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Impact of Egg Storage and Prewarming Duration on Embryo Viability, Hatchability and Chick Quality

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ABSTRACT: This study investigates the effects of storage time and pre-incubation warming duration on egg weight loss, hatchability, hatching synchrony, and chick quality. A total of 3000 eggs from Cobb 500 broiler breeder stock randomly devided into two storage times (4 and 10 days) and two pre-incubation warming durations (8 and 10 hours) at 25°C. The study was conducted in a commercial hatchery with 5 replicate trays per subgroup. Results showed that neither storage time nor preincubation warming duration had a significant effect on hatchability, embryonic mortality, or chick quality metrics, except for a slight improvement in the Pasgar score with a longer prewarming duration. A key finding was the significantly improved hatching synchronization and reduced average hatching time, in eggs stored for 10 days with a 10-hour prewarming duration.which reflected by significant interaction between storage time and prewarming duration.These results confirmed that extended storage increases egg weight loss, the preincubation warming duration of 10 hours may promote to improving hatching outcomes in commercial conditions.

Keyword: Egg incubation; storage time; preincubation warming; hatchability; chick quality

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INTRODUCTION

The duration of egg storage fluctuates between a few days and several weeks due to the poultry industry's fluctuating demand for day-old chicks and the limitations of hatchery capacity. Extended egg storage (>7 days) has been demonstrated to have detrimental effects on various egg quality parameters, increased embryonic abnormalities, mortality, and decreased hatchability **(Fasenko et al., 2001b; Boerjan, 2002; Tona et al., 2003a; Decuypere and Bruggeman, 2007; Schmidt et al., 2009)**. Furthermore, advanced breeder age exacerbates these negative impacts, **(Elibol and Brake, 2003; Tona et al., 2004; Joseph and Moran, 2005; Zakaria et al., 2009; Nasri et al., 2020)**. Eggs stored beyond 7 days also display prolonged hatching time, reduced hatchling weight and diminished chick quality **(Tona et al., 2003a; Reijrink et al., 2009; Mather and Laughlin, 1976; Shiranjang et al., 2018; Yildirim, 2005; Christensen et al., 2002)**. **Tona et al. (2003)** observed adverse effects of prolonged egg storage on chick quality at hatch, evaluating physical attributes such as appearance, activity, and the quality of the navel area, while also assessing relative growth during the initial week of life **(Willemsen et al., 2008)** and weekly body weight until slaughter age **(Tona et al., 2004)**. In addition to body weight and other physical parameters, quantitative methods such as measuring yolk-free body mass **(Wolanski et al., 2004; Lourens et al., 2005; Boussada and Ouachem, 2018; Boussaada et al., 2020)** or chick length **(Hill, 2001; Wolanski et al., 2004; Molenaar et al., 2008)** provide avenues to assess the adverse effects of prolonged egg storage on chick quality at hatch.

At the start of incubation, it is accepted that eggs need to smooth transition from their storage temperature to the incubation temperature **(Boerjan, 2010; Molenaar et al., 2010)**, a process known as pre-incubation warming. Preincubation warming minimizes variations in embryo temperature and results in a more uniform development of embryos **(Boerjan, 2010)**. Typically, the prewarming temperature falls within the range between the storage temperature (12°C–18°C) and the incubation temperature (37.8°C). Gradual preincubation warming is advocated to prevent condensation on eggs at the start of incubation **(Reijrink et al., 2010),** minimize temperature shock to embryos **(Hodgetts, 1999)** and boost uniformity of embryos' developmental stage **(Renema et al.,2006).** Previous studies have reported varying durations for the prewarming stage, ranging from 3 to 24 hours **(Elibol et al., 2002; 2009; Kamanli et al., 2009; Piestun et al., 2013; Yousaf et al., 2017);** and temperature **(Meijerhof et al., 1994; Kamanli et al., 2009; Lin et al., 2017)**. Industry guidelines recommend prewarming temperatures between 25°C and 27°C, with humidity levels of 50–55% and a minimum duration of 8 hours **(Hubbard Breeders, 2017)**. These temperatures are commonly used in hatchery operations to ensure consistent pre-warming of eggs prior to incubation, thereby promoting uniform embryonic development and reducing the risk of temperature-related stress.

