Research Note: Effects of turning and short period of incubation during long-term egg storage on embryonic development and hatchability of eggs from young and old broiler grandparent flocks[1]

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ABSTRACT Longer egg storage times (>7 d) are common in broiler parent and grandparent hatcheries to obtain the requested flock size. However, prolonged storage is known to decrease hatchability. The aim of this study was to investigate the effects of turning and short period of incubation during egg storage (**SPIDES**) for 14 d on the stage of blastoderm development, embryonic mortality, and hatchability of eggs from young and old grandparent flocks. Hatching eggs were obtained from Ross female line grandparent flocks aged 29 wk (young) and 58 wk (old). Eggs were stored at 15°C, and turned 90° 0 or 4 times daily during storage. On day 5 after egg collection, the eggs were either held in the storage room (control) or subjected to SPIDES treatment. The development of the blastoderm in sample eggs was determined immediately after collection on a farm and again after the SPIDES treatment. Each of the 8 subtreatments was tested on 6 replicate trays of 150 eggs (900 eggs per subtreatment) with 7,200 hatching eggs set in a single-stage setter and hatcher for the trial. The stage of blastoderm development was advanced by the old flock, by SPIDES, and by turning 4 times daily during egg storage ($P \leq 0.05$). There was a significant interaction effect of flock age × turning during storage on embryonic development, which suggested that turning advanced the stage of blastoderm development only in eggs from the old flock ($P \leq 0.05$). Eggs from the young flock had a better hatchability than eggs from the old flock ($P \leq 0.05$). Hatchability was increased by turning 4 times/day during the storage period compared with no turning because of a decrease in the percentage of late embryonic mortality ($P \leq 0.05$). SPIDES decreased early and late embryonic mortality as well as the percentage of second-grade chicks ($P \leq 0.05$), which increased the hatchability of fertile eggs at both flock ages ($P \leq 0.05$). The results of this study showed that a combination of turning eggs 4 times daily along with one SPIDES treatment during 14 d of storage resulted in the highest hatchability in both young and old broiler grandparent flocks.

Key words: hatching egg storage, SPIDES, turning, blastoderm, hatchability

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INTRODUCTION

Long-term storage of hatching eggs has been widely investigated over the last 100 yr. It is well documented that hatching egg storage longer than 7 d is associated with poorer hatchability (Fasenko, 2007; Okur et al., 2018) and chick quality (Reijrink et al., 2009; Ipek and Sozcu, 2015), as well as delays in the hatch time (Meir and Ar, 1998; Elibol et al., 2002a; Yildirim, 2005; Dymond et al., 2013).

Several interventions have been shown to compensate for the effect of long storage on hatchability and chick quality. One of the options used is to turn eggs in the egg store. Turning broiler hatching eggs 4 times daily during extended storage improved hatchability of fertile eggs in older flock eggs, but a consistent result was not found for younger flock eggs (Elibol et al., 2002b). Recently, Damaziak et al. (2018) reported that turning of eggs every 12 h during 12-day storage had a positive

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effect on the development of embryos in the initial incubation period, shortening the incubation time, but had no effect on the hatchability of apparent fertile eggs.

Another option investigated in trials performed over many years is the preincubation heating of hatching eggs, which can reduce the detrimental effects of periods of storage longer than 7 d (Fasenko et al., 2001; Reijrink et al., 2009; Gucbilmez et al., 2013). Several recent practical studies with eggs stored for very long periods in primary breeder facilities have examined short period of incubation during egg storage (**SPIDES**) in greater depth to try and establish robust techniques for commercial use (French et al., 2011; Nicholson et al., 2011, 2013) and reported beneficial effects on hatchability and chick quality.

Extended storage is a more common practice to broiler grandparent hatcheries where order patterns may be uneven and egg storage unavoidable. However, most of the previous studies on SPIDES and turning during extended storage have been conducted with the eggs of broiler parent stock flocks, and little is known with eggs of grandparent flocks. Therefore, the objective of the present study was to examine the interaction of turning during storage, SPIDES, and flock age on the embryonic development and mortality and hatchability of grandparent eggs stored for 14 d.

MATERIALS AND METHODS

Experimental Design

The experiment was designed as a 2 × 2 × 2 factorial arrangement with 2 flock ages (29 wk and 58 wk), 2 turning treatments (0 and 4 times/day) during storage, and 2 preincubation heating treatments (no heating-control and SPIDES).

