G.J.A.S.R

GLOBAL JOURNAL OF ANIMAL SCIENTIFIC RESEARCH

Journal homepage: www.gjasr.com

Print ISSN:2345-4377 *Online ISSN:2345-4385*

Original Article

Effects of Short Periods of Incubation During Egg Storage (SPIDES) in Prolonged Stored Eggs of Late DeKalb Breeder on Hatchability, Embryonic Mortality and Chick Quality

Atif A. Hamza[1], Osama E. Yassin[2], Elfadil A. Adam[2], Badr H. Eljack[2], Abubakr. S. Ali[2] and Mutaz S. Babiker[3]*

[1]Poultry Research Section, Animal Production Research Center, Animal Resources Research Corporation, Sudan
[2]Sudan University of Science and Technology, College of Animal Production Science and Technology, Sudan
[3]Department of Poultry Production, Faculty of Animal Production, University of Gezira, Sudan

ABSTRACT

The objective of this study was to study the effects of short periods of incubation during egg storage (SPIDES) of late DeKalb breeder, age and storage period on the hatchability, embryo mortality and chick quality. A total of 810 fertile eggs were randomly selected and allocated into three groups (270 eggs each). Each group was distributed in a 3 x 3 x 3 factorial experiment in a complete randomized design with three warming treatments (0, 60 and 120 minutes daily at 37.5° C and 53% RH), age (75, 80, and 85 weeks) and three storage periods (4, 9 and 14 days 18° C and 75%). Each treatment was replicated three times with ten eggs each. Fertility and hatchability of both fertile and total eggs were determined. Embryo mortality was determined in non-hatched eggs. All hatched chicks were weighed and graded to first or second grade chick. Results indicated that SPIDES for 60 minutes significantly improved the hatchability, reduced early dead embryos and total unhatched eggs and chick quality as compared to non-heated eggs or SPIDES for 120 minutes. The obtained results indicated that the daily SPIDES for 60 minutes significantly ($P \leq 0.01$) reduced embryonic mortality during the three incubation periods. The lower embryonic mortality was observed for 75 week old breeders and for eggs stored for 4 days. Moreover, SPIDES eggs for 60 minutes significantly ($P \leq 0.01$) improved hatchability and chick quality percentages. Also the best hatchability and chick quality

Corresponding Author: Mutaz S. Babiker < mutaz_babiker@yahoo.com>

Cite this Article: Hamza A, Yassin O, Adam E, Eljack B, Ali A, Mahmoud MSB. (2020). Effects of Short Periods of Incubation During Egg Storage (SPIDES) in Prolonged Stored Eggs of Late DeKalb Breeder on Hatchability, Embryonic Mortality and Chick Quality. *Global Journal of Animal Scientific Research*, 8(2), 68-82.
Retrieved from http://www.gjasr.com/index.php/GJASR/article/view/49
Article History: Received: 2020-07-04 Accepted: 2020-08-22

percentages were observed for the breeder of 75 week of age. Eggs stored for 4 days reported the best hatchability and chick quality percentages. In conclusion, daily SPIDES of late DeKalb breeder hatching eggs for 60 minutes at 37.5° C and 53% RH could be used by the poultry industry as a method to improve hatchability and increased the number of saleable first grade chicks which by far increases profits of eggs stored for longer periods.

Keywords: Incubation, SPIDES, Egg storage periods, Embryonic mortality, Hatchability, Chick quality

INTRODUCTION

The storage of hatching eggs is a common routine in hatcheries and varying in duration depending on hatchery capacity and the demand of day old chicks (Gharib, 2013). In some cases, the need of extending the period of storage more than seven days may takes place. Hatching eggs are stored, generally, to minimize transport cost or to collect enough eggs for high capacity incubators. Egg storage for more than 7 days deteriorated the egg white, increased embryonic mortality and chicks' abnormalities (Van de Ven, 2004) reduced hatchability percentage and post-hatch performance (Petek and Dikmen, 2006; Ruiz and Lunam, 2002; Tona *et al.*, 2003). It is also responsible for impairing embryo development and livability with a lag in embryo development due to change of metabolism rate (Christensen *et al.*, 2001; Elibol et al, 2002; Fasenko and O'Dea., 2009), and decline in hatchability (Yassin *et al.*, 2008). Many studies have been applied to enhance the hatching of eggs stored more than 7 days. Many suggested methods to modify the eggs storage conditions in order to prolong the period in which they maintain their high ability to hatch well. Pre-incubation (PI), i.e., temporary warming of eggs, which is supposed to imitate the natural conditions that the birds provide to the eggs before they start to hatch, turned out to be the most effective method (Fasenko *et al.*, (2001a, b) and demonstrated that a single (PI) allows the embryos to finish the formation of hypoblasts, which increases the ability of hatching up to 14 d of eggs storage. Dymond *et al.*, (2013) modified this method by analyzing an effect of short periods of incubation during egg storage (SPIDES). The most promised one is to warm eggs before or during storage (Anonymous, 2000). Warming of eggs before storage has been reported to increase hatchability and reduce embryonic mortality (Fasenko *et al.*, 2001ab). The objective of this study is to illustrate the role of (SPIDES) (60 and 120 minutes) of eggs during egg storage (4, 9, and 14days) and flock age (75, 80, and 85 weeks) on subsequent hatchability traits.

