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Effects of Pre-storage Incubation of Red-Legged Partridge (*Alectoris rufa*) Eggs on Hatchability and Incubation Length

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Abstract

This is the first study to investigate whether pre-storage incubation (PRESI) of red-legged partridge (*Alectoris rufa*) eggs improves hatchability. To this aim, 420 red-legged partridge eggs were organized in a 2 × 3 factorial design consisting of two levels of storage length (storage for 7 and 42 d) and three levels of PRESI (incubation for 0, 6 and 12 h at 37.8°C and 55% RH), resulting in six treatments consisting of 10 replications of 7 eggs each. For each treatment, egg weight losses during conservation and incubation, hatchability, chick weight at hatch, incubation length, and developmental stage at embryonic mortality were measured. It was found that 6 or 12 h of PRESI did not influence on hatchability of the fertile eggs stored during 7 d, and 6 h of PRESI did not improve the hatching rate of 42-d stored fertile eggs. However, 12 h of PRESI highly deteriorated hatchability of 42-d stored fertile eggs, increasing embryo mortality at positive development stage. Pre-storage incubation and the interaction PRESI × storage length increased egg weight loss during the storage period, though they did not influence either incubation length or chick weight at hatch. Thus, in contrast with other poultry species, PRESI does not offset the detrimental effect of the storage length on the hatchability and performance of *A. rufa* eggs, but even aggravates it in case of 42 d of storage. © 2014 Friends Science Publishers

Keywords: *Alectoris rufa*; Red-legged partridge; Pre-storage incubation; Long-term storage; Hatchability

Introduction

A common practice in small red-legged partridge (*Alectoris rufa*) game farms is to store eggs to gather the amount of eggs required to fill an incubator to obtain a large enough number of day-old chicks to be raised together (Beer and Jenkinson, 1981; González-Redondo, 2010). Given the reproductive seasonality of *A. rufa*, the frequency of egg laying is low at the beginning and at the end of its breeding season (Pérez y Pérez, 1981; Bagliacca *et al*., 1988; González-Redondo, 2006). This feature could force to store eggs for long periods, exceeding the recommended time to keep eggs viability, which in *A. rufa* varies between 7 and 15 d (Beer and Jenkinson, 1981; Cancho, 1991). On the other hand, nowadays complete-cycle farms coexist with other specialized only in rearing and preparing partridges for their release (González-Redondo, 2010). These specialized farms demand fertile eggs for hatching, as well as small farms which do not obtain the expected breeding results. In this regard, eggs' long-term storage might also be useful, since eggs must be stored on the farms on which they are produced until delivery of batches of hatching eggs. Eggs may also be stored for long periods while laying rate and hatchability of the eggs peaks in May and June (Flores, 1979; García-Martín and Dalmau, 2003). At this moment, incubator space may be limited and surplus eggs are stored until incubator space is available. Thus, long storage periods might be useful to incubate the surplus eggs later on, and so distribute the batches of chicks and benefits along the year.

Previous studies show that storage periods up to 28 d, at 15-16ºC and 70-80% RH, do not impair hatchability of *A. rufa* (González-Redondo, 2010) and *A. chukar* (Woodard and Morzenti, 1975) eggs. However, at least in *A. rufa* eggs, hatchability tends to decrease sharply when storage period is lengthened under the same storage conditions (35 d, at 15-16ºC, 70-80% RH, and regular turning of eggs; González-Redondo, 2010). On the other hand, in the last decade several studies reported that 6 or 8 h of pre-storage incubation (PRESI) of broiler, turkey and quail eggs at the standard incubation temperature (37 – 37.8ºC depending on the species) before prolonged storage periods (11-15 d) improves hatchability over than that of the eggs stored without the PRESI treatment (Fasenko *et al*., 2001a, b; Petek and Dikmen, 2004; Reijrink *et al*., 2009; Lotfi *et al*., 2011). However, PRESI does not affect the hatching rate of broiler and turkey eggs in case of shorter storage periods (Fasenko *et al*., 2001a, b; Reijrink *et al*., 2009). Reijrink *et al*. (2008) suggested that PRESI treatment before long storage periods improves the developmental stage of the embryo, increasing the number of viable cells and this could

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be the reason why these batches obtained higher hatching rates. However, the effect of PRESI of red-legged partridge (*A. rufa)* eggs has not yet been evaluated. On that basis, the objective of this research work was to investigate whether 6 or 12 h of incubation before very long-term storage (42 d) improves the hatchability of red-legged partridge eggs compared to the hatchability of eggs stored for a standard period of 7 d.

