle incubation (E7–E14), and late incubation (E15–E17) by using the following equation: (dead embryos number/whole viable egg numbers) × 100, (the rate of chick culled was calculated as abnormal chicks number/whole viable chicks number at hatch) × 100. At hatching, the rates of hatchability were expressed as fertile egg percentages as described previously (Molenaar et al., 2011). The incubation time was determined as the number of hours from setting eggs in incubator up to hatch.

Physiological Body Reactions About 90 newly hatched quails (3 groups × 6 replicates × 5 quails) were used to determine physiological body reaction. An infrared thermometer was used to measure the temperatures of the wing, the shank, the head, and the back of chicks to calculate the body surface temperature (BST [°C]) in accordance with the following equation: BST (°C) = (0.12 × wing T) + (0.15 × shank T) + (0.03 × head T) + (0.70 × back T) as described by Richards (1971). A digital thermometer was used to measure the chicks' cloacal temperatures (°C) by inserting it 1 cm deep into the chick cloaca.

Productive Traits All hatched chicks, 444 chicks (153 in the first group, 144 in the second group and 147 in the third group) were weighed on the first day of the growing period and placed in floor wooden pens up to 21 d. At 21 d of age, 270 quails were kept in the battery cages up to 40 d of age (3 groups × 6 replicates × 15 birds). All chicks were fed a standard broiler starter ration (22.52% crude protein (CP) and metabolizable energy (ME) 12.78 MJ kg^{-1} of diet) from days 1 to 14, a grower diet (22.12% CP and ME 14.14 MJ kg^{-1} of diet) from days 15 to 28, and finally a finisher diet (21.0% CP and ME 13.56 MJ kg^{-1} of diet) from days 28 to 40. The feed and water were offered *ad libitum* during the experiment. The chicks were exposed to 23 h of light and 1 h of darkness (30–40 lux m^2) during the experiment. The number of chicks that had died was recorded daily, and the mortality rate was calculated. All chicks were weighed at hatch (initial) and 5 wk of age, whereas daily weight gain was measured in accordance with the following equation (the final body weight − initial body weight) divided by the number of days during the experimental period. Feed consumption (g) was weekly measured during the experiment, whereas feed conversion ratio was calculated for all periods by dividing feed consumption (g) by body weight gain (g)

of chicks. The quail chicks were considered sexually mature when the females attained 5% egg production level (Oguz et al., 2001).

Slaughter Traits At day 35 of the experiment, 36 female quails (3 groups × 6 replicates × 2 quails) were weighed and slaughtered by cervical dislocation to measure the percentages of blood, feather, legs, head, carcass, intestine, liver, gizzard, heart, spleen, ovary weight, and oviduct weight to life body weight.

Hematological Parameters At 35 d of age, 36 blood samples were collected from birds (3 groups × 6 replicates × 2 chicks) into heparinized and nonheparinized tubes for hematological analysis. Blood samples were centrifuged at 3,000 rpm for 15 min to obtain plasma for the colorimetric estimation of total protein, albumin, globulin according to Trinder (1969) and triiodothyronine (T3) hormone concentration determined by commercial kits. A hematological index analysis including hemoglobin concentration was spectrophotometric determined; red blood cell (RBC/10^6) number was counted using a hemocytometer as described previously by Schalm et al. (1975). Total white blood cells (WBC/10^3) and differential WBC counts as well as the heterophils-to-lymphocyte ratio were estimated as described by procedures described by Gross and Siegel (1983) and Parga et al. (2001).

Follicles Development At 35 d of age, in female quails, the oviducts and ovaries were removed and weighed to the nearest gram (g) by using a digital balance, each hierarchy of large yellow follicles were removed from the ovaries and classified by their sizes to F1, F2, F3, F4, and F5 (Kashmiri and Samples, 2011). All follicles were weighed to the nearest gram (g) and their diameters were measured across the stigma to the nearest centimeter (cm) using a digital caliper.

Statistical Analysis

The obtained data were analyzed using a general linear model of SAS (SAS Institute 2004 (SAS, 2004)) by applying the following linear model: Yij = μ +Ti + eij, where Yij = observation measured, μ = overall mean, Ti = effect of thermal stress (1, 2, and 3), Eij = random error component was normally distributed assumed. The differences between least squares means were tested at the level of $P < 0.05$ using Duncan's multiple range test (Duncan, 1955).