MATERIALS AND METHODS

Experimental design

The experiment was conducted at a hatchery unit of Coral Company for Feed and Chicks Production. A total of 810 fertile eggs, clean and without shell abnormalities from DeKalb White layer breeder flock at different ages (75, 80, and 85 weeks) were randomly selected and transported to the hatchery in three groups (270 eggs each). Each group was distributed in a 3 x 3 x 3 factorial arrangement in a complete randomized design, with three daily warming durations (SPIDES) (0, 60 and 120 minutes) at 37.5°C and 53% RH), age (75, 80, and 85 weeks) and three storage

periods (4, 9 and 14 days 18○ C and 75%) summing up twenty-seven treatments with three replicates ten eggs each placed in setting trays.

General management

Eggs were collected three times a day and transported to the hatchery and immediately disinfected by simple fumigation with 3.2g paraformaldehyde⁄m3 area for 20 minutes heated in an electric pan to 105°C in the fumigation room at 25°C and relative humidity 70%. After disinfection the room was ventilated with fresh air for 1.5 hrs to remove the fumigation residues. Hatching eggs in the control (0 minutes) were kept in the cooler at 18°C and relative humidity 75% during the entire storage period. The two treatments were placed in a setter (Pas Reform, type Corridor 57, 2002, Zeddam) operating at 37.5°C and 53% RH, removed after 60 and 120 minutes daily warming and transferred to the egg storage room. This protocol was repeated on the three storage periods (4, 9 and 14 days). After 4, 9 and 14 days of storage period the eggs were set in a setter (Pas Reform, type Corridor 57, 2002, Zeddam) at 37.5°C average temperature and 53% RH, eggs were hourly turned for 18 days using single stage incubation program of layer eggs. At day 18 of incubation, hatching eggs (HE) were candled and consequently the clear eggs were removed and opened to determine macroscopically infertile or stage of embryonic mortality to calculate true fertility. After candling, HE with living embryos were transferred to the hatchery baskets and were placed in the hatcher cabinets (Pas Reform, Tiros, 2002, Zeddam) in which the temperature and relative humidity were adjusted at 36.6°C and 75% RH.

Measured parameters:

At the end of the hatching process hatched chicks and pipped eggs were removed and counted. All chicks were classified as first or second grade chicks based on the physical parameters. A chick was classified as a first grade chick if it was clean, dry, free of deformities or lesions and had bright eyes. The other chicks were classified as second grade chicks. The remaining unhatched eggs were broken to determine the late stage of embryonic mortality. At 18 and 21 day of incubation the following periods and phases of embryonic mortality were used to classify the dead embryos. The main characteristics observed in the current study based on description of Tona *et al.*, (2004) who reported them as follows:

Days 1 – 7 (white membrane over the yolk, blood ring).

Days 8 – 14 (black eye visible, embryo without down).

Days 15 – 21 (small embryo with down, full grown embryo with yolk out or full grown dead embryo).

The fertility, hatchability and mortality records were reported according to (Erensayin, 2002) as follows:

True Fertility (%) = Number of fertile eggs ⁄ total number of eggs set X100

Hatchability eggs set (%) = Number of chicks hatched ⁄ total number of eggs set X 100

Hatchability of fertile eggs (%) = Number of chicks hatched ⁄ total number of fertile eggs X100

Early phase mortality (%) = Number of embryos dead in early phase ⁄ number of unhatched eggs X100

Middle phase mortality (%) = Number of embryos dead in middle phase ⁄ number of unhatched eggs X100
Late phase mortality (%) = Number of embryos dead in late phase ⁄ number of unhatched eggs ×100
First grade chicks (%) = Number of first grade chicks ⁄number of chicks hatched×100
Second grade chicks (%) = Number of second grade chicks ⁄number of chicks hatched×100

Statistical analysis

Data were subjected to ANOVA using the General Linear Model procedure of SPSS (2008). Duncan's multiple range test used to assess the significant differences among treatment means according to the method described by (Steel *et al.*, 1996).