Materials and Methods

Breeders and Husbandry

This trial used 420 hatching eggs gathered from 2 and 3 years old red-legged partridges, bred on a game farm in Southern Spain (El Ronquillo, province of Seville). All eggs were laid within the three days before the collection day. Breeding pairs were housed outdoors, in 50×65 cm cages, and fed with a balanced commercial feed (20% CP, 3.3% Ca; Avipacsa A-78®, Sanders, Dos Hermanas). Birds received natural light until December. The period of light was subsequently increased by a quarter of an hour per day until a maximum of 16 h per day (natural light + artificial light) was reached by January. Egg laying commenced in mid January.

Experimental Design

Data were organized in an experimental design consisting in 2 × 3 factorial model with two levels of storage length (7 and 42 d) before incubation and three levels of PRESI (0, 6 and 12 h). It resulted in six treatments consisting on 10 replications of 7 eggs each. As this species have a remarked reproductive seasonality (Pérez y Pérez, 1981; González-Redondo, 2006), sampling was carried out in the middle of the laying season, when fertility is higher than at its beginning and end. Batches of eggs to be stored for 42 d were collected on March 18th and those to be stored for 7 d were collected on April 22nd. Eggs to be pre-incubated were introduced (for 6, or 12 h) in the incubator (Masalles HS25®, Masalles, Ripollet), set at 37.8°C and 55% RH. Thereafter, pre-incubated eggs were cooled down by maintaining them for 2 h at room temperature. Then, the pre-incubated eggs and the control eggs (0 h pre-incubation) were introduced at the same time in a storage chamber (Vinotek®, Liebherr, Biberach an der Riss, Germany) where all eggs were located with the smallest end down. Storage conditions were 15ºC, 80% RH, and 45º turning of eggs every 12 h. After storage, eggs were kept during 12 h at room temperature (24ºC, 43% RH). Thereafter, all batches were introduced at the same time in the incubator (on April 30th). The incubator was set at 37.8°C, 55% RH, and eggs were turned 45º every hour. On day 21 from the beginning of the incubation the eggs were transferred to the hatcher (Maino Incubators 2-630 XHM®, Maino Enrico-Adriano S.n.c., Oltrona di San Mamette, Italy), which was set at 37.5ºC and 80% RH, without turning of eggs.

Data Recorded

In order to calculate eggs weight losses during storage and during incubation, eggs were individually weighed before storage (initial weight), at the end of the storage period, and on day 21 of incubation. For each egg, weight loss during the storage period was calculated as a percentage of its initial weight, and weight loss at day 21 of incubation was obtained as a percentage of its weight at the end of the storage period. Total egg weight loss (storage+incubation periods) was obtained for each single egg as a percentage of its initial weight. Starting from day 21 of incubation, hatching controls were carried out every 12 hours to determine incubation length, as the difference between the incubator loading and hatching date. At hatching, every chick was weighed. After the incubation period, to determine hatchability, hatched and unhatched eggs were counted, and unhatched eggs were opened to discern macroscopically infertility or embryonic mortality in the following categories: fertile without development (FND), positive development (PD), early abortion (EA), late abortion (LA), or pipped but not out of shell (P) (Juárez-Caratachea and Ortiz, 2001; Ernst *et al*., 2004).

Statistical Methods

Fertility, weight losses of the fertile eggs during storage and during the first 21 d of the incubation period, chick weight at hatch, incubation length, as well as hatchability of the incubated eggs, hatchability of the fertile eggs, and embryonic mortality of the fertile eggs as dependent variables, were analyzed using the univariate general linear model (GLM) procedure with storage length and PRESI as fixed effects. Interactions between the factors were also analyzed. When significant differences were found by the GLM analysis, Tukey's multiple range tests were used to separate means. All data have been expressed as mean and SEM. For all comparisons, $P < 0.05$ was considered as a level indicating statistical significance. Analyses were carried out using SPSS v. 15.0 software (SPSS Inc., 2006).

Results

Fertility

In the present study, the average fertility of the total eggs set was 50.24%. No significant differences ($P > 0.05$) were observed in the fertility of the total eggs set according to the storage length or to the different PRESI treatments (Table 1).