RESULTS

Impact of continuous and intermittent thermal stress during incubation period on the relative water loss and chick weight are illustrated in Table 1.

The obtained results revealed that the lowest ($P \leq 0.05$) egg weight (at day sixth of incubation) and chick weight at hatch were obtained in the CTS group. The decrease in egg weight and chick weight under the CTS group are associated with the highest ($P \leq 0.05$) relative water loss. The cloacal temperatures of the chicks in the CTS and ITS groups (40.15°C and 40.08°C, respectively) significantly ($P \leq 0.001$) increased compared with those (39.80°C) of the control group. The chicks body surface temperature in the CTS and ITS groups (33.12°C and 32.91°C, respectively), also increased significantly ($P \leq 0.05$) compared with the control group (32.12°C).

Impact of continuous and intermittent thermal stress during incubation period on the embryonic mortality and hatchability are presented in Table 2.

The results showed significant effects ($P \leq 0.05$) of continuous and intermitted incubation temperatures on middle embryonic mortality and the highest mortality rate was observed in embryos from eggs exposed to the CTS group. However, there are no significant differences in early and late embryonic mortalities among treatments.

Impact of continuous and intermittent thermal stress on the productive performance are presented in Table 3.

The results at 12 h after hatch revealed that initial chick weight in the study groups insignificantly changed and that could be explained due to compensatory growth of the control and ITS group embryos during incubation. The body weights at 5 wk, total weight gain (g), and daily weight gain in the CTS groups were significantly decreased compared with the control and intermittent groups. The early onset of maturity was observed in the chicks of the CTS group as well as the low body weight at sexual maturity was found in the CTS and ITS groups, and the lowest body weight was recorded in the CTS group. The weight of the first egg obtained from the study groups was insignificantly affected.

Impact of continuous and intermittent thermal stress on carcass characteristics are illustrated in Table 4.

The results revealed that the living body weight and the percentages of the liver, gizzard, and heart were

Table 1. Impact of continuous and intermittent thermal stress on relative water loss, chick weight, and physiological body reactions of quails at hatch.

Traits	First group (control)	Second group (CTS/39.5°C)	Third group (ITS/39.5°C)	SEM	*P*-value
Initial egg weight (g)	11.83[a]	11.70[a]	11.93[a]	0.097	0.2683
Egg weight/6 d (g)	11.26[a,b]	11.00[b]	11.31[a]	0.099	0.0546
Relative water loss (RWL%)	4.81[b]	5.983[a]	5.196[a,b]	0.299	0.0151
Chick weight at hatch (g)	8.15[a]	7.87[b]	8.12[a]	0.082	0.0448
Relative chick weight (%)	72.62[a]	72.23[a]	72.56[a]	0.846	0.9401
Cloacal temperature (CLT/°C)	39.80[b]	40.15[a]	40.08[a]	0.105	0.0538
Body surface temperature (BST/°C)	32.18[b]	33.12[a]	32.91[a]	0.114	0.0001

[a,b]Means with different superscripts in the same row are significantly different ($P \leq 0.05$).
Abbreviations: CTS, continuous thermal stress; ITS, intermittent thermal stress.

Table 2. Impact of continuous and intermittent thermal stress on embryonic mortality and hatchability.

Traits	First group (control)	Second group (CTS/39.5°C)	Third group (ITS/39.5°C)	SEM	*P*-value
Embryonic mortality rate					
0–6 d	2.12[a]	2.73[a]	2.50[a]	0.273	0.3056
7–14 d	2.65[b]	3.53[a,b]	3.01[a]	0.267	0.0964
14–17 d	3.77[a]	4.79[a]	4.35[a]	0.391	0.2094
Dead after piping (%)	3.12[a]	4.03[a]	3.57[a]	0.304	0.1377
Chick cull rate (%)	1.32[b]	2.29[a]	2.14[a]	0.142	0.0005
Hatchability (%)	87.02[a]	82.62[b]	84.43[b]	0.622	0.0006
Incubation time (h)	395.33	388.66	391.83	6.704	0.7840
TEPH (H)	15.50[a]	11.92[b]	12.67[b]	0.659	0.0039

[a,b]Means with different superscripts in the same row are significantly different ($P \leq 0.05$).
Abbreviations: CTS, continuous thermal stress; ITS, intermittent thermal stress; TEPH, time between external piping and hatching.

significantly lower ($P \leq 0.05$) in the CTS and ITS groups than in the control group. The percentages of the blood, legs, head, and carcass, intestine, and spleen were insignificantly affected by thermal manipulation among the study groups.

