

Physiological responses of broiler embryos to in ovo implantation of temperature transponders^{1,2}

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ABSTRACT Previous techniques that have been used to monitor broiler embryonic temperature and metabolism during incubation either have been invasive to the embryo or have not directly determined internal egg temperature. Therefore, the current investigation was conducted to determine the earliest day of incubation on which temperature transponder implantation in the egg air cell could be performed with minimal invasiveness to broiler embryogenesis. Eggs from young broiler breeder hens (Ross 308; 27 wk of age) were weighed and set on 3 tray levels (approximately 120 eggs per tray level) of a single incubator. Randomly, on each tray level and on each day between d 10 and 14 of incubation, 4 embryonated eggs were assigned to an invasive control (IC) treatment (shell perforation only) and 4 other embryonated eggs were assigned to a transponder implantation (TI) treatment (shell perforation, followed by transponder implantation in the air cell). Four embryonated eggs per tray were also assigned to a noninvasive control treatment on d 10 of incubation.

Percentage of embryo survivability through d 18 of incubation, and percentages of mean daily incubational egg weight loss between d 18 and each respective day of implantation were calculated. Furthermore, various internal organ and tissue parameters were examined on d 18 of incubation. Embryo survivability was reduced because of transponder implantation on d 10 and 11 of incubation; however, embryo survivability was not different between eggs in the noninvasive control and IC treatments and the eggs that received the TI treatment on d 12, 13, and 14 of incubation. In comparison with eggs in the IC treatment, those in the TI treatment had a higher percentage of embryo weight. Conversely, yolk sac moisture was significantly higher in IC-treated eggs than in TI-treated eggs. It was concluded that temperature transponders may be inserted into the air cells of broiler hatching eggs between d 12 and 14 of incubation without adversely affecting eggshell porosity or embryogenesis and without causing physiological stress to growing broiler embryos.

Key words: broiler, egg, embryogenesis, temperature, transponder

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INTRODUCTION

Incubation temperature has a major influence on avian embryonic temperature (French, 1997), with subsequent influences on embryonic heat production (Janke et al., 2002) and embryonic development, hatchability, and posthatch performance (Wilson, 1991). Lourens et al. (2005) noted specific relationships between broiler embryo temperature and development, and subsequent

hatchability and posthatch performance. Therefore, accurately monitoring the temperature of broiler embryos in response to incubation temperature can be used as a management tool to modify incubation conditions to optimize embryogenesis and posthatch broiler performance (Bamelis et al., 2005).

Internal egg temperature has been used as measure of chick embryo heat production and body temperature (Janke et al., 2004). Researchers have previously used various methods to determine internal egg or embryo temperature during incubation (Turner, 1990; Janke et al., 2004; Lourens et al., 2006a,b; Renema et al., 2006). Thermistors or thermocouples (connected to data loggers) attached to the eggshell (Lourens et al., 2005) or implanted in the allantoic fluid (Janke et al., 2004), in the yolk sac (Renema et al., 2006), or inside the egg contents (Turner, 1990) have been used. However, those methods have exhibited certain limitations. Among these limitations are physiological invasiveness, increased egg

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contamination (Turner, 1990), interference with the egg turning mechanism (Lourens et al., 2006a,b), altered embryo metabolism (Janke et al., 2004), and increased embryonic mortality (Janke et al., 2004). Conversely, preliminary trials in our laboratory (unpublished data) suggest that temperature transponder implantation in the air cells of embryonated broiler hatching eggs and the subsequent closure of the eggshell perforation with a sealant can be safely performed. However, the potential harm that may be caused by such implantation to embryonic metabolism, development, and hydration status have yet to be studied in detail. These responses may be assessed by examining the relative weights and moisture contents of embryos and their supply (liver and heart), storage (yolk sac), and demand (pipping muscle) organs (Christensen et al., 2002). Furthermore, the embryonic stress response resulting from the continued presence of transponders in the egg air cells may be assessed by examining the relative weights of lymphoid organs such as the spleen and bursa of Fabricius (Glick, 1967; Puvadolpirod and Thaxton, 2000a,b,c).