RESULTS AND DISCUSSION

Effect of Short period incubation during storage (SPIDES) treatment, breeder's age and storage period on embryonic mortality

Early death, mid death, late death and total embryonic mortality percentages were significantly influenced by the experimental treatments (Table 1). Short period incubation (SPIDES) treatment for (60 minutes) resulted in significantly ($P \leq 0.01$) the lowest percentages of early, late and total embryonic mortality when compared with the other short period incubation (120 minutes) or (zero minutes) group, while Mid death was not influenced by the (SPIDES) treatment. Early death, late death and total embryonic mortality percentages were significantly ($P \leq 0.01$) increased by breeder's age, while no significant effect in mid death. Four days of storage period resulted in significantly ($P \leq 0.01$) reduction in early death and total embryonic mortality followed by nine. Mid death results showed a significant ($P \leq 0.05$) reduction for eggs stored for four days compared to nine and fourteen days of storage period. Late death was not influenced by the storage period. The reduction in embryonic viability during egg storage is due to the apoptosis (cell death) in the egg (Bakst, 2016). Until seven days of proper egg storage, the number of embryonic cells remains stable, then after seven days, the number of dead and abnormal cells started to increase. Maintaining lower temperature and higher humidity during egg storage can dramatically improve cell viability for eggs to be stored long term (Fasenko, 2007). Similar findings w reported by Gharib (2013) who found that significantly higher rate of late embryonic mortality for egg stored for10 and 14 d compared to 4 and 7 d of storage. Hamidu *et al.*, (2011) explained the deleterious effect of prolonged storage on broiler and layer blast dermal cell viability, cell death and embryo survival. Significant interactions were also detected between the SPIDES treatment duration and storage period on all embryonic mortality rates (Table 2). The results indicated that SPIDES treatment for 60 minutes significantly decreased embryonic mortality within all storage periods as compared to those not warmed or warmed for 120 minutes at 75, 80 and 85 week of age breeder's eggs respectively except for mid death which showed no significant differences. When eggs were stored for more than four days, total embryonic mortality rates were significantly lower when eggs were exposed to SPIDES treatment for 60 minutes, as compared to those not heated or heated for 120 minutes at 75, 80 and 85 weeks old breeder's eggs respectively. This results are in agreement with the previous

reports on broilers, turkey and Japanese quail chicks (Anonymous, 2000; Fasenko *et al.*, 2001a, b) warming eggs before or during storage was reported to increase hatchability and reduce embryonic mortality. Tag EL-Din, *et al.*, (2017) recommended that when storage of eggs to more than seven days, one should warm eggs for 2.5 h every five days to minimize the harmful impact of storage. These results are in agreement with the present study. Reijrink *et al.*, (2010) reported that significantly higher late embryonic mortality rate observed for egg stored for 10 and 14 d compared to 4 and 7 d storage.

Effect of Short period incubation during storage (SPIDES), breeder's age and storage period on fertility and hatchability:

The results of the true fertility, hatchability of total and fertile eggs were shown in Table 3. There were no significant effects of Short period incubation (SPIDES) treatment for (0, 60 and 120 minutes), storage period (4, 9 and 14 days) on the true fertility percentage. Storage heating eggs did not affect apparent fertility. Fertility should not have been affected by the two main treatments because fertilization would or would not have occurred before the eggs were exposed to the treatments. Similar suggestions were reported by Fasenko *et al.*, (2001a) in chicken eggs, and Petek and Dikmen (2004) in quail eggs. They found that the differences for the apparent fertility among the main groups of pre-storage heating and storage duration were not significant. On the other hand, true fertility percentage was significantly ($P \leq 0.01$) affected due to breeder's age. The highest values obtained from eggs produced by 75 weeks old breeders followed by eggs produced from 80 weeks old breeders and the lowest values obtained from eggs produced from 85 week old breeders. True fertility percentage was not affected by the all interactions between factors. These results are in agreement with those reported by Fasenko *et al.*, (2001) who showed that fertility of broiler breeder eggs was not affected by the interaction as fertilization would or would have not occurred before the eggs were exposed to the pre-storage incubation (0, 12 or 18 hrs) or by storage periods (4 or 14 days). Similarly, Elibol *et al.*, (2002) did not find any significant effects on the apparent fertility when they stored eggs for four, seven, ten and fourteen days at 18°C and 75% RH. Pre-warming treatment did not show any significant effect on the number of fertile eggs and fertility%.