Egg Weights and Egg Weight Losses

Table 2 shows mean values found for recently-laid eggs weight, percentage of fertile eggs weight loss during the storage period and during incubation, and overall percentage of egg weight loss as a function of PRESI treatment and storage length. Mean weight found for recently-laid eggs

Table 1: Fertility and hatchability of red-legged partridge eggs, according to the length of the storage period and pre-storage incubation[1]

Item	n[2]	Fertility[3] (%)	Hatchability[4] (%)	Hatchability of the fertile eggs[5] (%)
Storage time (d)				
7	30	54.29	45.71[a]	83.61[a]
42	30	46.19	16.19[b]	31.39[b]
Pre-storage incubation (h)				
0	20	54.29	33.57	62.75[a]
6	20	52.86	34.29	63.50[a]
12	20	43.57	25.00	46.25[b]
Storage time (d) × pre-storage incubation (h)				
7-0	10	50.00[ab]	38.57[abc]	75.00[a]
7-6	10	58.57[a]	48.57[ab]	83.33[a]
7-12	10	54.29[ab]	50.00[a]	92.50[a]
42-0	10	58.57[a]	28.57[bc]	50.50[b]
42-6	10	47.14[ab]	20.00[cd]	43.66[b]
42-12	10	32.86[b]	0.00[d]	0.00[c]
SEM		2.46	3.00	4.53
P-value				
Storage time		0.081	< 0.001	< 0.001
Pre-storage incubation		0.123	0.133	0.002
Interaction		0.030	0.001	< 0.001

[1]Mean; [2]Number of replications of 7 eggs each; [3]Percentage of incubated eggs that were fertile; [4]Percentage of incubated eggs that hatched; [5]Percentage of fertile eggs that hatched; [a-d]Values in the same column with different superscripts are significantly different ($P < 0.05$)

Table 2: Eggs' weight losses during storage and incubation periods in red-legged partridge fertile eggs according to the length of the storage period and pre-storage incubation[1]

Item	n[2]	Egg weight before storage (g)	Egg weight loss during storage[3] (%)	Egg weight loss after 21 d of incubation[4] (%)	Total egg weight loss[5] (%)
Storage time (d)					
7	30	19.68	1.39[b]	10.72	12.11[b]
42	30	19.91	2.70[a]	10.41	13.11[a]
Pre-storage incubation (h)					
0	20	19,92	2.01[b]	10.56	12.57
6	20	19,69	1.87[b]	10.30	12.17
12	20	19,78	2.25[a]	10.84	13.09
Storage time (d) × pre-storage incubation (h)					
7-0	10	20.18	1.16[c]	10.51	11.69
7-6	10	19.54	1.30[c]	10.27	11.52
7-12	10	20.01	1.81[b]	11.39	13.12
42-0	10	19.65	2.84[a]	10.61	13.45
42-6	10	19.84	2.48[a]	10.33	12.82
42-12	10	19.55	2.77[a]	10.29	13.06
SEM		0.11	0.10	0.14	0.19
P-value					
Storage time		0.296	< 0.001	0.271	0.004
Pre-storage incubation		0.705	0.001	0.294	0.084
Interaction		0.235	0.005	0.145	0.074

[1]Mean; [2]Number of replications of 7 eggs each;[3]Values are expressed as a percentage of egg weight at the beginning of storage period; [4]Values are expressed as a percentage of egg weight at the beginning of incubation; [5]Values are expressed as a percentage of egg weight loss between the beginning of the storage period and 21 d of incubation; [a-b]Values in the same column with different superscripts are significantly different ($P < 0.05$)

was 19.80 g. No differences ($P > 0.05$) were observed, between batches, in the initial weight of the fertile eggs.

Mean value found for the percentage of fertile eggs weight loss during storage was 2.05%. This variable was highly influenced by the storage length ($P < 0.001$) and by the PRESI treatment ($P = 0.001$). Thus, 7-d stored eggs lost 1.39% of their initial weight during storage, while those stored for 42 d lost 2.70% of their initial weight, and 0 and 6-h PRESI batches lost 1.94% of their initial weight during storage, while 12-h PRESI batches lost 2.70% of their initial weight. We found very significant PRESI × storage length interaction for percentage of fertile eggs weight loss during storage ($P = 0.005$). So, in case of 7-d storage period, eggs pre-incubated for 12 h showed a higher percentage of weight loss during storage (1.81% of its initial weight) than eggs with 0 or 6 h of PRESI (1.23%). In contrast, within the 42-d storage batches, no significant difference was observed among 0, 6, and 12 h of PRESI.

During the first 21 d of incubation, we found no significant differences in the percentage of fertile eggs weight loss ($P > 0.05$) according to the storage time or PRESI. No interaction ($P > 0.05$) was observed between the two factors for the percentage of fertile eggs weight loss during the first 21 d of incubation.