A methodology is needed that would allow for the practical assessment of broiler embryo temperature with confirmed minimal physiological invasiveness. Therefore, the objective of the current research was to examine the effects of the timing of temperature transponder implantation in the air cells of broiler hatching eggs, and the subsequent closure of the site of perforation by a porous sealant on consecutive days during the second week of incubation, on the physiological responses of embryos. To this end, various physiological parameters of embryos, including those described above, were assessed to determine the relative invasiveness of the implantation procedure and to further determine a period in incubation during which optimal embryonic development can occur in implanted eggs.

MATERIALS AND METHODS

General

Approximately 720 broiler hatching eggs were collected from a 27-wk-old Ross 308 breeder flock. The eggs were held under standard storage conditions for 3 d before being set. Eggs that were contaminated, misshaped, cracked, or with malformed shells were discarded from the experiment. Moreover, eggs with weights that were not within 10% of the mean for all 720 eggs were discarded. On d 0, eggs were randomly labeled and weighed, and at least 120 eggs were set on each of the 3 tray levels of an NOM 45 incubator (NatureForm Inc., Jacksonville, FL). The eggs were incubated under standard commercial conditions at 28.8°C wet bulb and 37.5°C dry bulb temperatures.

On d 10 of incubation, the eggs were candled, and those not containing live embryos or that contained slanted air cells were discarded. Randomly, on each tray level and on each day between 10 and 14 d of incuba-

tion, 4 embryonated eggs were assigned to an invasive control (**IC**) treatment (shell perforation only), and 4 other embryonated eggs were assigned to a transponder implantation (**TI**) treatment (shell perforation, followed by transponder implantation in the air cell). In addition, 4 embryonated eggs per tray were assigned to a noninvasive control (**NC**) treatment on d 10 of incubation.

Implantation Procedure

On each day of implantation, eggs were weighed and candled. The eggshell surface area surrounding the site of eggshell perforation (2 cm in diameter) was disinfected with 70% isopropyl alcohol. The eggshell of each of the live embryonated treatment (**IC** and **TI**) eggs was then perforated (3-mm-diameter hole) at a point offset approximately 1 cm from the center of the large end of the egg. In each of the **TI**-treated eggs, a sterile transponder was aseptically inserted into its air cell without disrupting the air cell membrane overlaying the embryo. Subsequently, the perforation was covered with a sterile sealant (8-hydroxyquinoline and pyroxylin solution; New-Skin Liquid Bandage, Prestige Brands Inc., Irvington, NY) that is porous and allows for gaseous exchange. Egg weight (**EW**) was determined before and after implantation and sealant application to allow for the deduction of both transponder and sealant weights from the observed **EW** for accurate determination of percentage **EW** loss for the entire 0 to 18 d incubational period and after each day of implantation through d 18 of incubation.

Transponders

Each implantable, programmable temperature transponder (IPTT-300, Bio Medic Data Systems, Inc., Seaford, DE) was 14 mm long and 2 mm in diameter and was precalibrated by the manufacturer for an accuracy of $\pm 0.1^\circ\text{C}$. Temperature readings could be detected within 5 cm of each transponder using a handheld reader (DAS-6006/7 Smart Probe, Bio Medic Data Systems Inc.). The transponder readings could subsequently be transferred to the spreadsheet of a computer by using a wireless adaptor.

Data Collection and Sample Analyses

On d 18 of incubation (i.e., approximately 24 h before the beginning of the hatching process), the eggs were weighed and broken out to extract embryos and subsequently to record embryo survivability. To confirm that transponders were contained within the air cell of all eggs used for embryo extraction, transponder location was recorded for each **TI**-treated egg. Live embryos extracted from the **NC**-, **IC**-, and **TI**-treated eggs were analyzed to determine weights of the embryo, yolk sac, liver, pipping muscle, heart, spleen, and bursa of

RESULTS

General

Fabricius. Embryos and organs were dried at 85°C for 96 h or until moisture loss ceased, and they were then cooled to room temperature to determine their dry weights as described by Peebles et al. (1998). The wet and dry weights thus obtained were used to determine moisture concentrations $\{\% \text{ moisture} = [(\text{wet weight} - \text{dry weight}) / \text{wet weight}] \times 100\}$ of the yolk sac, liver, pipping muscle, and embryo carcass devoid of the yolk sac, liver, and pipping muscle.