Hatchability of fertile and total eggs was significantly ($P \leq 0.01$) affected by the experimental factors. The results showed that higher percentages of both hatchability of fertile or total eggs set were observed for groups exposed to short period incubation (SPIDES) treatment for 60 minutes followed by those SPIDES for 120 minutes and the poorest values observed for control group (0 minutes). The highest values for the two parameters obtained from eggs produced by 75 weeks old breeders followed by those produced by 80 weeks old breeders and the lowest values obtained from eggs produced from 85 week old breeders. Longer period of egg storage resulted in a linear significant decrease in the hatchability of fertile and total eggs. A significant ($P \leq 0.01$) improvement in hatchability from total eggs and hatchability from fertile eggs was observed for eggs stored for 4 days followed by those eggs stored for 9 days and the lowest values stand for eggs stored for 14 days. The interaction between SPIDES treatment (minutes) and storage period days showed a highly significant ($P \leq 0.01$) effect on both hatchability on fertile and total eggs. SPIDES treatment for 60 minutes

significantly increased hatchability in eggs stored for more than 4 days as compared to those not heated or heated for 120 minutes at 75, 80 and 85 week of old breeder's eggs respectively (Table 4). Lower rates of fertilization, hatchability, and higher embryonic mortality at various incubation periods of older hens' eggs are caused by a number of biological factors such as decreased sperm retention in the uterovaginal sperm host glands (Fasenko *et al.,* 1992) and deteriorating egg quality (Reijrink *et al.,* 2008). In this study, short period incubation during storage (SPIDES) treatment for 60 minutes for 4 days of eggs storage allowed for an improvement of hatchability, mainly from eggs of older hens and may be considered as a good practice to improve incubation results of eggs stored for short, intermediate, and long periods. These results are consistent with previous reports by Reijrink *et al.,* (2010), who improved hatching from eggs from older hens stored for 11 d and treated with PI. Short period of incubation during storage (SPIDES) treatment for 60 minutes may be considered as a good practice to improve incubation results of eggs stored for short, intermediate, and long periods. Tag EL-Din *et al.,* (2017) reported that warming egg at 2.5 and 5 hours showed the highest hatchability from total eggs and hatchability from fertile eggs. In further contrast to previous studies by Fasenko *et al.,* (2001b) and Reijrink *et al.,* (2009) found a positive effect on hatchability when advancing SPIDES embryos to early primitive streak formation over several short pre-incubation, advancement of embryos to hypoblast formation or primitive streak formation in 6- or 12-h pre-incubation, respectively, showed a detrimental effect. Dymond *et al.,* (2013) have shown that three-to-four 'short periods of incubation during egg storage' or 'SPIDES' of 21 days increased hatchability and reduced hatching time when compared with eggs stored for similar periods of 21 days (controls). These findings are in agreement with Damaziak *et al.,* (2018) who demonstrated that the 2 × 4 h pre-incubation during 12 d of eggs storage allowed for an improvement of hatchability, mainly from eggs of older hens.

Effect of Short period incubation during storage (SPIDES) treatment, breeder's age and storage period on chick quality:

Commercial chick quality grading was used for measuring the chick quality. Chick quality grade studied were significantly **(**$P \leq 0.01$) affected by short period incubation (SPIDES) treatment for (0, 60 and 120 minutes), breeder's age (75, 80 and 85week) and storage period (4, 9 and 14 days) (Table 5). Short period incubation (SPIDES) treatment for 60 minutes resulted in significant **(**$P \leq 0.01$) improvement in both chick quality grades followed by short period incubation (SPIDES) treatment for 120 minutes, as compared to non-heated eggs. Breeder's age significantly **(**$P \leq 0.01$) affected the chick quality. Chicks produced from 85 weeks of age breeders were significantly **(**$P \leq 0.01$) lower in quality (lower percentage of first grade chicks and higher percentage of second grade chicks) compared to those chicks hatched from 75 and 80 week of age of breeders. No significant differences between chicks hatched from 75 and 80 weeks of age breeders. Long storage period 14 days resulted in significant **(**$P \leq 0.01$) lower quality hatched chicks compared to those hatched from eggs stored for 4 or 9 days. No significant differences in chick's quality between chicks hatched from eggs stored for 4 or 9 days. Longer periods of storage affected the vitality of the embryo, causing increased early and late embryonic mortality, a delay

in hatch and reduced chick quality (Fasenko, 2007; Dymond, 2013)**.** There were significant interactions between the storage period and SPIDES treatment duration for chicks' grade (Table 6). The obtained data indicated that the chicks produced from SPIDES treatment for 60 minutes and stored for 4, 9 and14 days had significantly higher percentages of grade (A) chicks, as compared to non-warmed eggs at 75, 80 and 85 week old breeder's eggs respectively (Table 3). Tag EL-Din, *et al.*, (2017) reported that warming egg at 2.5 and 5 hours showed highest significance for chick quality. Damaziak *et al.*, *(*2018) showed that pre incubation had increased the hatchability of the set and apparently fertilized eggs, decreased the number of unhatched eggs, and improved chick's quality.