Furthermore, pre-incubation of hatching eggs, either before or during storage, has been shown to mitigate the adverse effects of storage periods exceeding 7 days **(Fasenko et al., 2001a,b; Reijrink et al., 2009)**. However, the impact of pre-incubation warming duration on embryo viability remains uncertain. It is conceivable that the increase in cell death over time results in stored embryos having fewer viable cells at the onset of incubation compared to non-stored embryos. Embryos with fewer viable cells at the outset of incubation might be more susceptible to suboptimal pre-incubation warming profiles. Studies by **Reijrink et al. (2010)** and **van Roovert-Reijrink et al. (2018)** indicate that gradual prewarming benefits hatchability during extended storage, with a prewarming profile exceeding 29.4°C (84.92°F) reducing early embryonic mortality in prime breeder flock eggs. Conversely, some argue for rapid prewarming of eggs from younger breeder flocks to the desired temperature, as prolonged exposure to temperatures below 35°C may elevate embryonic mortality or result in abnormal embryonic development **(Wilson, 1990; Renema et al., 2006)**.

The impact of preincubation on hatchability is influenced by both the duration of egg storage and the developmental stage of the embryo before **(Reijrink et al., 2009)** or after **(Kosin, 1956; Fasenko et al., 2001a)** the heating treatment.

Eggs from younger flocks are typically subjected to prolonged storage as a regular practice, primarily because of lower egg production and inadequate hatching egg weight during the early stages of egg production **(Gucbilmez et al., 2013)**. Similarly, eggs from older breeder flocks may also require prolonged storage due to reduced egg production, which necessitates additional time to accumulate a sufficient quantity of eggs for setting. For older breeder eggs have lower fertility rates and more sensitive embryos to temperature **(Meijerhof et al., 1994)** which makes them more susceptible longer storage times.

During the study period, Algeria had faced a significant problem with importing breeding chicks, leading to a supply shortage. This situation forced breeders to keep their stocks for an extended period, resulting in the use of older breeder eggs and the need for longer storage to meet the operational requirements of the hatchery. Therefore, the aim of this research was to examine if the beneficial effects of pre-incubation warming on embryo viability, hatchability, hatch time, and chick quality would change with pre-incubation warming duration of 8 or 10 h when eggs from older (69-71 weeks of age) breeders stored for short (4 d) or long (10 d) periods under the commercial conditions.

MATERIALS AND METHODS

The study has been approved by the Scientific and Technical Research Centre for Arid Areas (CRSTRA) in Biskra, Algeria, and all experimental protocols adhered to the European Union Directive on Legislation concerning the welfare of animals used for scientific research, 2010-63-EU.

Experimental Design

The experiment was conducted in a commercial hatchery, reflecting real-world conditions. The experimental setup followed a 2 × 2 factorial design, involving two storage periods (short, 4 days and long, 10 days) and two pre-incubation warming duration (8 or 10 hours at 25°C). A total of 3000 eggs were used for the experiment. Each experimental replicate comprised a tray containing 150 eggs, with 5 replicate trays assigned to each preincubation warming treatment for every storage duration, resulting in a total of 750 eggs per treatment.

Egg collection and egg storage

Hatching eggs were obtained from broiler breeder flocks (Cobb 500) aged 71 weeks. Eggs were stored in the hatchery under consistent environmental conditions: a temperature range of 17-18°C and a relative humidity of 70%. The study were conducted at the Ouchelouz commercial hatchery, located in Batna, Algeria.