Eggshell respiratory function was determined by calculating the 0- to 18-d percentage of incubational EW loss [PEWL, calculated as (g of EW loss/g of set EW) \times 100; Peebles et al., 2005]. Average daily PEWL (DPEWL) for the 0- to 18-d incubation period, and PEWL and DPEWL in the 10- to 18-d, 11- to 18-d, 12- to 18-d, 13- to 18-d, and 14- to 18-d incubation periods were also calculated. Furthermore, relative yolk sac-free embryo weight (EMW) and yolk sac weight (percentages of set EW); yolk sac:embryo weight ratio; percentage concentrations of embryo carcass moisture (EM) and yolk sac moisture (YSM); relative weights (percentages of embryo weight devoid of the yolk sac) of the liver, pipping muscle, heart, spleen, and bursa of Fabricius; percentage moisture concentrations of the liver and pipping muscle; and DM:moisture ratios of yolk sac-free embryos (EDM:EM) and yolk sacs (YDM:YM) were determined on d 18 of incubation. In addition, on d 18 of incubation, whole-blood samples were collected from each live embryo for determination of hematocrit, which was expressed as percentage of packed blood cell volume, by using microcapillary tubes that were subsequently centrifuged in a microhematocrit centrifuge and read with a microcapillary reader.

Statistical Analysis

Each egg within each of the treatments (NC, IC, or TI) was considered a replicate unit. The effects of the NC treatment; the IC treatment at 10, 11, 12, 13, and 14 d of incubation; and the TI treatment at 10, 11, 12, 13, and 14 d of incubation on PEWL and DPEWL for the 0- to 18-d incubation period; PEWL and DPEWL in the 10- to 18-d, 11- to 18-d, 12- to 18-d, 13- to 18-d, and 14- to 18-d incubation periods; and d 18 embryo, organ, and hematocrit data were analyzed by 1-way ANOVA, where treatment was designated as a fixed effect and tray as a random effect. In addition, a 2 (IC and TI treatments) \times 5 (day of implantation during incubation) factorial analysis was used to determine the main and interactive effects of implantation and their timing on all the aforementioned parameters, where both day and treatment were designated as fixed effects and tray was designated as a random effect. Least squares means were compared in the event of significant global effects (Steel and Torrie, 1980). All data were analyzed using SAS version 9.1 (SAS Institute, 2003). Global effects and differences among least squares means were considered significant at $P \leq 0.05$.

In approximately 86.7% (52 out of total 60) of the TI-treated eggs on d 18 of incubation, transponders remained within the air cell without penetrating the underlying shell membrane or subsequently entering the egg contents. More important, in these eggs, the transponder implantation did not disrupt the vasculature of the chorioallantoic membrane under the air cell and did not cause contamination of the egg contents.

Percentage of Incubational EW Loss

Factorial analysis between day of implantation (10, 11, 12, 13, or 14 d of incubation) and implantation treatment [IC (shell perforated but transponder not implanted) vs. TI (transponder implanted)] revealed no significant main or interaction effects for implantation treatment and its timing on PEWL or DPEWL in the 10- to 18-d, 11- to 18-d, 12- to 18-d, 13- to 18-d, 14- to 18-d, or 0- to 18-d incubational intervals. Furthermore, 1-way ANOVA revealed no significant differences between the NC treatment group, and the IC and TI treatment groups at 10, 11, 12, 13, and 14 d for these same parameters.