In conclusion short periods of incubation during egg storage daily for 4- 9 days of late DeKalb breeder's (75-80 weeks) hatching eggs for 60- 120 minutes at 37.5° C and 53% RH could be used by the poultry industry as a method to improve hatchability, decrease embryonic mortality percentage and increase the number of saleable first grade chicks which by far increases profits of eggs store for longer periods.

Table 1. Effect of short period of incubation during storage (SPIDES), breeder's age and storage period on embryonic mortality

	Embryonic mortality (%)			
Main factors	Early death	Mid death	Late death	Total mortality
Overall mean	19.42	3.41	7.20	30.04
SEM	0.359	0.148	0.236	0.434
SPIDES (minutes)				
0	25.19^{a}	3.58	8.77^{a}	37.53^{a}
60	14.32^{c}	2.96	5.93^{c}	23.21^{c}
120	18.77^{b}	3.70	6.92^{b}	29.38^{b}
SEM	0.621	0.257	0.41	0.751
Significant	**	NS	**	**
Age (week)				
75	14.32^{c}	3.83	5.93^{c}	24.07^{c}
80	19.01^{b}	2.96	7.16^{b}	29.14^{b}
85	24.94^{a}	3.45	8.52^{a}	36.91^{a}
SEM	0.621	0.257	0.41	0.751
Significant	**	NS	**	**
Storage (days)				
4	11.36^{c}	2.84^{b}	6.79	20.99^{c}
9	18.89^{b}	3.45^{a}	6.79	29.13^{b}
14	28.03^{a}	3.95^{a}	8.03	40.00^{a}
SEM	0.621	0.257	0.41	0.751
Significant	**	*	NS	**

N=27/treatment, SEM=standard error of mean. Different superscript letters under the same factor in the same column means significant differences **=significant difference at P<0.01, *=significant difference at P<0.05, NS=No significant differences

Table 2. Interaction effects of short period of incubation during storage (SPIDES), breeder's age and storage period on embryonic mortality (%)

	Embryonic mortality (%) at different breeder's age (week)											
	Early death %			Mid death %			Late death (%)			Total mortality (%)		
SPIDES × Storage	75	80	85	75	80	85	75	80	85	75	80	85
0hr × 4 days	14.82^{def}	15.56^{d}	15.56^{ef}	2.59^{b}	1.11^{b}	3.33	8.15^{ab}	8.89	8.89^{b}	25.55^{de}	25.56^{cde}	27.78^{e}
60mins × 4 days	7.78^{g}	7.78^{f}	7.78^{g}	2.59^{b}	1.11^{b}	3.33	5.56^{b}	6.67	7.78b^{c}	15.93^{f}	15.56^{f}	18.89^{f}
120mins × 4 days	11.48^{fg}	11.11^{ef}	12.22^{fg}	3.33^{ab}	3.33^{a}	3.33	6.67^{b}	7.78	7.78^{bc}	21.48^{ef}	22.22^{e}	23.33^{ef}
0hr × 9 days	24.07^{bc}	20.00^{c}	28.89^{cd}	3.7^{ab}	3.33^{a}	4.44	7.78^{b}	7.78	10.00^{ab}	35.55^{bc}	31.11^{c}	43.33^{c}
60mins × 9 days	14.07^{efg}	13.33^{de}	20.00^{e}	3.33^{ab}	3.33^{a}	3.33	5.56^{b}	6.67	5.56^{c}	22.96^{ef}	23.33^{de}	28.89^{e}
120mins × 9 days	18.52^{cde}	15.56^{d}	26.67^{d}	3.33^{ab}	3.33^{a}	3.33	7.04^{b}	8.89	5.56^{c}	28.89^{cde}	27.78^{cde}	35.55^{d}
0hr × 14 days	36.67^{a}	37.78^{a}	43.33^{a}	4.44^{a}	4.44^{a}	4.44	10.37^{a}	8.89	12.22^{a}	51.48^{a}	51.11^{a}	60.00^{a}
60mins × 14 days	21.11^{bcd}	21.11^{c}	33.33^{bc}	2.96^{ab}	3.33^{a}	2.22	6.67^{b}	4.44	8.89^{b}	30.74^{bcd}	28.88cd	44.44^{bc}
120mins × 14 days	26.30^{b}	28.89^{b}	36.67^{b}	4.44^{a}	3.33^{a}	3.33	7.04^{b}	4.45	10.00^{ab}	37.78^{b}	36.67^{b}	50.00^{b}
SEM	1.166	1.769	2.253	0.165	0.272	0.217	0.312	0.494	0.482	1.358	1.946	2.559
Significant	**	**	**	*	*	NS	**	NS	**	**	**	**