Pre-incubation warming and incubation conditions

Eggs were incubated at a commercial hatchery using two identical Royal Pas Reform incubators (model SmartSetPro™, capacity 57,600 eggs each) in which the setter and hatcher were separate units. Pre-incubation warming regimen of 25°C for either 8 or 10 hours was applied in two identical setters. The setters were set to 25°C, and the humidity was maintained at 60% during the pre-incubation period. Throughout the pre-incubation warming duration, the eggs remained horizontally positioned and were not subject to rotation.

There were 5 replicate trays per treatment containing 150 eggs, totaling 750 eggs per treatment. To mitigate potential minor machine position effects resulting from slight disparities in airflow, trays from each treatment were dispersed across all positions in both the setter and hatcher.

For the first 5 days of the incubation period, the temperature was maintained at 37.8°C, with a humidity ranging 60% to 50% and CO_2 levels between 0.1 and 0.2%. From day 5 to day 18, the temperature gradually reduced from 37.8°C to 36.7°C with humidity levels of 50% to 45%. Dataloggers were employed to monitor incubator air temperature and relative humidity during both the prewarming and incubation stages. Eggs were rotated by 90° every hour until day 18 of the incubation period.

At the 18th day of incubation, eggs were moved to hatcher baskets. The eggs from each replicate (setter tray) was transferred to two hatcher baskets and were placed together in the same hatcher. The temperature within the hatcher was set at 36.7°C. Relative humidity levels fluctuated between 50 and 55%, with the inlet and outlet valves of the hatcher regulated to maintain CO_2 levels below 0.35%.

Egg weight loss during storage and incubation

Egg weight loss was measured both for storage and incubation period. At the beginning of the experiment, the trays were weighed empty and then reweighed when filled with eggs to calculate the average egg weight based on the number of eggs in each tray. After the storage period, the trays were reweighed, and the reduction in egg weight was calculated as a percentage of the initial average egg weight.

On day 18 of the incubation process, egg trays were weighed again and the average weight of hatching eggs was calculated. The weight loss of eggs from the end of storage to day 18 of incubation was calculated as a percentage of the egg weight at the end of storage. The total weight loss of the eggs was calculated by summing the weight losses that occurred during both the storage and incubation periods.

Embryo Mortality

After the chicks were removed from the hatcher at 504 hours of incubation, all of the unhatched eggs were opened and examined by a single individual, blinded to the treatments, to determine the stage of embryonic mortality. The assessment distinguished only between embryonic and pipping (embryo piped the shell but failed to hatch) mortality. Mortality percentages were calculated based on the number of fertile eggs.

Hatchability and Hatching Time

Starting from 468 hours of incubation, hatched chicks were recorded every 6 hours to determine the duration of incubation and the synchronization of hatching for each treatment group. To avoid prolonging the hatching period, the number of hatched chicks was determined from photographs taken with a camera and analyzed on a laptop screen. The hatching phase was segmented into three intervals: early (468–480 hours), middle (480–492 hours), and late (492–504 hours). Chicks were removed from the hatchers after hatching was fully completed. These intervals were computed as a percentage of the total number of hatched chicks for each group. All chicks were extracted from the hatching baskets at 508 hours of incubation after the chicks getting dry.

Chick Quality

Chick quality was assessed using various metrics including Pasgar score, chick-hatching weight, chick yield, and chick length. A total of 100 chicks per treatment group were randomly selected for Pasgar scoring, wherein they received scores out of a maximum of 10 based on criteria such as activity, navel condition, leg and beak confirmation, and yolk membrane status (**Boerjan, 2002**). Subsequently, these chicks were measured for weight, yield (calculated for each group using the formula: Chick yield = (average chick weight / average hatching egg weight before incubation) × 100.), and length. Chick length is defined as the distance from the tip of the beak to the implantation of the nail on the middle toe (**Hill, 2001; Willemsen et al., 2008**).