Embryo Parameters

Analysis for the main and interaction effects of day of implantation (10, 11, 12, 13, or 14 d of incubation) and implantation treatment (IC vs. TI) on d 18 percentage of embryo survivability revealed only a significant ($P \leq 0.0008$) main effect attributable to implantation treatment. Percentage of embryo survivability in the IC treatment group was higher than that in the TI treatment group. Percentage of embryo survivability for the IC and TI treatments were 96.7 and 70.0%, respectively (pooled SEM = 4.71%). Percentages of embryo survivability on d 18 of incubation in all 11 treatments, including the NC treatment group and the IC and TI treatment groups on each of d 10, 11, 12, 13, and 14, are provided in Table 1 for reference. Embryo survivability comparisons between these 11 individual treatment groups also revealed significant ($P \leq 0.008$) differences. Survivability was significantly lower in eggs that received implants (TI) on d 11 of incubation in comparison with all other treatment groups, except for those that received implants (TI) on d 10. Furthermore, percentage of embryo survivability in the d 10 TI group was lower than that of all other treatment groups except for the TI treatments on d 11 to 14. Approximately 83.3% embryo survivability was achieved with the TI treatment if applied on d 12, 13, or 14 of incubation. Furthermore, the level of survivability in the TI treatment group on d 12, 13, and 14 was not statistically different from that of the IC treatment (100%) on those same days (Table 1).

Table 1. Percentage survivability of embryos by d 18 of incubation for the following treatments: noninvasive control (NC) treatment, and invasive control (IC) treatment [shell perforation (hole) but transponder not inserted] and transponder implantation (TI) treatment [shell perforation (hole) with transponder inserted in the air cell] on d 10, 11, 12, 13, and 14 of incubation¹

Treatment	Survivability
NC	100.0 ^a
IC on d 10	91.7 ^a
IC on d 11	91.7 ^a
IC on d 12	100.0 ^a
IC on d 13	100.0 ^a
IC on d 14	100.0 ^a
TI on d 10	58.3 ^{bc}
TI on d 11	41.7 ^c
TI on d 12	83.3 ^{ab}
TI on d 13	83.3 ^{ab}
TI on d 14	83.3 ^{ab}
SEM	10.05

^{a-c}Means with no common superscript differ significantly ($P \leq 0.05$).

¹Approximately 12 individual eggs were used for the calculation of each mean for the NC treatment and the IC and TI treatments on each of d 10, 11, 12, 13, and 14 of incubation.

Main or interaction effects involving day of implantation (10, 11, 12, 13, or 14 d of incubation) and implantation treatment (IC vs. TI) were not significant for yolk sac:embryo weight, EMW, and EDM:EM. However, main effects attributable to treatment (IC vs. TI) were significant for EMW ($P \leq 0.009$), YSM ($P \leq 0.02$), and YDM:YM ($P \leq 0.02$) on d 18 of incubation (Table 2). Day 18 EMW and YDM:YM were higher in TI-treated than in IC-treated eggs. Conversely, YSM was lower in TI-treated than in IC-treated eggs. Nonetheless, day of implantation had no effect on EMW, YSM, or YDM:YM. When EMW, YSM, and YDM:YM were compared for all 11 treatment groups, including NC, significant ($P \leq 0.02$) differences between the treatments were found only for EMW (Table 3). No significant differences were found between treatments for YSM or YDM:YM. The EMW in the treatment group that was subjected to TI treatment on d 14 was significantly greater than those in the NC treatment group and in the treatment groups that were subjected to IC treatment on d 10 and 12. In addition, the EMW in the

Table 2. Relative yolk sac-free embryo weight (EMW; % of set egg weight), yolk sac moisture concentration (YSM; %), and yolk sac DM-to-moisture ratio (YDM:YM) on d 18 of incubation for the following treatments: invasive control (IC) treatment [shell perforation (hole) but transponder not inserted] and transponder implantation (TI) treatment [shell perforation (hole) with transponder inserted in the air cell] on d 10, 11, 12, 13, and 14 of incubation¹

Item	IC	TI	SEM
EMW	50.4 ^b	52.0 ^a	0.39
YSM	47.0 ^a	45.6 ^b	0.39
YDM:YM	1.13 ^b	1.20 ^a	0.019

^{a,b}Means within a row with no common superscript differ significantly ($P \leq 0.05$).

¹Approximately 30 individual eggs each in the IC and TI treatment groups were used for the calculation of each treatment mean.

treatment group that received the TI treatment on d 11 and 12 was greater than the EMW in the treatment groups that received the IC treatment on d 13 and 14 and the treatment group that received the TI treatment on d 13 of incubation.