Impact of continuous and intermittent thermal stress on blood proteins, hematological parameters, and T3 hormone concentrations of quails are presented in Table 5.

The total protein, albumin, and globulin levels in the CTS and ITS groups were insignificantly influenced compared with the control group. The lowest concentration of the T3 hormone ($\mu g\ L^{-1}$) for quails was found in the CTS group, whereas the highest value was recorded in the control group ($P \leq 0.05$). The globulin level, lymphocytes (%), and WBC (10^3) of quails produced from eggs exposed to thermal stress during the early incubation period were significantly ($P \leq 0.05$) reduced compared with the control. the corticosterone level increased in response to heat stress which caused reduction in the total leucocyte count. Also, there are higher heterophils (%) in relation to the other leukocytes. However, eosinophils (%) in thermal-treated groups were insignificantly reduced in comparison with the control group. A no significant reduction of monocyte (%) was observed. A nonsignificant increase in RBC count (10^6) was observed in the CTS and ITS groups, whereas hemoglobin ($g\ dL^{-1}$), eosinophils (%), basophils (%), and heterophils-to-lymphocyte ratio were not affected among treated groups.

The impact of continuous and intermittent thermal stress on ovary weight and follicles development is presented in Table 6.

In all groups at 5 wk old, females are displaying normally appearing ovary, as well as the ovary weight (g), ovary (%), oviduct weight (g), and oviduct (%) are nonsignificantly decreased in the CTS and ITS groups compared with the control group. The highest follicles weights (first, second, third, fourth, and fifth) were observed in the control group and the lowest corresponding weights were found in the CTS group. The first, second, third, and fourth follicle diameters in all groups were not affected only the fifth follicle (known as the large follicle) diameter were significantly decreased in the thermal-treated groups.

DISCUSSION

The obtained results indicated that both thermal-treated groups induced acceleration in embryo development which associated with increase embryo metabolic rate and water loss, although embryos exposed to continuous thermal treatments were significantly less developed than the control and ITS groups. It was reported that the high incubation temperature resulted in increases in the evaporation of water and embryo metabolic rate (Walstra et al., 2010; Abuoghaba, 2017). Eggs exposed to continuous thermal treatments showed a significant decrease in egg weight and chick weight as well as a significant increase in water loss with no change with relative chick weight compared with the control and ITS groups. The decreased chick weight at hatch in the CTS group compared with other groups could be attributed to increased relative water loss than those of the other groups. The high incubation temperature

Table 3. Impact of continuous and intermittent thermal stress on the productive performance of quails.

Traits	First group (control)	Second group (CTS/39.5°C)	Third group (ITS/39.5°C)	SEM	*P*-value
Chick weight at hatch (g)	8.13[a]	7.97[a]	8.19[a]	0.104	0.3288
Body weight (5 wk)	261.58[a]	247.10[b]	256.57[a]	2.154	0.0001
Total weight gain (g)	253.45[a]	239.14[b]	248.38[a]	2.157	0.0001
Daily weight gain (g)	7.24[a]	6.83[b]	7.10[a]	0.062	0.0001
Total feed intake (g)	783.81[a]	747.96[b]	760.96[c]	2.875	0.0001
Daily feed intake (g)	22.39[a]	21.37[c]	21.74[b]	0.082	0.0001
Feed conversion ratio	3.11[a]	3.16[a]	3.10[a]	0.033	0.4153
ASM (d)	36.05[a]	34.75[b]	35.112[a]	0.314	0.0174
BWSM (g)	281.56[a]	267.89[b]	275.73[a,b]	3.261	0.0198
Weight of first egg (g)	10.48[a]	9.80[a]	9.90[a]	0.3548	0.3485

[a,b]Means with different superscripts in the same row are significantly different ($P \leq 0.05$).
Abbreviations: ASM (d), age at sexual maturity; BWSM (g), body weight at sexual maturity; CTS, continuous thermal stress; CW (g), chick weight; ITS, intermittent thermal stress.