Egg Collection and Storage Conditions

Hatching eggs were obtained from Ross female line grandparent breeder flocks at 29 wk (young) and 58 wk (old) of age. In both flocks, all the eggs were cleared from the nests immediately after the lights were turned on in the houses before egg collection, to ensure that the eggs laid late on the previous day were not included. The freshly collected hatching eggs (a total of 7,358 eggs) from the second collection of the day were stored for 1 d in a farm egg storage room at 17°C and 65-70% RH. The next day, eggs were transported (approximately 2 h) to a hatchery (Aviagen, Ankara, Turkey) in a temperature-controlled vehicle and stored on setter trays in a storage room at a temperature of 15°C and RH of 70 to 75%. The storage duration was 14 d. The eggs were divided at random into 2 groups that were either turned 4 times a day (held in the setter buggies, and the turning angle was 90° as in the setter) or not turned (held parallel to the floor in the setter buggies) during the storage period. The eggs were then either held in the storage room (control) or were subjected to a SPIDES in a Petersime Re-Store (Petersime, Zulte, Belgium) machine on day 5 after egg collection. Thereafter, eggs were again stored in the same storage room as the control eggs. During the SPIDES treatment, the eggshell temperature was over 32°C for 3.5 h and remained at 35°C for 2.5 h. All eggs were held parallel to the floor and were not turned during the SPIDES process. The eggshell temperature was recorded with Tiny Tags (Gemini Data Loggers, West Sussex, England).

Incubation

A total of 7,200 eggs were incubated together in a single incubator and hatcher with a capacity of 57,600 and 19,200 eggs, respectively (PasReform Hatchery Technology, Zeddam, the Netherlands). The rest of the incubator and hatcher were filled with hatching eggs that were not part of the experiment to ensure uniform air flow across the eggs. A single-stage incubation program with a gradually decreasing set-point temperature of 38.1°C at embryonic day (**E**) 1 to 37.5°C at E19 was used. RH was set at 53 ± 2% during the entire incubation process. The eggs were turned once every hour until E19 of incubation. At E19, eggs were transferred to hatcher baskets and placed in a hatcher. The hatcher began at a set point temperature of 37.2°C that was gradually decreased to 36.8°C at E21. The trays representing each flock age, turning treatment, and preincubation heat treatment were distributed throughout all the positions in the setter and hatcher to minimize effects of machine position. Each tray of 150 eggs was considered to be a replicate, and there were 6 replicate trays per subtreatment group and 24 replicate trays per treatment, such as a flock age, a turning treatment, or a preincubation heat treatment.

Measurements

Embryo Collection and Staging To monitor the initial blastoderm development of eggs collected from young and old flock ages, a random subset of 19 embryos were examined on the collection day for each flock age. A total of 120 eggs were selected (15 eggs per subtreatment group; flock age × turning treatment × heat treatment) to be used to identify the stage of blastoderm development after SPIDES. In this present study, a total of 158 hatching eggs were opened to determine the stage of embryo development either at collection or after SPIDES on day 5 of 14-day storage. The embryos were isolated from the yolk by using the filter ring technique as described in the study by Gupta and Bakst (1993). The dorsal and ventral sides of the embryo were examined with a stereomicroscope (Leica S6D; Leica Microsystems GmbH, Wetzlar, Germany) to determine the stage of the embryonic development according to the classification table of Eyal Giladi and Kochav (1976), with scores of 8-14 corresponding to stages EGKVIII-XIV, respectively.

Embryonic Mortality, Hatchability, and Second-Grade Chicks Infertile eggs and early deaths (0–7 d) were identified by candling and removed on day 10,

followed by macroscopic identification of the opened eggs. When the chicks were removed from the hatcher, all the remaining unhatched eggs were opened and examined macroscopically by a single experienced individual to determine the remaining embryonic mortality (middle [8–17 d] or late [18–21 d] plus pipped). The percentage hatchability of the fertile eggs was calculated as the number of saleable chicks hatched per 100 fertile eggs set. The number of saleable (clean, dry, and without deformities) and second-grade (unhealed navels, splayed legs, and so on; Tona et al., 2004) chicks as judged by experienced hatchery staff were determined for each treatment group at the time of the final pull.