Statistical Analysis

The data were analyzed using a 2 × 2 completely randomized design, with storage time and pre-incubation warming duration as the two main factors. A setter tray containing 150 eggs served as the experimental unit for egg weight loss (%), hatchability and mortality parameters (%). For chick quality parameters, the individual chick was the experimental unit. Normality assumptions of the linear model were checked.

A general linear model (GLM) was applied using IBM SPSS Statistics 22 to assess the effects of storage time, preincubation warming duration, and their interaction on the traits measured. Storage time, preincubation warming duration, and their interaction were included as fixed effects in the model. To further explore significant differences, post-hoc analyses conducted using the Least Significant Difference (LSD) test. All analyses were performed at a significance level of $\alpha = 0.05$.

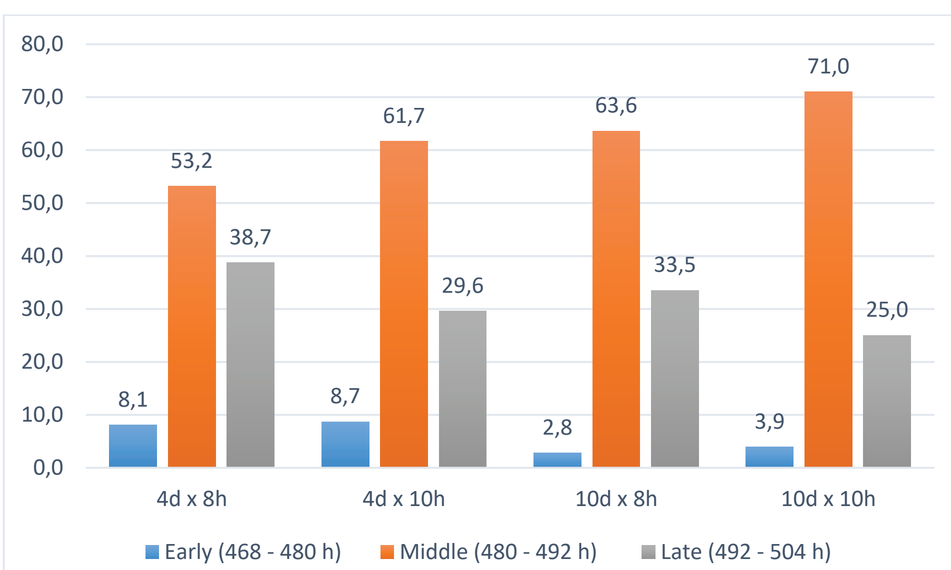

Figure 1. Percentage of hatched chicks at each control time.

RESULTS

Egg Weight and Egg Weight Loss

The effects of treatments on egg weight and egg weight loss are presented in Table 1. The weight of fresh eggs was consistent across both storage times (P = 0,106). The weight loss significantly differed with the storage time (P<0.05) being 0.31% and 0.79% for 4 and 10 days storage, respectively. During the incubation, eggs stored for 10 days lost 1.14% more weight compared to those stored for 4 days (P=0.001). The total egg weight loss was 1.56% higher for eggs stored for 10 days than for those stored for 4 days (P<0.001). The egg weight loss per storage day was 0.077 ± 0.009% for the 4-day storage period and 0.079 ± 0.009% for the 10-day storage period. The duration of preincubation warming did not affect egg weight loss during storage, incubation, or the total egg weight loss.

Hatchability and Embryo Viability

The impact of storage time and preincubation warming duration on hatchability parameters (%) is shown in Table 2. The percentage of infertile eggs were not varied among the experimental groups and ranged between the 7,73 and 10,80%. Storage time and pre-incubation warming duration did not affect hatchability, total mortality and embryonic mortality stages during the incubation (Table 2).