Organ Parameters and Hematocrit

Except for YSM, main or interaction effects involving day of implantation and implantation treatment were not significant for the relative weights of the embryonic organs and their moisture concentrations. Yolk sac moisture concentration on d 18 was higher ($P \leq 0.02$) in eggs that were subjected to the IC treatment in comparison with those that were subjected to the TI treatment. Mean YSM were not significantly different for each of the 11 individual treatment groups.

Main or interaction effects for day of implantation and implantation treatment were not significant for the relative weights of the yolk sac, liver, pipping muscle, heart, spleen, and bursa of Fabricius; hematocrit; and moisture concentrations of the liver, pipping muscle, and bodies of yolk sac-free embryos on d 18 of incubation. Furthermore, for all the aforementioned parameters, differences between the 11 individual treatment groups were not significant.

DISCUSSION

The determination of actual internal egg temperature is essential for the accurate derivation of internal egg water vapor pressure and the subsequent calculation of eggshell water vapor conductance (Ar and Rahn, 1978). An assessment of actual internal egg temperature would also allow for a more precise measure of embryo body temperature for the determination of the relative level

Table 3. Relative yolk free embryo weight (EMW; % of set egg weight) on d 18 of incubation for the following treatments: noninvasive control (NC) treatment, and invasive control (IC) treatment [shell perforation (hole) but transponder not inserted] and transponder implantation (TI) treatment [shell perforation (hole) with transponder inserted in the air cell] on d 10, 11, 12, 13, and 14 of incubation¹

Treatment	EMW	SEM
NC	51.3 ^{bc}	0.69
IC on d 10	50.7 ^{bc}	0.85
IC on d 11	51.8 ^{abc}	0.85
IC on d 12	50.1 ^{bc}	0.79
IC on d 13	49.5 ^c	0.85
IC on d 14	49.7 ^c	0.79
TI on d 10	51.8 ^{abc}	0.85
TI on d 11	52.7 ^{ab}	1.04
TI on d 12	52.1 ^{ab}	0.85
TI on d 13	49.5 ^c	0.85
TI on d 14	53.9 ^a	0.85

^{a-c}Means with no common superscript differ significantly ($P \leq 0.05$).

¹Approximately 9 individual eggs in the NC treatment group and approximately 6 individual eggs each in the IC and TI treatment groups, on each of d 10, 11, 12, 13, and 14 of incubation, were used for the calculation of each treatment mean.

of heat production of the embryo and the subsequent estimation of its metabolic rate. Therefore, a minimally invasive method to assess actual internal egg temperature would be a useful tool in avian research and hatchery management. The implantation of temperature transponders in the air cells of broiler hatching eggs was used in this study as a potential method by which to assess internal egg temperature with minimal physiological stress to the embryo. Evaluations were performed to determine more specifically the effects of transponder implantation between 10 and 14 d of incubation on the physiological properties of the eggshell and on embryo development and survivability.

The current findings showed no significant effects of treatment (NC, IC, and TI) or day of treatment (10, 11, 12, 13, and 14 d of incubation) on PEWL in the 10- to 18-d, 11- to 18-d, 12- to 18-d, 13- to 18-d, and 14- to 18-d incubational time periods or on DPEWL across the entire 0- to 18-d incubational time period. The lack of any treatment effects on PEWL and DPEWL suggests that eggshell porosity and the overall hydration status of the eggs were not affected by eggshell perforation or by the further implantation of transponders. Although the water content of the embryo contributes only approximately 18 to 20% of the total water content of the embryonated egg on d 18 of incubation (Ar, 1991), the NC, IC, and TI treatment groups were not significantly different for EM and EDM:EM, thereby indicating that eggshell perforation and further transponder implantation also did not affect embryo moisture levels. Moreover, the sealant (a liquid bandage) used to cover the 3-mm-diameter hole in the large end of the egg was effective in controlling water loss from the egg so that the hydration status of the whole egg and that of the embryo were preserved. Hematocrit or the packed cell volume of blood has been reported to increase as a result of dehydration (Boyd, 1981). Therefore, the lack of any changes in the hematocrit of embryos on d 18 of incubation in response to shell perforation and transponder implantation provides further evidence that the hydration status of the embryos was undisturbed. Mean values of YSM were significantly lower and YDM:YM were higher in the TI treatment groups compared with the IC treatment groups. However, in this experiment, the YSM were not significantly different between any of the 11 treatment groups, including NC. Furthermore, on d 18 of incubation, the yolk and albumen contribute only 5% and 1.5 to 2.0%, respectively, of the total water content of the egg (Ar, 1991). Therefore, a lower YSM in TI-treated eggs compared with IC-treated eggs would not be expected to have an appreciable influence on the viability or hydration status of the embryo.