Table 4. Impact of continuous and intermittent thermal stress on carcass characteristics of female quails.

Traits	First group (control)	Second group (CTS/39.5°C)	Third group (ITS/39.5°C)	SEM	*P*-value
Life body weight (g)	276.72[a]	249.67[b]	262.89[a,b]	5.68	0.0082
Blood (%)	2.59	2.78	2.44	0.27	0.6723
Feather (%)	6.55	7.99	6.78	0.49	0.0996
Legs (%)	2.45	2.44	2.48	0.08	0.9263
Head (%)	4.94	4.70	4.53	0.15	0.1728
Carcass (%)	69.76	66.85	67.75	1.69	0.4685
Intestine (%)	4.25	4.09	4.20	0.19	0.8357
Liver (%)	4.98[a]	4.06[b]	4.14[a,b]	0.30	0.0521
Gizzard (%)	2.65[a]	2.28[b]	2.37[a,b]	0.11	0.0516
Heart (%)	0.959[a]	0.771[b]	0.803[b]	0.043	0.0106
Spleen (%)	0.097	0.084	0.093	0.005	0.1580

[a,b]Means with different superscripts in the same row are significantly different ($P \leq 0.05$). All percentages to life body weight.
Abbreviations: CTS, continuous thermal stress; ITS, intermittent thermal stress.

resulted in increases in the evaporation of water and embryo metabolic rate (Walstra et al., 2010; Abuoghaba, 2017). In addition, the increased chick weights in the control group may be due to the increased yolk sac uptake of the embryos into the abdomen, which provides their nutrients requirements during the first few days of the chick life (Meijerhof, 2009; Abuoghaba, 2017). Similarly, the higher chick and yolk sac weights at 12 h after hatch were in the control group, whereas the lower corresponding values were recorded in the chronic high group (Sozcu and Ipek, 2015). In addition, the increased cloacal and body surface temperatures may be due to the deleterious effects of the high incubation temperature on the chicks, which reflect by increasing the chicks physiological body reactions. Similar results were also found by Abuoghaba (2017), who found that the broilers body surface and cloacal temperatures in the chronic group (40°C) significantly ($P \leq 0.05$) increased compared with those of the control (37.5°C).

Recent studies documented that increase the temperature during early, middle, and late embryogenesis had a negative impact on mortality and hatchability in bobwhite quails (Reyna, 2019). Similar findings found by Walstra et al. (2010) in the broiler exposed to thermal stress reflect the broiler hatchability. However, the previous report by Alkan et al. (2013) showed no significant changes in embryonic mortality and hatchability percentages in quails exposed to thermal manipulation during early and late embryogenesis. Raising incubation temperature result in increasing the mortality rates due to insufficient egg heat loss and egg water loss which leads to hemostasis disturbance and dehydration, respectively (Ono et al., 1994). Previous studies applied in quails found that the application of thermal stress reflected in the loss of the egg weight (Aygun and Sert 2012, 2013). The loss of water also resulted in a decrease in the hatchability rate in broiler chickens (Morita et al., 2009). Furthermore, the high temperature leads to ascites development which increased the embryonic mortality rate (Balog et al., 2003). Thus, the increased embryonic mortality rate in the CTS group could be accepted. The lowest hatchability in the CTS and ITS groups may be due to the higher water loss and insufficient egg contents required for embryonic development. This finding agreed with the present study, which recorded significant decrease in hatchability rate at temperatures higher than that of the control for broiler chickens (Morita et al., 2009). The obtained findings showed that the embryos in the thermal stress groups insignificantly hatch early as compared with the control group. These results disagreed with Sgavioli et al. (2015). No significant changes were found in the

Table 5. Impact of continuous and intermittent thermal stress on blood proteins, hematological parameters, and T3 hormone concentrations of female quails.