Hatching Time and Hatching Synchrony

The effects of storage time and preincubation warming duration on hatched chicks percentage (%) and average hatching time (h) are presented in Table 3. Storage time did not influence the percentage of early, middle, or late-hatched chicks, nor the average hatching time (hours). However, a longer prewarming duration significantly enhanced hatching synchronization. Specifically, eggs subjected to a longer prewarming duration had a higher percentage of early- and middle-hatched chicks (P ≤ 0.001) and reduced average hatching time by 1.03 hours compared to those with a shorter prewarming duration (P < 0.001).

An interaction between storage time and pre-incubation warming duration was observed for early-hatched (P < 0.001), middle-hatched (P = 0.006), and late-hatched chicks (P = 0.003), as well as for average hatching time (P < 0.001). For the same storage time, a longer prewarming duration significantly increased the percentages

Table 1. Effect of storage time and preincubation warming duration on egg weight (g) and egg weight Loss (%).

Treatment	FEW (g)	EWLI (%)	TEWL (%)
Storage time (d)			
4	69,70 ± 0,17	12,09 ± 0,19	12,36 ± 0,17
10	69,27 ± 0,17	13,23 ± 0,19	13,92 ± 0,17
Preincubation warming duration			
8	69,65 ± 0,17	12,91 ± 0,19	13,39 ± 0,17
10	69,32 ± 0,17	12,41 ± 0,19	12,89 ± 0,17
Storage time x Preincubation warming duration			
4 x 8	69,76 ± 0,38	12,39 ± 0,51	12,74 ± 0,45
4 x 10	69,64 ± 0,38	11,78 ± 0,36	11,99 ± 0,36
10 x 8	69,53 ± 0,91	13,43 ± 0,41	14,04 ± 0,34
10 x 10	69,01 ± 0,35	13,03 ± 0,99	13,79 ± 0,86
P-value			
Storage time	0,106	0,001	<0,001
Preincubation warming duration	0,214	0,08	0,057
Interaction	0,428	0,704	0,317

FEW: Fresh egg weight (g)

EWLI: Egg weight loss during incubation (%)

TEWL: Total egg weight loss (%)

Table 2. Effect of storage time and preincubation warming duration on hatchability parameters (%)

Treatment	Hatchability (%)	Total Mortality (%)	Embryonic mortality (%)	Pipping mortality (%)	Infertile eggs (%)
Storage time (d)					
4	87,33 ± 1,22	4,13 ± 0,69	1,73 ± 0,50	2,40 ± 0,32	8,53 ± 0,97
10	84,73 ± 1,22	6,00 ± 0,69	3,67 ± 0,50	2,33 ± 0,32	9,27 ± 0,97
Preincubation warming duration					
8	85,07 ± 1,22	5,60 ± 0,69	2,80 ± 0,50	2,80 ± 0,32	9,33 ± 0,97
10	87,00 ± 1,22	4,53 ± 0,69	2,60 ± 0,50	1,93 ± 0,32	8,47 ± 0,97
Storage time (d) x Preincubation warming duration					
4 x 8	87,60 ± 2,61	4,53 ± 1,45	1,73 ± 0,71	2,80 ± 0,87	7,87 ± 1,79
4 x 10	87,07 ± 2,93	3,73 ± 1,21	1,73 ± 0,71	2,00 ± 0,67	9,20 ± 2,42
10 x 8	82,53 ± 5,84	6,67 ± 2,71	3,87 ± 0,71	2,80 ± 1,37	10,80 ± 5,22
10 x 10	86,93 ± 3,11	5,33 ± 2,87	3,47 ± 0,71	1,87 ± 0,99	7,73 ± 1,01
P-value					
Storage time	0,150	0,074	0,536	0,882	0,597
Preincubation warming duration	0,278	0,291	0,201	0,072	0,534
Interaction	0,171	0,788	0,406	0,885	0,127

Table 3. Effect of storage time and pre-incubation warming duration on hatched chicks percentage (%) and average hatching time (h).