N=27/treatment, SEM=standard error of mean

Different superscript letters under the same factor in the same column means significant differences

NS=No significant differences **=significant difference at P<0.01, *=significant difference at P<0.05

Table 3. Effect of short period of incubation during storage (SPIDES), breeder's age and storage period on fertility and hatchability percentage

	%		
Main factors	True fertility	Hatchability from total eggs	Hatchability from fertile eggs
Overall mean	67.41	37.24	54.50
SEM	0.485	0.414	0.495
SPIDES (minutes)			
0	67.04	29.26^{c}	42.87^{c}
60	67.16	43.95^{a}	64.76^{a}
120	68.03	38.52^{b}	55.88^{b}
SEM	0.840	0.716	0.857
Significant	NS	**	**
Age (weeks)			
75	73.83^{a}	49.88^{a}	67.58^{a}
80	66.54^{b}	37.04^{b}	55.70^{b}
85	61.85^{c}	24.82^{c}	40.23^{c}
SEM	0.840	0.716	0.857
Significant	**	**	**
Storage (days)			
4	66.91	45.93^{a}	68.31^{a}
9	67.41	37.78^{b}	55.34^{b}
14	67.90	28.02^{c}	39.86^{c}
SEM	0.840	0.716	0.857
Significant	NS	**	**

N=27/treatment, SEM=standard error of mean. Different superscript letters under the same factor in the same column means significant differences **=significant difference at P<0.01, NS=No significant differences

Table 4. Interaction effects of short period of incubation during storage (SPIDES), breeder's age and storage period on fertility and hatchability percentage

	fertility and hatchability percentage at different breeders' age (week)								
	True fertility (%)			Hatchability from total eggs (%)			Hatchability from fertile eggs (%)		
SPIDES × Storage	75	80	85	75	80	85	75	80	85
0hr × 4 days	67.04	66.67	62.22	41.48^{abcd}	41.11^{bc}	34.45^{bc}	61.54^{b}	61.69^{bc}	55.28^{c}
60mins × 4 days	66.67	66.67	60.00	50.74^{a}	51.11^{a}	41.11^{a}	75.77^{a}	76.88^{a}	68.56^{a}
120mins × 4 days	67.04	65.56	61.11	45.56^{ab}	43.33^{b}	37.78^{ab}	67.64^{ab}	66.11^{b}	61.94^{b}
0hr × 9 days	67.41	66.67	63.33	31.11^{d}	35.55^{c}	17.78^{ef}	45.62	53.42^{d}	28.03^{e}
60mins × 9 days	67.04	66.67	61.11	43.70^{abc}	42.22^{b}	32.22^{c}	64.50^{ab}	63.44^{bc}	52.65^{c}
120mins × 9 days	67.78	66.67	61.11	38.52^{abcd}	37.78^{bc}	25.55^{d}	55.89^{bc}	56.76^{cd}	41.69^{d}
0hr × 14 days	66.67	66.67	60.00	15.19^{e}	15.56^{e}	0.00^{g}	21.44^{d}	23.28^{f}	0.00^{g}
60mins × 14 days	67.78	66.67	63.33	37.41^{bcd}	37.78^{bc}	20.00^{e}	54.02^{bc}	56.59^{cd}	31.54^{e}
120mins × 14 days	69.26	66.67	64.45	31.48^{cd}	28.89^{d}	14.44^{f}	44.12^{c}	43.15^{e}	22.41^{f}
SEM	0.693	0.576	0.782	1.662	1.927	2.468	2.223	2.910	4.061
Significant	NS	NS	NS	**	**	**	**	**	**

N=27/treatment, SEM=standard error of mean

Different superscript letters under the same factor in the same column means significant differences

NS=No significant differences **=significant difference at P<0.01, *=significant difference at P<0.05.

