

Effects of breeder age and pre-incubation storage of eggs on hatchability, time of hatch and relative organ weight of quail chicks at hatch

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Abstract

Two thousand four hundred quail (*Coturnix coturnix japonica*) hatching eggs were used to determine the effects of breeder age and pre-incubation storage time on egg traits, hatching traits and the growth of some selected organs in newly hatched chicks. Eggs from two non-commercial flocks (aged 10 vs. 40 weeks) of the same strain were incubated. Eggs were stored for 4 or 14 days prior to incubation. All eggs were set in an experimental setter and incubated under uniform conditions for approximately 19 days (448 h). Fresh egg weight, chick weight and percentage yolk weight were significantly higher in eggs obtained from breeders at 40 weeks of age than from those of 10 weeks of age, whereas percentage albumen weight was significantly higher in eggs obtained from 10 wk old breeders. There were significant storage period x hen age interactions for water loss in chicks, early embryonic death, late embryonic death, deaths during internal and external pipping, and hatchability of fertile eggs. From 405 h to 441 h of incubation, the percentage of hatched chicks was influenced by the storage period x breeder age interaction. The jejunum length of chicks from 40 wk old breeders was longer when eggs were stored for 4 days compared to 14 days. At hatch, chick liver weight as a percentage of live weight was higher when eggs obtained from 10 wk old breeders were stored for 4 days compared to 14 days. It was concluded that the effects of extended storage time on hatching traits were different for eggs laid by young compared to old breeders.

Keywords: Hatchability, breeder age, egg storage, organ weights, quail

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Introduction

Fertilised eggs during pre-incubation are often stored at cool temperatures for extended periods. Egg storage occurs at the broiler breeder farm as well as at the hatchery. Storage at hatcheries occurs for two main reasons: hatching eggs are stored until enough eggs are available to fill large incubator racks; and stockpiling of eggs occurs in anticipation of fluctuations in egg production or demand during the production year (Fasenko *et al.*, 2001). The effects of pre-incubational egg storage on embryonic viability depend on storage time, environmental conditions, hen age and strain (Brake *et al.*, 1997). Butler (1991) indicated that parental age can influence the extent of embryonic development at oviposition. Eggs laid by older birds were more developed, both at oviposition and after 24 h and 42 h of incubation than those from younger birds. In explanation of these differences, the author identified three possible contributing factors. Firstly, changes may occur in the inherent growth rate of embryos. Secondly, eggs in older birds may spend a longer period in the oviduct, either because the oviduct is longer or because passage time is reduced or thirdly, clutch or sequence effect may be a factor. Wilson (1991a) stated that incubation time was positively correlated with egg weight but that eggs from older flocks, which generally are larger than those from younger hens, have been reported to hatch earlier. Suarez *et al.* (1997) stated that chicks from young flocks hatch late and are underdeveloped. Fasenko (1996) documented that incubation period lengthens when hatching eggs are stored for extended periods. There are a number of conflicting reports concerning the appropriate storage conditions for optimal hatchability and it remains unclear how these storage conditions affect water vapour loss during incubation, chick weight at hatch and hatching time (Ruiz & Lunam, 2002). The objective of this study was to determine the influences of storage time and breeder age on hatchability of fertile eggs, embryonic mortalities at different stages of incubation, hatching time and the weights of some organs in quail (*Coturnix coturnix japonica*) chicks at hatch.

Materials and Methods

Fertile eggs produced by two non-commercial strains of quail breeder hens of two age groups (10 and 40 weeks of age) were collected. The breeders were fed a standard diet (200 g CP/kg, 12.6 MJ ME/kg). Water was available *ad libitum* and natural daylight was supplemented with artificial light to give a 16 h photoperiod. Within parent age group freshly laid eggs were collected twice daily (9:00 and 16:00), weighed and stored for 14 days (designated the 14 d treatment). Excreta-contaminated eggshells and eggshells with visible cracks were discarded. The eggs were stored at an average of 12 °C and a relative humidity (RH) of 75%. Ten days after the previous group, a second group of eggs was collected over a period of one day, weighed and stored for 4 days (designated the 4 d treatment). The eggs in this group were stored at an average of 18 °C and 75% RH. The storage length of 4 d (representing the experimental control) was chosen based on practices in the industry in which hatching eggs are commonly stored for three to seven days prior to incubation while storage periods of longer than seven days are avoided. The 14 d storage treatment was chosen to simulate a worst-case scenario.