When considering various methods for the determination of embryonic body temperature and heat production (Janke et al., 2004), the effects they have on embryonic mortality are a major concern. Increased embryo mortality was a problem that limited the successful use of thermistors in the experiments performed by Janke et al. (2004). Conversely, in the current experiment, d

18 embryo survivability values were not significantly different between the NC and IC treatment groups and the treatment groups that received the TI treatment on d 12, 13, and 14. Lower embryo survivability in the d 10 and 11 TI treatment groups may be attributed to a smaller air cell size and a greater chance of developmental disruption by transponders of the underlying embryo and surrounding extra-embryonic membranes. Moreover, survivability levels in the d 12, 13, and 14 TI treatment groups were numerically higher than those in the d 10 TI treatment group and significantly higher than those in the d 11 TI treatment group, confirming that the transponders could be implanted on 12, 13, or 14 d of incubation in the egg air cells without harming the growing embryo.

Reductions in BW (Puvadolpirod and Thaxton, 2000a,b,c) and size of the immunological organs (Glick, 1967; Puvadolpirod and Thaxton, 2000a,b,c) are the generalized responses of avian species to physiological stress. No consistent change was observed in relative embryo BW on d 18 of incubation in response to the IC or TI treatment or the age at which the treatments were imposed, which suggests that transponder implantation did not influence embryogenesis. Mean values of EMW were significantly higher in the TI treatment groups compared with the IC treatment groups. In addition, the d 14 TI treatment group had a significantly higher EMW on d 18 of incubation than did the NC treatment group. The higher mean EMW for all the TI treatment groups across day of treatment can be attributed to the higher EMW in the TI treatments that were specifically performed on d 11, 12, and 14. It is unclear why implantation caused an increase in EMW because EDM:EM did not differ between the IC and TI treatment groups. However, because a decrease in BW generally occurs in birds in response to stressful conditions (Puvadolpirod and Thaxton, 2000a,b,c), and no decrease in BW was observed in the TI treatment group in comparison with the IC treatment group, it is apparent that no appreciable physiological stress occurred in the embryos of the implanted eggs.

The liver is the center of metabolism in the body, and its optimal growth and functioning are critical for the active supply of various nutrients to tissues such as the pipping muscle, in preparation for the subsequent emergence of the embryo from the eggshell (Pulikanti et al., 2010). Limiting gaseous exchange across the eggshell has been shown to retard growth (Metcalf et al., 1981) and reduce heart and liver development (McCutcheon et al., 1982) in avian embryos. However, on d 18, the lymphoid (spleen and bursa of Fabricius), supply (liver and heart), storage (yolk sac), and demand (pipping muscle) organ weight data were not different among the treatment groups tested in the current study. Therefore, these results confirm that transponder implantation in the air cells of the eggs did not interfere with embryo growth, homeostasis, or the functionality of their vital organ systems. Furthermore, the sealant (a liquid bandage) used for covering the shell perforation

did not significantly interfere with embryonic growth or development of the heart and liver.

In conclusion, temperature transponders may be inserted into the air cells of broiler hatching eggs between d 12 and 14 of incubation, to monitor internal egg temperature and embryo metabolism, without causing eggshell dysfunction or physiological stress, and growth inhibition in broiler embryos. However, further refinement of the shell perforation and transponder implantation procedures before d 12 of incubation may be needed to reduce embryonic mortality.

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