Table 5. Effect of short period of incubation during storage (SPIDES), breeder's age and storage period on chick quality

Main factors	First Grade (%)	Second Grade (%)
Overall mean	85.21	14.79
SEM	0.703	0.703
SPIDES (minutes)		
0	69.66^{c}	30.34^{a}
60	95.10^{a}	4.90^{c}
120	90.88^{b}	9.12^{b}
SEM	1.217	1.217
Significant	**	**
Age (weeks)		
75	91.33^{a}	8.67^{b}
80	89.05^{a}	10.96^{b}
85	75.27^{b}	24.73^{a}
SEM	1.217	1.217
Significant	**	**
Storage period (day)		
4	90.72^{a}	9.28^{b}
9	87.96^{a}	12.04^{b}
14	76.96^{b}	23.04^{a}
SEM	1.217	1.217
Significant	**	**

N=27/treatment, SEM=standard error of mean. Different superscript letters under the same factor in the same column means significant differences. NS=No significant differences **=significant difference at P<0.01, *=significant difference at P<0.05

Table 6. Interaction effect of short period of incubation during storage (SPIDES), breeder's age and storage period on chick quality

SPIDES × Storage	chick quality percentage at different breeders' age (week)					
	1st Grade			2nd Grade		
	75	80	85	75	80	85
0hr × 4 days	83.11^{ab}	89.32^{a}	73.74^{c}	16.89^{ab}	10.68^{b}	26.26^{a}
60mins × 4 days	95.97^{a}	97.78^{a}	91.88^{a}	4.03^{c}	2.22^{b}	8.12^{d}
120mins × 4 days	93.08^{a}	95.06^{a}	88.13^{abc}	6.92^{c}	4.94^{b}	11.87^{abc}
0hr × 9 days	77.08^{b}	75.00^{b}	75.56^{bc}	22.92^{a}	25.00^{a}	24.44^{ab}
60mins × 9 days	94.92^{a}	94.87^{a}	93.94^{a}	5.08^{c}	5.13^{b}	6.06^{d}
120mins × 9 days	91.88^{a}	94.19^{a}	87.83^{abc}	8.12^{c}	5.81^{b}	12.17^{abc}
0hr × 14 days	48.80^{c}	65.00^{b}	0.00^{d}	17.87^{ab}	35.00^{a}	0.00^{d}
60mins × 14 days	94.40^{a}	97.22^{a}	89.68^{ab}	5.60^{c}	2.78^{b}	10.32^{bc}
120mins × 14 days	87.69^{ab}	92.96^{a}	76.67^{bc}	12.31^{bc}	7.04^{b}	23.33^{ab}
SEM	2.183	2.302	5.542	1.156	2.302	2.091
Significant	**	**	**	**	**	**

N=27/treatment, SEM=standard error of mean

Different superscript letters under the same factor in the same column means significant differences

**=significant difference at P<0.01

REFERENCES

Anonymous (2000). Preheating of ostrich eggs. Int.Hatch. Pract., 14 (Abstract), 32.

Bakst M. R. Welch, G. R. Fetterer, R. and Miska, K. (2016). Impact of broiler egg storage on the relative expression of selected blastoderm genes associated with apoptosis, oxidative stress, and fatty acid metabolism. *Poult. Sci.*, 95:1411–1417.

Christensen, V. L., Wineland, M. J., Fasenko, G. M. and Donaldson, W. E. (2001). Egg storage effects on plasma glucose and supply and demand tissue glycogen concentrations of broiler embryos. *Poult. Sci.*, 80:1729–1735.

Damaziak, K., Paweska, M., Gozdowski, D. and Niemiec, J. (2018). Short periods of incubation, egg turning during storage and broiler breeder hens age for early development of embryos, hatching results, chicks quality and juvenile growth. *Poultry Science*, 97:3264-3276.

Dymond, J., Vinyard, B., Nicholson, A. D., French, N. A. and. Bakst, M. R. (2013). Short periods of incubation during egg storage increase hatchability and chick quality in long-stored broiler eggs. *Poult. Sci.* 92:2977–2987.

Elibol, O., Peak, S. D. and Brake, J. (2002). Effect of flock age, length of egg storage and frequency of turning during storage on hatchability of broiler hatching eggs. *Poult. Sci*., 81:945–950.

Erensayin, C. (2000). Scientific Technical Practical Poultry Broiler breeding and hatchability. Nobel Publication. Ankra, Turkey in Turkish English abstract. Vol.1.2nd rev.ed.

Fasenko, G. M. (2007). Egg storage and the embryo. *Poult. Sci*., 86 (5), 1020-1024.