Prior to storage a random of 10 eggs from each flock-age (10 and 40 wk) was used to measure the pH of the albumen and yolk to the nearest 0.01 units, using a model HI 8424 combination pH microelectrode FC 200 B. Furthermore, 10 eggs from each age group (10 and 40 wk) were randomly selected, weighed (nearest 0.01 g) and eggshell conductance (G) and conductance constant (k) were determined, using the technique of Christensen *et al.* (2001). Functional and physical characteristics of each egg were determined using the procedures of Rahn *et al.* (1981). Each egg was assigned a number. The desiccators used to measure eggshell conductance contained 10 eggs for each age group. Only apparently normal eggs were selected for determination of eggshell conductance. The weights of the wet yolk, albumen and the eggshell of 10 eggs per breeder age were measured. Only apparently normal eggs were selected for determination of these egg components. Each selected egg was weighed and then broken. Yolk and albumen were separated from the shell and from each other.

After the required storage period, the eggs were prewarmed in an incubator for eight hours at around 24 °C and 65% RH, and fumigated (triple strength formaldehyde gas: 3X) in the incubator on the day of setting. The 4 d and 14 d stored eggs were weighed, randomly placed according to their age group in a laboratory setter at an average temperature of 37.6 °C and 60% RH. The eggs were turned hourly through 90°. At day 15 the eggs were transferred to four identical laboratory hatchers operating at 37.2 °C and 75% RH. A total of 2400 eggs were used in the study.

To determine the effects of holding time in hatchers on chick water loss (WL), starting at hour 405 of incubation, a random of 10 chicks from each storage and age treatment groups was weighed, wing banded and returned to the hatchers. At the completion of the incubation period (pull time) the same chicks were weighed again. Thereafter, WL was calculated as: chick weight at hatch - chick weight at pull time divided by chick weight at hatch and multiplied by 100, thus expressed as a percentage.

The number of hatched chicks was recorded from 405 h till 447 h of incubation divided over six hour periods. All chicks that hatched during a six hour period of incubation were counted. Percentage of hatched chicks (PHC) during a six hour interval was calculated as: number of saleable chicks hatched ÷ number of fertile eggs set per treatment X 100. At pull-time (removal of chicks from the hatchers at the completion of 447 h of incubation) the chicks were weighed, euthanized via decapitation, and livers, hearts, pancreas and jejunums were removed and weighed. From the data, hatchability (number of saleable chicks hatched ÷ per number of fertile eggs set X 100) was calculated. At completion of 447 h of incubation all unhatched eggs were opened, examined macroscopically and assigned to one of the following categories: infertile, early dead (ED) (1 to 7 days), late dead (LD) (8 to 19 days) and pips (IP - Internal pip: beak in air cell; EP - External pip: beak through shell). Total incubation time was calculated as the time between setting and emergence.

Data were analyzed as completely randomized designs, according to a 2 x 2 factorial arrangement of treatments. The main factors were two ages of breeders (10 vs. 40 wk) and two egg storage (4-d and 14-d) periods. A total of 2400 eggs were used in the study. There were four replicate-trays for each age group in every storage period, containing 150 hatching eggs per each tray for the hatching results. All main effects and interactions were tested for significance ($P < 0.05$) using the GLM procedure of Minitab (1998). All percentage data were transformed using *arc sine* transformations prior to analysis. Means were compared using Fisher's Protected Least Significance Difference (Steel & Torrie, 1980).

Results

Egg characteristics were measured to determine if variations in egg components existed between breeder age groups prior to storage. The effects of flock age on egg characteristics are presented in Table 1. Wet eggshell weight as a percentage of total egg weight, albumen pH, yolk pH, conductance (G) and conductance constant (k) were not significantly influenced by flock age. Yolk weight (as a percentage of total egg weight) was higher ($P < 0.05$) in old breeder eggs than in that from the young breeders. Conversely, albumen weight (as a percentage of total egg weight) was lower ($P < 0.05$) in eggs obtained from breeders at 40 wk of age than of 10 wk of age.