Treatment	Hatch time (h)	Hatched chicks (%)		
Storage time (d)		Early hatching	Mid hatching	Late hatching
4	493,44 ± 0,68	8,40 ± 2,03	57,43 ± 3,81	34,17 ± 3,95
10	493,43 ± 0,68	3,39 ± 2,03	67,33 ± 3,81	29,28 ± 3,95
Preincubation warming duration				
8	493,95 ± 0,68	5,47[b] ± 2,03	58,39 [b] ± 3,81	36,14 [a] ± 3,95
10	492,92 ± 0,68	6,32 [a] ± 2,03	66,36 [a] ± 3,81	27,32 [b] ± 3,95
Storage time (d) x Preincubation warming duration				
4 x 8	493,92 ± 0,96	8,08 ± 9,77	53,17 ± 4,80	38,74 ± 14,20
4 x 10	492,97 ± 0,96	8,71 ± 6,74	61,69 ± 11,48	29,60 ± 6,09
10 x 8	493,98 ± 0,96	2,85 ± 2,13	63,62 ± 16,86	33,53 ± 16,99
10 x 10	492,88 ± 0,96	3,93 ± 4,44	71,03 ± 11,89	25,04 ± 9,77
P-value				
Storage time	0,991	0,100	0,112	0,327
Preincubation warming duration	<0,001	0,001	<0,001	<0,001
Interaction	<0,001	<0,001	0,006	0,003

The early hatch time was 468–480 h, the middle hatch time was 480–492 h, and the late hatch time was 492-504 h.

of early-hatched (P < 0.001) and middle-hatched chicks (P = 0.006).

Among the treatments, eggs stored for 10 days with a 10-hour preincubation warming duration (10 x 10) exhibited the highest percentage of middle-hatched chicks (70.86%) and the lowest percentage of late-hatched chicks (25.15%). In contrast, the highest percentage of late-hatched chicks (38.96%) was observed in eggs stored for 4 days with an 8-hour preincubation warming duration (4 x 8).

Chick Quality

Storage time had no impact on any of the chick quality variables examined (Table 4). The Pasgar score of chicks from eggs exposed to a 10-hour preincubation warming duration was 0.34 points higher than that of chicks from eggs exposed to an 8-hour duration (P = 0.001). However, preincubation warming duration did not affect other chick quality metrics, such as weight or yield. No interaction between storage time and preincubation warming duration was observed for chick weight, chick yield, or Pasgar score.

DISCUSSION

Egg Weight and Egg Weight Loss

Our study provides insights into the effects of storage time and preincubation warming duration on egg weight and weight loss. The results show that egg weight loss increased with longer storage time which likely exacerbate moisture loss. Specifically, eggs stored for 10 days lost 0.48% more weight during storage and 1.14% more weight during incubation compared to those stored for 4 days. These findings are consistent with the results of **Reijrink et al. (2010)**, who observed increased egg weight loss with longer storage durations.

However, our study did not find a significant effect of pre-incubation warming duration on egg weight loss during storage, incubation, or overall weight loss. This contrasts with the findings of **Reijrink et al. (2010)** and **Walsh et al. (1995)**, who reported that longer pre-incubation warming durations (24 hours) resulted in greater egg weight loss compared to shorter durations (4 hours). The discrepancy in our results may be due to the shorter pre-incubation warming durations (8 and 10 hours) used in our study, which might not have been long enough to produce a measurable effect on egg weight loss between the pre-incubation warming durations.