Statistical Analyses

The present study was a 2 × 2 × 2 factorial design with 2 flock ages, 2 turning treatments, and 2 preincubation heat treatments. Data were analyzed via a factorial ANOVA using the GLM procedure in SAS (SAS, 2004). The model used for the statistical analyses of blastoderm stage, hatchability of fertile eggs, embryonic mortalities, and second-grade chick percentage was $Y_{ijkl} = \mu + A_i + T_j + P_k + (AT)_{ij} + (AP)_{ik} + (TP)_{jk} + (ATP)_{ijk} + e_{ijkl}$, where Y_{ijkl} is the dependent variable, μ is the overall mean, A_i is the flock age (i = young or old), T_j is the turning treatment (j = 0 or 4 times/day), P_k is the preincubation heat treatment (k = control or SPIDES), AT_{ij} is the interaction between the flock age and turning, AP_{ik} is the interaction between the flock age and preincubation heat treatment, TP_{jk} is the interaction between the turning and preincubation heat treatment, ATP_{ijk} is the interaction between the flock age, turning, and preincubation heat treatment, and e_{ijkl} is the error term. The statements of statistical significance were based on $P \leq 0.05$, unless otherwise indicated.

RESULTS AND DISCUSSION

Embryonic Development

According to Meir and Ar (1998) and Reijrink et al. (2009), on the collection day, embryos at the pregastrula stage (<EGK10) are sensitive to prolonged egg storage. In the present study, the average development stage of the embryos on the day the eggs were laid was below EGK10 in both flocks, with the stage of blastoderm on average EGK9.71 and EGK9.92 for young and old flock ages, respectively ($P > 0.05$).

It has been reported that the stage of blastoderm development is more advanced both before and after storage for eggs from an old flock than for the eggs from a young flock (Özlü et al., 2018a). As demonstrated in Table 1, on day 5 after egg collection, the stage of blastoderm development was more advanced for the eggs from the old flock (EGK11.10) than for the eggs from the young flock (EGK10.27) ($P < 0.001$).

In the present study, embryos after the SPIDES treatment were more advanced ($P < 0.001$) than the control embryos (Table 1). This result was in agreement with previous studies (Meir and Ar, 1998; Fasenko et al., 2001; Reijrink et al., 2009, 2010; Dymond et al., 2013).

Table 1. The effect of flock age, preincubation heat treatment, and turning during storage on the stage of blastoderm development on day 5 after egg collection.

Item		n[1]	Average stage (EGK)
Flock age[2]			
Young		60	10.27[b]
Old		60	11.10[a]
Preincubation heat treatment[3]			
Control		60	10.24[b]
SPIDES		60	11.13[a]
Turning treatment[4]			
0 times/day		60	10.47[b]
4 times/day		60	10.90[a]
SEM			0.131
Flock age × turning treatment			
Young	0 times/day	30	10.25[a]
	4 times/day	30	10.29[a]
Old	0 times/day	30	10.68[a]
	4 times/day	30	11.52[b]
SEM			0.185
P value			
FA			<0.001
PHT			<0.001
TT			0.020
FA × PHT			0.179
FA × TT			0.035
PHT × TT			0.532
FA × PHT × TT			0.778

[a,b]Means with different superscripts differ significantly ($P \leq 0.05$).

EGK = developmental stage of the embryo according to Eyal-Giladi and Kochav (1976).

[1]There were 60 eggs opened for flock age, heat treatment, and turning treatment; 30 eggs for a flock age × turning treatment group.

[2]Flock age (FA): young = 29 wk, old = 58 wk.

[3]Preincubation heat treatment (PHT): control = not heated during storage, and short period of incubation during egg storage (SPIDES) = heated over 32°C EST for 3.5 h and remained at 35°C for 2.5 h on day 5 after egg collection.

[4]Turning treatment (TT): 0 or 4 times/day during the storage period.

On day 5 after egg collection, average developmental stages EGK10.90 and 10.47 were observed from the turning 4 times daily and no turning groups, respectively ($P = 0.02$; Table 1). Recently Damaziak et al. (2018) showed that turning 2 times a day (every 12 h) during the 12-day storage period advanced embryonic development measured at 48 h of incubation and shortened the incubation time. In this present study, there was a significant interaction of flock age × turning during storage for embryonic development, which suggested that turning advanced the stage of blastoderm development only in older flock eggs ($P \leq 0.05$; Table 1). One possible mechanism for the beneficial effect of turning eggs from older flocks during extended storage may lie in the juxtaposition of the albumen and shell membrane due to significant changes in albumen pH, albumen thickness, and water loss during storage as documented by Brake et al. (1997). These changes could create an environment that would not be optimally receptive to the growth of the chorioallantoic membrane but

Table 2. The effect of flock age, preincubation heat treatment, and turning during 14 d storage on hatchability of fertile eggs, early, middle, and late embryonic mortality, second-grade chick, and contaminated egg percentages.