Traits	First group (control)	Second group (CTS/39.5°C)	Third group (ITS/39.5°C)	SEM	*P*-value
Total protein (mg/dL)	4.651	4.108	4.245	0.1848	0.4542
Albumin (mg/dL)	2.902	2.778	2.819	0.1956	0.9023
Globulin (mg/dL)	1.749[a]	1.330[b]	1.426[b]	0.110	0.0276
A/G ratio	1.730	2.230	2.195	0.225	0.8063
T3 (μg L^{-1})	82.01[a]	65.67[b]	70.41[a,b]	4.432	0.0385
Hemoglobin (g/dL)	11.31	9.63	10.36	0.5799	0.2195
Heterophils (%)	51.28[b]	54.67[a]	53.67[a]	0.624	0.0017
Lymphocytes (%)	37.01[a]	34.00[b]	35.08[b]	0.543	0.0016
Eosinophils (%)	3.29	3.160	3.119	0.2113	0.2470
Monocytes (%)	3.222	3.146	3.066	0.214	0.8892
Basophils (%)	5.187	5.133	5.166	0.7178	0.8768
H/L ratio	1.386	1.618	1.534	0.0351	0.1287
WBC (10^3)	26.416[a]	23.333[b]	24.666[a,b]	0.853	0.0500
RBC (10^6)	2.935	3.077	3.010	0.227	0.9065

[a,b]Means with different superscripts in the same row are significantly different ($P \leq 0.05$).
Abbreviations: CTS, continuous thermal stress; H/L, heterophils-to-lymphocyte; ITS, intermittent thermal stress; RBC, red blood cell; T3, triiodothyronine; WBC, white blood cell.

Table 6. Impact of continuous and intermittent thermal stress on ovary weight, follicle development of female quails.

Traits	First group (control)	Second group (CTS/39.5°C)	Third group (ITS/39.5°C)	SEM	*P*-value
Life body weight (g)	287.43[a]	258.17[c]	272.85[b]	4.024	0.0001
Ovary weight (g)	6.75	4.55	4.94	0.785	0.1215
Ovary (%)	2.37	1.77	1.76	0.274	0.2135
Oviduct weight (g)	7.41	4.50	5.43	1.111	0.1846
Oviduct (%)	2.60	1.74	1.92	0.390	0.2730
Follicle weights (g)					
First	1.67[a]	1.35[a,b]	1.04[b]	0.198	0.0957
Second	1.50[a]	0.98[b]	0.87[b]	0.153	0.0142
Third	1.30[a]	0.82[b]	0.77[b]	0.154	0.0382
Fourth	0.95[a]	0.54[b]	0.59[b]	0.105	0.0191
Fifth	0.58[a]	0.35[b]	0.38[b]	0.064	0.0334
Follicles diameters (cm)					
First	1.15	0.94	0.98	0.097	0.2887
Second	1.02	0.75	0.80	0.099	0.1446
Third	0.91	0.65	0.72	0.102	0.1958
Fourth	0.75	0.52	0.66	0.078	0.1447
Fifth	0.57[a]	0.34[b]	0.52[a,b]	0.067	0.0456

[a–c]Means with different superscripts in the same row are significantly different ($P \leq 0.05$).

Abbreviations: ASM (d), age at sexual maturity; BWSM (g), BW at sexual maturity; CTS, continuous thermal stress; ITS, intermittent thermal stress.

incubation period and the percentage of the dead after piping between the groups. In addition, chick cull was significantly increased ($P \leq 0.05$) in the thermally treated groups. In addition, the time between external piping and hatching and hatchability percentages were significantly decreased ($P \leq 0.05$) by incubation thermal manipulation compared with the control group. These results are in agreement with results obtained by Reyna (2019).

That initial chick weight in the study groups insignificantly changed and that could be explained due to compensatory growth of the control and ITS group embryos during incubation. These results agree with the results obtained by Reyna and Burggren (2012) and Reyna (2019). The body weights at 5 wk, total weight gain (g), daily weight gain in the CTS groups were significantly decreased compared with the control and intermittent groups. This reduction could be attributed to the lowest feed intake; these results may be related to atrophy of digestive organs including the gizzard, intestine, and liver as their weights were significantly decreased in response to heat stress. In harmony with the obtained findings, the higher chick weight was in the control group, whereas the lower chick weight was obtained in the thermal group (Guo et al., 1998). By contrast, the thermal manipulation influences the quail's chick body weight at 35 d old and the highest body weight in the quail exposed to high temperature during the early embryogenesis (Alkan et al., 2013).

The early onset of maturity was observed in the chicks of the CTS group as well as the low body weight at sexual maturity was found in the CTS and ITS groups, and the lowest body weight was recorded in the CTS group. This results could be attributed to the disturbance in the thyroid hormone level in the thermally treated groups as the thyroid involved in regulating the onset of puberty in birds and the disturbance in its activity reflects on the bird's reproductive performance (Elnagar et al., 2010; Lara and Rostagno, 2013).