The effects of treatments on water loss of chicks, ED, LD, IP, EP and hatchability of fertile eggs are shown in Table 2. Chicks from breeders at 10 wk of age exhibited relatively high WL irrespective of storage time compared to those from 40 wk old breeders. When stored for 14 d the ED was higher among embryos in eggs from the young breeders than from the old breeders. Conversely, there were no differences in ED in eggs stored for 4 d ($P < 0.05$), irrespective of breeder age. The long storage period increased ED in embryos compared to those stored for 4 d. The incidence of LD was higher ($P < 0.05$) in embryos of eggs stored for 14 d than for 4 d. In general, the occurrence of IP and EP increased ($P < 0.05$) in embryos obtained from young breeders when stored for 14 d compared to 4 d. Hatchability of fertile eggs was improved by approximately 7.4% when eggs were stored for 4 d compared to 14 d prior to incubation. During the short (4 d) storage period, no significant effects on hatchability were found between age groups. The negative impacts were most pronounced when eggs from the young breeders were stored for 14 d.

Table 1 Albumen and yolk pH, weight of albumen, yolk and eggshell as a percentage of total egg weight, eggshell conductance (G) and conductance constant (k) values prior to storage from quail breeders at 40 and 10 weeks of age (Mean^{*} ± s.e.)

Breeder age (wk)	Albumen pH	Yolk pH	Component weight as % of total egg weight			G (mg H ₂ O /d/torr)	k (G/g)
			Albumen %	Yolk %	Eggshell %		
40	8.6 ± 0.1	6.0 ± 0.4	54.4 ^b ± 0.8	31.4 ^a ± 0.4	14.2 ± 0.6	2.8 ± 0.2	1.2 ± 0.1
10	8.7 ± 0.3	6.0 ± 0.4	56.8 ^a ± 0.6	30.1 ^b ± 0.3	13.1 ± 0.4	2.4 ± 0.1	1.1 ± 0.1

^{a,b} Means within a column with no common superscripts differ significantly ($P < 0.05$)

* 10 eggs per age group

Table 2 The effect of duration of pre-incubation egg storage and age of breeders on water loss (WL) of chicks and embryo mortality (Mean ± s.e.)

Storage time* (d)	Breeder age (wk)	WL as percentage of chick weight	Percentage embryo mortality at stages of development				Hatchability of fertile eggs (%)
			ED	LD	IP	EP	
14	40	13.7 ^b ± 0.5	9.0 ^b ± 1.3	3.4 ^{ab} ± 1.0	0.3 ^b ± 0.2	0.2 ^b ± 0.5	88.1 ^b ± 1.8
	10	14.6 ^a ± 0.6	11.5 ^a ± 1.0	4.3 ^a ± 0.7	1.4 ^a ± 0.3	1.8 ^a ± 0.4	80.7 ^c ± 1.3
4	40	13.0 ^b ± 0.5	4.7 ^c ± 0.4	2.1 ^{bc} ± 0.4	0.0 ^b ± 0.0	1.0 ^{ab} ± 0.4	92.1 ^a ± 0.1
	10	14.9 ^a ± 0.6	5.7 ^c ± 1.0	1.6 ^c ± 0.4	0.2 ^b ± 0.0	0.9 ^b ± 0.5	91.6 ^a ± 1.2

^{a,b} Means within a column with no common superscripts differ significantly ($P < 0.05$)

ED - early dead (1 to 7 d); LD - late dead; IP - Internal pip: beak in air cell; EP - External pip: beak through shell

* 4 trays per age group

The effects of treatments on PHC at different periods of incubation are presented in Table 3. At 405 h of incubation, the PHC from old breeders was higher ($P < 0.05$) at 81.5% when stored for 4 d compared to the other treatment groups. The lowest PHC (16.6%) occurred in eggs from the young breeder group stored for 14 d. At 411 h of incubation, the PHC at 35.9% was the highest ($P < 0.05$) for young breeders compared to the other treatments. At 417 h of incubation, PHC was highest ($P < 0.05$) in eggs from the young breeders stored for 14 d compared to other treatment groups. At 423 h of incubation, PHC was higher in embryos stored for 14 d irrespective of parent age, compared to those stored for 4 d. Although the PHC was significantly different between treatment groups in the remaining hours of incubation, most of the hatching had been completed at this stage in all treatments. Generally, the old breeder eggs and those stored for 4 d had required less time to hatch than the other treatments (Table 3).