Item	n[1]	Hatchability of fertile eggs	Embryonic mortality			Cull[2]	Cont[3]
			Early	Middle	Late		
Flock age[4]		(%)					
Young	24	81.60[a]	10.16[a]	1.27[b]	5.43[b]	1.30[b]	0.23[b]
Old	24	79.24[b]	7.90[b]	2.10[a]	6.68[a]	2.39[a]	1.68[a]
Preincubation heat treatment[5]							
Control	24	78.50[b]	9.81[a]	1.73	6.65[a]	2.34[a]	0.97
SPIDES	24	82.34[a]	8.26[b]	1.64	5.46[b]	1.36[b]	0.94
Turning treatment[6]							
0 times/day	24	79.57[b]	8.74	1.84	6.78[a]	2.08	0.92
4 times/day	24	81.28[a]	9.33	1.53	5.33[b]	1.62	1.00
SEM		0.616	0.351	0.169	0.338	0.261	0.124
P value							
FA		0.008	0.001	0.001	0.010	0.004	0.001
PHT		0.001	0.002	0.722	0.015	0.009	0.865
TT		0.045	0.240	0.200	0.003	0.213	0.664
FA × PHT		0.689	0.174	0.710	0.763	0.512	0.415
FA × TT		0.268	0.421	0.884	0.053	0.460	0.263
PHT × TT		0.726	0.702	0.718	0.738	0.971	0.868
FA × PHT × TT		0.583	0.338	0.412	0.595	0.254	0.869

[a,b]Means within a column with different superscripts differ significantly ($P \leq 0.05$).
[1]There were 24 replicate trays for per treatment.
[2]Cull is the second-grade chick percentage, as calculated based on the number of fertile eggs in each replicate tray.
[3]Contaminated egg percentages were calculated based upon the number of fertile eggs in each replicate tray.
[4]Flock age (FA): young = 29 wk, old = 58 wk.
[5]Preincubation heat treatment (PHT): control = not heated during storage, and short period of incubation during egg storage (SPIDES) = heated over 32°C EST for 3.5 h and remained at 35°C for 2.5 h on day 5 after egg collection.
[6]Turning treatment (TT): 0 or 4 times/day during the storage period.

could have been overcome by turning 4 times/day. An associated mechanism may lie in the role of the albumen in buffering or sequestering ammonia, a toxic waste product produced by the blastoderm (Benton and Brake, 2000). During long-term storage, the buffering capacity of the albumen will be reduced particularly in older flock eggs, and the transblastoderm gradient increased such that turning 4 times/day may have allowed the blastoderm to interact with a greater quantity of fresh albumen in such a manner as to promote embryonic development.