Hatchability and Embryo Viability

Contrary to previous findings, our results indicate that neither storage time nor preincubation warming

Table 4. Effect of storage time and preincubation warming duration on chick weight (g), chick yield (%) and Pasgar score

Treatment	Chick Weight (g)	Chick Yield (%)	Pasgar score
Storage time (d)			
4	47,43 ± 0,27	68,08 ± 0,38	8,75 ± 0,74
10	47,45 ± 0,27	68,52 ± 0,38	8,67 ± 0,74
SEM			
Preincubation warming duration			
8	47,25 ± 0,27	68,09 ± 0,38	8,54 ± 0,74
10	47,63 ± 0,27	68,51 ± 0,38	8,88 ± 0,74
Storage time (d) x Preincubation warming duration			
4 x 8	47,22 ± 3,72	67,54 ± 0,53	8,60 ± 1,19
4 x 10	47,64 ± 3,86	68,61 ± 0,53	8,89 ± 0,92
10 x 8	47,28 ± 4,21	68,63 ± 0,53	8,48 ± 1,10
10 x 10	47,61 ± 3,41	68,41 ± 0,53	8,86 ± 0,93
P-value			
Storage time	0,651	0,414	0,473
Preincubation warming duration	0,855	0,435	0.001
Interaction	0,152	0,243	0.666

duration significantly affected hatchability or stages of embryo mortality.

Previous research has shown that storage time can influence hatchability and embryonic development **(Boerjan, 2010; Reijrink et al., 2010).**

In contrast, our findings did not reveal any significant effects of storage time on hatchability or embryonic mortality, which could be due to the relatively shorter storage times used in this study. **Reijrink et al. (2010)** suggested that eggs stored for shorter durations might not impacted similarly to pre-incubation warming profiles as the eggs stored for a longer time. This suggests that for shorter storage times, the benefits of preincubation warming on hatchability may not be pronounced.

Regarding preincubation warming duration, our results indicate no significant impact on hatchability, embryonic mortality, or other parameters. The benefits of pre-incubation warming can be summarized as following: the gradual increase in temperature during warming period allows embryos to compensate for cell death that occurs during storage, thus improves viability; it prevents condensation on the eggs at setter; and reduces stress on the embryos due to abrupt temperature changes **(Reijnik et al, 2010, Renema et al, 2006; Boerjan, 2010)**.

Our study's lack of significant findings could be due to the shorter preincubation warming durations tested (8 and 10 hours), which may not have been long enough to show measurable differences in hatchability outcomes. This supports the idea that the benefits of preincubation warming profiles may become more apparent with extended warming periods and longer storage times, as noted by **Reijrink et al. (2010)** and **Proudfoot (1966)**.

The role of temperature in embryonic development during preincubation warming is a key consideration in understanding our results. Previous studies have shown that gradual warming can enhance embryonic viability by allowing the embryo to recover from storage-induced cell damage. **Funk and Biellier (1944)** noted that embryonic development begins at temperatures above 27°C, and **Arora and Kosin (1968)** demonstrated that mitotic activity in the embryo increases as the temperature rises from 7.2°C to 18.3°C. **Reijrink et al. (2010)** hypothesized that during the early hours of a slow preincubation warming profile, embryos may activate mitotic activity without initiating full morphological development, potentially compensating for cell death during storage.

In our study, the preincubation warming durations (8 and 10 hours) might not have provided sufficient time for these compensatory mechanisms to take effect, particularly for eggs stored for shorter periods. As a result, the effects of preincubation warming on hatchability and embryonic mortality were minimal. This suggests that longer preincubation warming profiles, such as the 24-hour profiles tested by **Reijrink et al. (2010)**, may be necessary to observe improvements in embryonic viability and hatchability, particularly for eggs stored for longer durations.

Hatching Time and Hatching Synchrony

In our study, we observed that storage time did not significantly influence the percentage of early-, middle-, or late-hatched chicks or the average hatching time. However, significant interaction between storage time and prewarming duration highlights the potential for optimizing incubation practices to mitigate the effects of prolonged storage. In this regard, most profound effect of longer prewarming duration of 10 h was more synchronized hatching and with a reduced overall hatching time by 1.03 hours. A longer prewarming duration increased the percentage of early- and middle-hatched chicks and reduced the percentage of late hatched chicks. This result is consistent with findings from several studies in the literature.