The living body weight and the percentages of the liver, gizzard, and heart were significantly lower ($P \leq 0.05$) in the CTS and ITS groups than in the control group. The results confirm the previous findings from embryonic exposure of quail to thermal manipulations (Morita et al., 2009). Such a reduction effect on the heart weight at 5 wk of age in the CTS group may be due to the anomalous development of the cardiovascular system (Leksrisompong et al., 2007). Furthermore, the liver percentage reduction may be due to retarded liver development (Balog et al., 2003). In accordance with the present finding the gastrointestinal tract development in birds has a major role in the growth of chick after hatching (Palo et al., 1995a, 1995b).

The lowest concentration of the T3 hormone for quails was found in the CTS group, whereas the highest value was recorded in the control group ($P \leq 0.05$). The findings are in agreement with those which explained that the exposure to chronic heat stress resulted in a decrease of T3 levels (Badran et al., 2012). Similarly, exposing incubated eggs to high incubation temperature resulted in the reduction of the T3 level of broiler hatched chicks (Abuoghaba, 2017).

The globulin level, lymphocytes (%), and WBC (10^3) of quails produced from eggs exposed to thermal stress during the early incubation period were significantly ($P \leq 0.05$) reduced compared with the control. These results could be explained by the atrophy of lymphoid organs as the liver weight was significantly reduced by thermal stress and these results are in accordance with those of McFarlane and Curtis (1989); in addition, the corticosterone level increased in response to heat stress which caused reduction in the total leucocyte count. Also, there are higher heterophils (%) in relation to the other leukocytes; Maxwell and Robertson (1998) mentioned that the moderate and high stress leads to increase in the heterophils (%). However, eosinophils (%) in thermal-treated groups were insignificantly reduced in comparison with the control group. Similar results

indicated that the exposure young chicks 10 to 17 d old to hot environment resulted in an insignificant decrease in eosinophils percentage (McFarlane and Curtis, 1989; Altan et al., 2000). A no significant reduction of monocyte (%) was observed; however, the exposing broilers to heat stress caused a significant decrease in monocyte (Altan et al., 2000).

The results showed that there are negative effects on the studied female reproductive organs after treatment with continuous and intermittent temperatures. In accordance, heat stress affects female reproductive organ development in quail embryos. The highest follicles weights (first, second, third, fourth, and fifth) were observed in the control group and the lowest corresponding weights were found in the CTS group. The first, second, third, and fourth follicle diameters in all the groups were not affected only the fifth follicle (known as the large follicle) diameter were significantly decreased in the thermal-treated groups. The results of heat stimulation on female reproductive organ development in the present study confirm the negative effects of heat stress on the bird's reproduction performance. As described previously by Etches et al. (2008), the impairments in a domestic bird's reproductive performance resulted from the exposure to the heat stress and this could be explained by the negative effects of the heat in the gonadotropin-releasing hormones content and the circulating luteinizing hormone level (Donoghue et al., 1989).

CONCLUSION

From these findings could be summarized as follows:

(i) There were significant adverse effects from continuous and intermittent thermal stress (39.5°C) during embryogenesis on relative water loss, embryonic mortality, and hatchability.
(ii) Cloacal temperature (°C) and BST (°C) in the treated thermal stress significantly increased compared with the control group.
(iii) Exposing incubated eggs to continuous thermal stress significantly decreased body weight (5 wk), weight gain (g), age, and body weight at sexual maturity compared with the intermittent and control group.

Thus, from these results, it could be noted that using continuous thermal stress during the early incubation period significantly affects hatchability, T3 hormone, physiological body reactions, and follicle weights of quails compared with other groups.

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This study was performed at Experimental Poultry Farm, Poultry production Department, Faculty of Agriculture, Sohag University, Egypt.

Ethics Statement: The study was carried out according to Sohag University Ethics Committee for the Care and Using Experimental Animals. All quails were monitored 3 times daily during experimental period to detect any signs of stress and suffering birds. Humane endpoints could not be used due to the continuous severity assessment within the procedure of the study showed that all aspects of the study do not cause quails severe pain or suffering or lasting harm.

DISCLOSURES

The authors certify that the material discussed in the article was not conflicting of interest with any financial organization.

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