Table 3 The effect of duration of pre-incubation egg storage and age of breeders on percentage of chicks hatched during six hours intervals between 405 and 447 hours of incubation (Mean \pm s.e.)

Storage time* (d)	Breeder age (wk)	Percentage of chicks hatched							
		Hours of incubation							
		405 h	411 h	417 h	423 h	429 h	435 h	441 h	447 h
14	40	46.4 ^b \pm 10.2	17.3 ^c \pm 4.1	8.5 ^b \pm 5.2	6.1 ^a \pm 1.8	4.3 ^a \pm 0.3	3.3 ^a \pm 1.1	0.7 ^a \pm 0.2	1.4 \pm 0.3
	10	16.6 ^c \pm 8.6	29.3 ^b \pm 8.5	26.7 ^a \pm 9.8	4.9 ^a \pm 2.2	0.9 ^b \pm 0.6	1.1 ^b \pm 0.4	0	1.1 \pm 0.2
4	40	81.5 ^a \pm 3.1	4.5 ^d \pm 2.4	2.6 ^b \pm 0.6	1.4 ^b \pm 0.8	0.9 ^b \pm 0.4	0	0	1.2 \pm 0.4
	10	44.6 ^b \pm 5.6	35.9 ^a \pm 5.7	9.3 ^b \pm 1.5	0.4 ^b \pm 0.3	0	0.2 ^b \pm 0.2	0.2 ^b \pm 0.2	0.9 \pm 0.5

Main Effects

Storage time** (d)									
14	31.5 ^b \pm 8.4	23.3 \pm 4.9	17.6 ^a \pm 6.2	5.5 ^a \pm 1.3	2.6 \pm 0.7	2.2 \pm 0.7	0.4 \pm 0.2	1.3 \pm 0.2	
4	63.0 ^a \pm 7.6	20.2 \pm 6.6	6.0 ^b \pm 1.5	0.9 ^b \pm 0.4	0.5 \pm 0.2	0.1 \pm 0.1	0.1 \pm 0.1	1.0 \pm 0.3	
Breeder age** (wk)									
40	63.9 ^a \pm 8.2	10.9 ^b \pm 3.2	5.6 ^b \pm 2.7	3.7 \pm 1.2	2.6 \pm 0.7	1.7 \pm 0.8	0.4 \pm 0.2	1.3 \pm 0.2	
10	30.6 ^b \pm 7.1	32.6 ^a \pm 4.9	18.0 ^a \pm 5.6	2.7 \pm 1.3	0.4 \pm 0.3	0.7 \pm 0.3	0.1 \pm 0.1	1.0 \pm 0.3	

^{a,b} Means within a column within effects with no common superscripts differ significantly ($P < 0.05$)

* n = 4; ** n = 8

An interaction was measured between storage time and breeder age in jejunum length and liver weight as a percentage of chick weight (Table 4). Fresh egg weight and chick weight were higher in the 40 wk breeder age group compared to the young group. The jejunum length of chicks from 40 wk old breeders was longer when eggs were stored for 4 d rather than for 14 d. At hatch, percentage liver weight per chick weight from 10 wk old breeders was higher in chicks when eggs were stored for 4 d than for 14 d. Heart weight as a percentage of chick weight was higher ($P < 0.05$) in chicks from 10 wk than from 40 wk old breeders.

Discussion

The hypothesis was tested that breeder age and duration of egg storage prior to incubation would affect hatching traits, time of hatch of quail eggs, and organ weights of newly hatched chicks. Data in the current study showed that breeder age and storage time and their interactions affected hatching traits and other variables. Fresh egg weight, chick weight and percentage yolk weight increased as breeder age increased. Conversely, percentage albumen weight increased in eggs from young breeders compared to old breeders. These results supported the findings of Shanawany (1984) who stated that egg sizes increased as hens got older. Therefore, the embryos and chicks from older hens would be heavier than those from younger ones. Similarly, Cunningham *et al.* (1960) stated that the weight and volume of the yolk increased with hen age. No significant differences were found in the pH of albumen and yolk between breeder age groups. Brake *et*

al. (1997) reported that albumen pH at oviposition was about 7.6 and rose to about 9.0 during storage. The albumen pH was slightly higher than commonly recorded. The possible reason of the high albumen pH in the present study might be the six hour delay in eggs leaving the research farm to the laboratory.