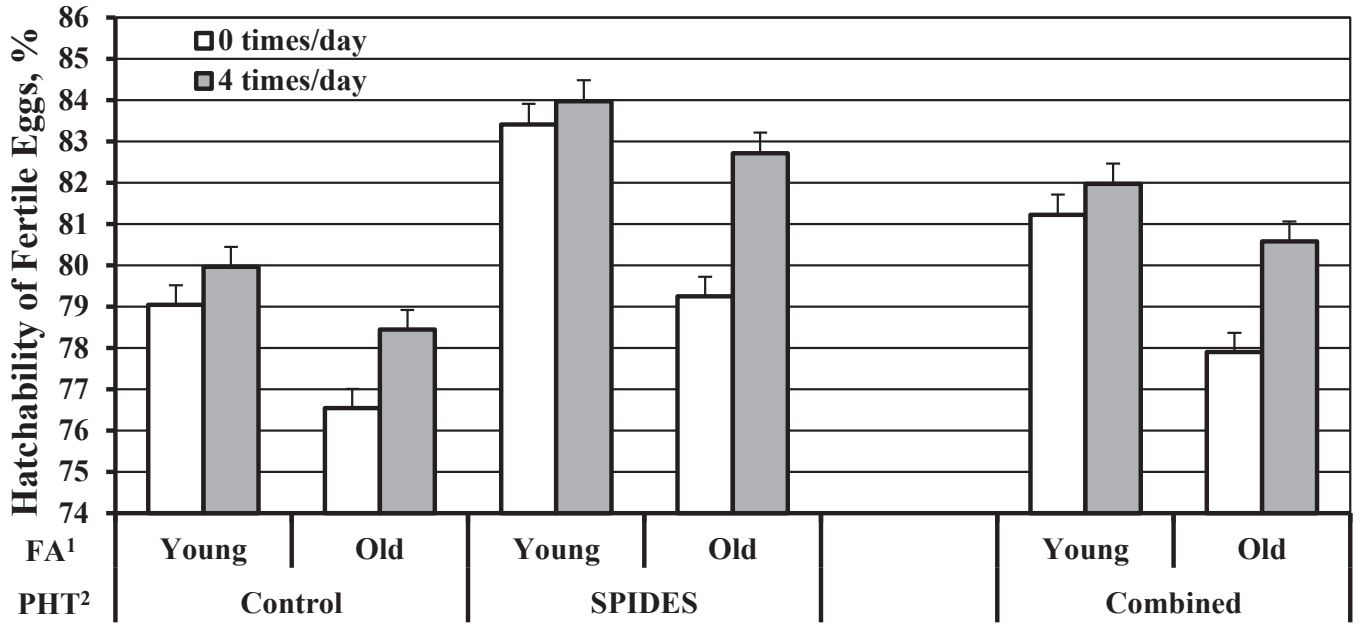

Figure 1. The effect of flock age, preincubation heat treatment, and turning during 14-day storage on the hatchability of fertile eggs. [1]Flock age (FA): young = 29 wk, old = 58 wk. [2]Preincubation heat treatment (PHT): control = not heated during storage, SPIDES = heated over 32°C EST for 3.5 h and remained at 35°C for 2.5 h on day 5 after egg collection, and combined = control and SPIDES treatments. Turning treatment: 0 or 4 times/day turning during the storage period.

Hatchability and Embryonic Mortality

In this present study, no interactions were observed among treatments for embryonic mortality and hatchability ($P > 0.05$) (Table 2). The hatchability of fertile eggs was significantly increased (by 3.8%) in SPIDES-treated eggs compared with that of the control eggs because of less early and late embryonic mortality and fewer second-grade chicks ($P \leq 0.05$) (Table 2). Similarly, Ebeid et al. (2017) reported that preincubation heat treatment improves hatchability because of a reduction in both early and late embryonic mortality in eggs from young and old flocks. More commonly, authors have reported that preincubation heat treatment decreased early embryonic mortality (Fasenko et al., 2001; Reijrink et al., 2009, 2010; Gucbilmez et al., 2013) compared with the control.

In the present study, at collection day, a higher proportion of less developed embryos were determined in young and old flocks (57.9 and 47.3% of the embryos below EGK10), and SPIDES improved hatchability in eggs from both flock ages in comparison to the control treatment. This result was consistent with that of Meir and Ar (1998) and Reijrink et al. (2009) that SPIDES would be most beneficial when the majority of the embryos are below the stage EGK10 of blastoderm development at egg collection.

In a recent study, turning eggs 2 times per day during a 12-day storage period had no effect on the hatchability of apparent fertile eggs (Damaziak et al., 2018). However, in this present study, hatchability was increased by turning 4 times/day during the storage period compared with no turning because of a decrease in the percentage of late embryonic mortality ($P < 0.05$) (Table 2). This turning effect was more evident in eggs from the older flock. Turning increased the hatchability of fertile eggs by 0.8 or 2.7% in eggs collected from a young or an old flock, respectively (Figure 1). This was in agreement with the study by Elibol et al. (2002b), who observed that turning broiler hatching eggs 4 times daily during extended storage improved hatchability of fertile eggs only in older flock eggs with lower fertility. Therefore, it was hypothesized that turning during storage would be the most beneficial when the eggs were produced by an older breeder flock because of egg quality changes with flock age. Eggs with poor quality albumen or increased shell porosity (e.g., eggs from older flocks) would be expected to lose water vapor more rapidly and cause the albumen to change more rapidly as well (Brake et al., 1997).

The results of this study showed that a combination of turning eggs 4 times daily along with one SPIDES treatment during 14 d of storage resulted in the highest hatchability in both young and old broiler grandparent flocks (Figure 1).

Second-Grade Chick Percentage

In this present study, there was no significant effect on second-grade chick percentage because of turning 4 times daily during a 14-day storage period, whereas the SPIDES treatment reduced the number of second-grade chicks when compared with the control (1.36 vs. 2.34%) ($P \leq 0.05$) (Table 2). Previous studies reported that longer storage resulted in an increase in the incubation time (Meir and Ar, 1998; Elibol et al., 2002a; Dymond et al., 2013), and long egg storage caused some live chicks to be rejected at take-off because they hatched too late (Nicholson et al., 2013). Moreover, the second-grade chick percentage was significantly ($P \leq 0.05$) higher, and the live performance was reduced in the late-hatched chicks compared with that for the early- and middle-hatched chicks (Özlü et al., 2018b). Preincubation heating of the eggs during extended storage reduced the incubation time and percentage of the late-hatched chicks (Reijrink et al., 2010; Dymond et al., 2013). Some of the improvement in the second-grade chick percentage by SPIDES was attributed to the reduction in the incubation time.

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DISCLOSURES

All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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