Özlü (2021) found that eggs stored for extended periods exhibited longer incubation times, with an increase of approximately 4 hours in eggs stored for 11 days compared to those stored for 4 days. However, our results suggest that within the storage times studied (4 and 10 days), storage duration alone did not affect hatching time distribution, supporting earlier work by **Tona et al. (2003)**, who also found that storage time did not significantly alter hatching time distribution for shorter storage periods. The lack of impact on hatch timing from shorter storage times aligns with the findings of **Mather and Laughlin (1976)**, who observed that storage for 14 days extended the incubation period but did not affect hatch synchrony.

Our findings also revealed an interaction between storage time and preincubation warming duration, where longer prewarming durations (10 hours) significantly enhanced hatch synchrony, particularly for eggs stored for 10 days. This is in agreement with **Elibol et al. (2002)**, who reported that prewarming for 10 to 18 hours significantly reduced the percentage of late-hatched chicks after extended storage. Similarly, **Özlü (2021)** demonstrated that higher prewarming temperatures could reduce late hatching and improve chick quality, particularly for eggs subjected to longer storage. The improved hatching synchronization and reduced late-hatch percentages observed with longer prewarming durations in our study mirror these findings.

Furthermore, eggs stored for 10 days with a 10-hour prewarming duration showed the highest percentage of middle-hatched chicks and the lowest percentage of late-hatched chicks, suggesting that prewarming can offset some of the negative effects of prolonged storage. This supports research by **Nicholson (2012)**, who noted that late-hatching chicks often face rejection and poorer quality, highlighting the importance of prewarming in managing hatching outcomes. Additionally, studies by **Özlü et al. (2018)** and **Shiranjang et al. (2018)** have demonstrated that late-hatched chicks often have reduced performance and lower quality compared to earlier-hatched counterparts.

Chick Quality

In our study, we found that storage time had no significant effect on chick quality variables, such as weight, yield, or Pasgar score. However, a longer preincubation warming duration (10 hours) slightly improved the Pasgar score by 0.34 points compared to the 8-hour duration. Despite this, no other chick quality metrics, such as weight or yield, were influenced by prewarming duration, nor was there any interaction between storage time and prewarming duration for these variables.

This aligns with **Meijerhof's (1994)** suggestion that while egg weight loss during storage may be important, its impact on hatchability and chick quality is minimal if the weight loss remains within moderate limits. Similarly, our finding that prewarming duration had little impact on key chick quality indicators supports earlier observations. **Reijrink et al. (2010)** reported that although preincubation warming improved hatchability for eggs stored for longer periods, it had no meaningful effect on chick quality, including metrics like yolk-free body mass or chick length.

The lack of negative impact from storage time in our results contrasts with some earlier studies. **Tona et al. (2003, 2004)** and **Reijrink et al. (2010)** both showed that prolonged storage could negatively affect chick quality by reducing physical parameters like yolk-free body mass and chick length. In **Reijrink et al.**'s study, longer storage periods were associated with a higher percentage of second-grade chicks. Despite these findings, the minor positive effect of prewarming on Pasgar score in our study may indicate that preincubation warming duration can mitigate some negative impacts of prolonged storage on chick vitality.

CONCLUSION

Results from this study did not indicate a pronounced effect of the storage time and the pre-incubation warming durations on hatchability and chick quality. This may be due to relatively moderate egg storage times and pre-incubation warming durations applied in this study. However, our study provides valuable insights into the effects of storage time and preincubation warming duration on hatching syncrony and chick quality. A 10 h preincubation warming duration, played a role in improving hatch synchrony by reducing the percentage of late hatched chicks, and slightly enhancing the Pasgar score, particularly when combined with longer storage times. Future research should focus on exploring a wider range of storage durations, preincubation warming profiles, and environmental factors such as relative humidity (RH) to further optimize egg handling and improve incubation outcomes.

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CONFLICT OF INTEREST

The authors declare no conflct of interest

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