Table 4 The effect of duration of pre-incubation egg storage and age of breeders on fresh egg weight, chick weight and organ weights as percentage of chick weight (Mean \pm s.e.)

Storage time* (d)	Breeder age (wk)	Fresh egg weight (g)	Chick weight (g)	Jejunum length (mm)	Organ weight as % of chick weight		
					Heart	Liver	Pancreas
14	40	13.3 ^a \pm 0.3	8.8 ^a \pm 0.2	72.0 ^b \pm 3.6	0.9 \pm 0.1	2.8 ^b \pm 0.1	0.3 \pm 0.1
	10	11.9 ^b \pm 0.2	7.1 ^b \pm 0.2	71.4 ^b \pm 2.7	1.0 \pm 0.1	2.7 ^b \pm 0.2	0.3 \pm 0.01
4	40	13.6 ^a \pm 0.4	8.6 ^a \pm 0.1	85.2 ^a \pm 3.4	1.0 \pm 0.1	2.7 ^b \pm 0.1	0.3 \pm 0.03
	10	12.0 ^b \pm 0.2	7.9 ^b \pm 0.1	73.6 ^b \pm 4.8	1.1 \pm 0.1	3.1 ^a \pm 0.2	0.4 \pm 0.1

Main effects							
Storage time** (d)							
14		12.6 \pm 0.2	7.9 \pm 0.3	71.7 ^b \pm 2.1	0.9 \pm 0.1	2.7 \pm 0.1	0.3 \pm 0.03
4		12.8 \pm 0.3	8.2 \pm 0.1	79.4 ^a \pm 3.4	1.0 \pm 0.1	2.9 \pm 0.1	0.4 \pm 0.03
Breeder age** (wk)							
40		12.6 \pm 0.2	7.9 \pm 0.3	71.7 ^b \pm 2.1	0.9 \pm 0.1	2.7 \pm 0.1	0.3 \pm 0.03
10		12.8 \pm 0.3	8.2 \pm 0.1	79.4 ^a \pm 3.4	1.0 \pm 0.1	2.9 \pm 0.1	0.4 \pm 0.03

^{a,b}; Means within a column within effects with no common superscripts differ significantly ($P < 0.05$)

* n = 10; ** n = 20

The present study demonstrated that early embryonic deaths were significantly higher in eggs stored for an extended period (14 d) than for 4 d. Furthermore, the deleterious effect of extended pre-incubation storage was more pronounced in embryos of eggs obtained from young than from older breeders. However, embryo mortality did not differ between breeder age groups in eggs which were stored for 4 d. It can be concluded that embryos in eggs stored for 14 d (particularly, from young breeders) were at a greater risk of ED than eggs stored for 4 d. These results partly agreed with Fassenko (1996) who found that early embryonic mortality increased when eggs were stored for extended periods of time. Similar results were also reported in domestic fowl and turkey eggs when their eggs were stored for up to two weeks (Bakst & Gupta, 1997).

Eggs from the young breeders had higher incidences of IP and EP in embryos when stored for 14 d prior to setting compared to the other treatment groups. There could be two possible explanations for this: Firstly, the maternal investment provided by young breeders might not have been sufficient to protect the embryo for the detrimental effects of long storage periods. Fassenko (1996) stated that long-term storage of eggs may result in an increase in energy requirements of the embryo and this could deplete glycogen resources below levels critical for survival. Secondly, the initiation of embryonic development in eggs stored for 14 d in young breeder eggs could have increased cell necrosis. Lapao *et al.* (1999) determined that pre-incubation egg storage lead to morphological changes in the blastoderm and malformations with increased cell necrosis. The results are also partly in agreement with Fassenko (2001) who observed that internal and external pipping were significantly higher in eggs stored for 14 d compared to 4 d.

In the current study it was not possible to correlate eggshell conductance with increasing IP and EP. Thus, high incidence of IP and EP in eggs from young breeders when exposed to 14 d storage could not be attributed to eggshell conductance. This is in contrast with Christensen *et al.* (1995) who found that chicken eggs with embryos that died during internal pipping had less permeability compared with eggs that hatched. However, these observations in the study do not discount the importance of eggshell conductance.