Fasenko, G. M. and O'Dea, E E. (2009). Evaluating broiler growth and mortality in chicks with minor level conditions at hatching. *Poult. Sci*., 87: 594 -597.

Fasenko, G. M., Christensen, V. L. Wineland, M. J. and Pettite, J. N. (2001b). Examining the effects of pre storage incubation of turkey breeder eggs on embryonic development and hatchability of eggs stored for four or fourteen days. *Poult. Sci.*, 80:132–138.

Fasenko, G. M., Hardin, R. T. and Robinson. F. E. (1992). Relationship of hen age and egg sequence position with fertility, hatchability, viability, and pre incubation embryonic development in broiler breeders. *Poult. Sci*. 71:1374–1383.

Fasenko, G. M., Robinson, F. E., Whelan, A. I., Kremeniuk, K. M. and Walker, J. A. (2001a). Pre storage incubation of long-term stored broiler breeder eggs: 1. Effects on hatchability. *Poult. Sci.*, 80:1406–1411.

Gharib H.B. (2013). Effect of pre-storage heating of broiler breeder eggs, stored for long periods, on hatchability and chick quality. *Egyptian J. Anim.Prod*. 50 (3):174 -184.

Hamidu, J. A., Rieger, A. M., Fasenko, G. M. and Barreda, D. R. (2010). Dissociation of chicken blastoderm for examination of apoptosisand necrosis by flow cytometry. *Poult. Sci*. 89:901–909.

Hamidu, J. A., Uddin, Z., Li, M., Fasenko, G. M., Guan, L. L. and Barreda. D. R. (2011). Broiler egg storage induces cell death andinfluences embryo quality. *Poult. Sci*. 90:1749–1757.

Petek, M. and Dikmen, S. (2004). The effects of prestorage incubation of quail breeder eggs on hatchability and subsequent growth performance of progeny. *Animal. Research*, 53: 527–534.

Petek, M. and Dikmen, S. (2006). The effect of pre storage incubation and length of storage of broiler breeder eggs on hatchability and subsequent growth performance of progeny. *Czech J.Sci*., Vol.51,(2):73-77.

Reijrink , I. A. , Berghmans, D., Meijerhof, R., Kemp, B. and van den Brand, H. (2010). Influence of egg storage time and pre incubation warming profile on embryonic development, hatchability, and chick quality. *Poult. Sci.,* 89:1225–1238.

Reijrink I. A. M., Meijerhof, R., Kemp, B. and van den Brand, H. (2008). The chicken embryo and its micro environment during egg storage and early incubation. *World's Poultry Science Journal.* 64:581-598.

Reijrink, I. A. M., Meijerhof, R., Kemp, B., Graat, E. A. M. and Van den Brand, H. (2009). Influence of pre storage incubation on Embryonic Development, Hatchability, and chick quality. *Poult. Sci.,* 88: 2649–2660

Ruiz, J. and Lunam, C.A. (2002). Effect of pre-incubation storage conditions on hatchability, chick weight at hatch and hatching time in broiler breeders. *British poultry science*, 43:374-383.

SPSS (2008). Statistical package for the social sciences, version 17.0. Chicago: SPSS Inc,.

Steel, R. G. D., Torrie, G. H. and Dickey, D. A. (1996). Principles and Procedures of Statistics: A Biometrical Approach, 3rd ed., p. 666.McGraw Hill Book Comp. Inc. New York

Tag EL–Din, T. H., Kalaba, Z. M., EL-Kholy, K. H. M .and Abd-EL-Maksoud, S. A. (2017). Effect of short period incubation during egg storage on hatchability, embryonic mortality and chick quality. *J. Anim. and Poultry Prod.,* 8 (7): 161 – 165.

Tona K, Bamelis F, De Ketelaere B, Bruggeman, V. Moraes VMB, Buyse J, Onagbesan O, and Decuypere E (2003). Effects of egg storage time on spread of hatch, chick quality, and chick juvenile growth. Poult. Sci. 82:736–741.

Tona K, Onagbesan O, DeKetelaere B, Decuypere E, Bruggeman V (2004). Effects of age of broiler breeders and egg storage on egg quality, hatchability, chick quality, chick weight, and post-hatch growth to forty-two days. Journal of Applied Poultry Research (13):10–18.

Van De Van, L. (2004). Storage of hatching eggs in the production process," International Hatchery Practice, vol. 18, pp. 27–31.

Yassin, H., A., Velthuis, G. J., Boerjan, M., Van Riel, J. and Huirne, R. B. M. (2008). Field study on broiler eggs hatchability. *Poult. Sci*., 87:2408–2417.