The interaction between breeder age and storage length influenced the hatchability of fertile eggs (Table 2). Hatchability of fertile eggs was depressed following extended storage of eggs (14 d), irrespective of breeder age compared to those stored for 4 d prior to setting. Additionally, the deleterious effects of long

storage on hatchability of fertile eggs were more pronounced for eggs from the young breeders than from the older breeders. These results agree with those of Kosin (1950), Sittman *et al.* (1971), Mayes & Takeballi (1984) and Meijerhof (1994) who reported that hatchability declined after seven or more days of storage. However, it disagrees with that of Christensen *et al.* (1993) who found that hatchability of fertile eggs declined as hens age. This discrepancy between the two studies might be attributable to differences in breeder age and species (turkey *vs.* quail).

Time of hatch is important because chicks held in incubator trays for 14 to 32 h post-hatch weighed 5 to 12% less than chicks removed promptly, and this reduction in weight persisted up to 49 days of age. As a consequence, lost weight may never be regained due to the age at which broilers are marketed (Swann & Brake, 1990). Time of hatch was significantly effected by an interaction between flock age and length of storage. The longer storage period (14 d) lengthened the incubation period in chicks, and this was more pronounced in eggs from the young breeders. The current study showed that incubation time extended as storage period increased. These findings are in general agreement with the results of Smith & Bohren (1975) who stated that after long storage, eggs from older hens tended to hatch earlier compared with those from younger hens. Likewise, Tona *et al.* (2003) found that an 18 d pre-incubation storage prolonged incubation by at least 15 h compared with eggs stored for 3 d. Furthermore, incubation time decreased as hens got older. Joseph (2004) stated that the relationship between breeder age and incubation length is poorly understood because it is not a function of egg weight. However, the results in the present study imply that egg weight is an important determinant for incubation length of quail eggs. This agrees with Wilson (1991b) who recorded that incubation time was positively correlated with egg weight. Butler (1991) found that the embryos of eggs laid by older birds were more developed and at a more advanced stage compared with embryos of eggs from young birds. Shanawany (1984) indicated that egg size increased as hens got older and total incubation period reduced with breeder age. Furthermore, Suarez *et al.* (1997) found that an increased breeder age from 29 wk to 41 or 47 wk resulted in a decreased duration of incubation.

Supply organs were defined as heart, liver, intestine (Christensen *et al.*, 2002), pancreas and gallbladder (North & Bell, 1990). Embryonic body and organ growth are highly conserved biological traits and embryos may regulate physiologically between growths and function when presented with life-threatening situations (Christensen *et al.*, 2002). Data in the current study showed that breeder age and egg storage period and their interactions affected embryonic organ weights differently. As storage period increased the jejunum length decreased in chicks from old breeders. These results are partly in agreement with Singal & Cosin (1969) who found a decrease in the frequency of dividing cells in the embryo spleen in stored eggs. They suggested that continual sub-optimal activity of the cells during storage might induce irreversible physiochemical changes in metabolism by lowering the capacity of the cell to maintain vital activities during subsequent incubation, such as protein synthesis.

As length of egg storage increased, percentage of liver weight decreased in chicks from eggs of the young breeders. This agrees partly with Christensen *et al.* (1996) who found that in turkeys liver weight expressed as a percentage of whole body weight was greatest in embryos from eggs produced by young hens compared of those from the oldest hens.

The heart weights were lower in chicks from old than from young breeders regardless of storage period. This may be the result of time of hatch. The chicks from old breeders hatched earlier and spent more time in hatcher compared with those from young breeders. Fasenko (1996) found that if poults stayed in the hatcher more than the normal period of time after hatch, this may act as a stressor, as indicated by depressed heart glycogen concentration and increased glucose level. Both are indications that glycogen has being mobilized to increase blood glucose levels in response to the stress of being kept in the hatcher.

Conclusion

Data in the current study demonstrated that the breeder age, storage time and their interactions have different effects on hatching results. The hatchability of fertile eggs declined as egg storage time increased. In addition, the responses to an extended storage period of embryos obtained from different age groups were not the same. This may be attributed to different egg components. Particularly, the young breeders invested less yolk to their eggs than old breeders. This raises the question of various pre-storage treatments to determine if different pre-storage treatments would be beneficial to increase hatchability in eggs obtained from young and old breeders when stored for extended periods.

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