Influenced by the egg weight loss during storage, we found very significant differences in the percentage of fertile eggs total weight loss according to the length of the storage period (P = 0.004). Thus, total egg weight losses were 13.11% for eggs stored during 42 d and 12.11% for eggs stored during 7 d. However, we have not found any differences in the total egg weight loss according to the PRESI, and no interaction PRESI × storage length was observed for it.

Chick Weight at Hatch

Mean value found in this study for chick weight at hatch was 13.95 g. We found no influence of the storage length and the PRESI treatment on the chick weight at hatch (P > 0.05, Table 3). In addition no interaction between the two factors was observed for this variable.

Incubation Length

Incubation length was highly influenced (P < 0.001) by storage length, but not by PRESI (Table 3). Eggs stored for 7 d took 23.29 d to hatch. By contrast, 42-d stored eggs achieved a highly significant increase in incubation length (24.17 d). No difference was found on the incubation length according to the PRESI, and no interaction PRESI × storage length was observed for this variable.

Hatchability

The average hatchability of the total eggs set found in this study was 30.95% (Table 1), highly influenced by the storage treatment (P < 0.001). So, hatchability of 42-d stored eggs was significantly lower (16.19%) than for 7-d stored eggs (45.71%). The PRESI treatment did not influence on the hatching rate (P > 0.05). However, we observed interaction (P = 0.001) between storage length and the PRESI treatment for hatchability of the total eggs set. Thus, in case of 42-d storage period, total hatchability decreased with increasing length of PRESI treatment (Table 1), though total hatchability was not affected by the PRESI treatment in case of 7-d storage period.

The average hatchability of the fertile eggs recorded in this research was 57.5%. Highly significant differences were found on this variable according to the storage length (P<0.001). So, hatchability of the 7-d stored batches was 83.61%, while hatchability of 42 d-stored batches was much lower (31.39%). The PRESI treatment influenced significantly on hatchability of fertile eggs (P < 0.01). Thus, hatchability of 12-h PRESI treatments (46.25%) was lower than that from 0 and 6-h PRESI treatments (62.75 and 63.50%, respectively). Interaction PRESI × storage length (P < 0.001) was also found for hatchability of fertile eggs. All batches of 7-d stored fertile eggs (0, 6 and 12 h of PRESI) showed higher hatchability than those stored for 42 d and submitted to treatments consisting of 0 and 6 h of PRESI, and these showed higher hatchability than the batch stored for 42 d with 12 h of PRESI.

Embryonic Mortality

Table 4 shows the embryonic mortality rates of the fertile eggs, divided into five groups depending on the embryonic development at death, according to the treatments. Storage length significantly increased the total embryonic mortality (P < 0.001), the rate of embryo mortality at positive development stage (P < 0.001), the rate of early abortions (P = 0.001) and the rate of late abortions (P = 0.001). Thus, the total embryonic mortality amounted to 15.56% for the 7-d storage treatment and 68.61% for the 42-d storage treatment.

Pre-storage incubation during 12 h increased significantly the total embryonic mortality (P = 0.005), particularly at the positive development stage (P < 0.001). Interaction between storage length and PRESI was found for the total embryonic mortality (P < 0.001) and for the mortality at positive development stage (P < 0.001). Thus, the total embryonic mortality increased up to 100% in the 12-h PRESI × 42-d storage treatment, while no difference was found among the embryonic mortality of the 7-d storage batches with different PRESI treatments. Embryonic mortality at the positive development stage increased significantly to 54.17% in the 12-h PRESI × 42-d storage treatment, and no significant differences were found among the other treatments, whose mortality at the positive development stage was 2.9%.

Discussion

Mean fertility achieved in this research (50.24%) was lower than the mean values reported by previous researches on *A. rufa* eggs (values ranging from 73.5% to 85.6%; Bagliacca *et al*., 1988; Paci *et al*., 1992; González-Redondo, 2006, 2010). The low fertility observed was not due to seasonal factors because the eggs used in this study were gathered in the middle of the reproductive season: half of the batches were collected in mid March and the other half in late April, when laying rate and fertility of red-legged partridge peaks (Flores, 1979; García-Martín and Dalmau, 2003). Moreover, the breeding partridges had a similar age (2-3 years), characterized by the maximum fertility (Mourão *et al*., 2010). So, the difference between eggs' fertility mean value obtained in this research and the mean values found in the literature could be due to other farming conditions such as differences in the breeding flocks, housing type or kind of feed used.

Mean weight found for recently-laid eggs (19.80 g) in this study was similar to that reported for partridges of the *A. rufa* species (Beer and Jenkinson, 1981; Pérez y Pérez, 1981; González-Redondo, 2010; Mourão *et al*., 2010) as well as for other *Alectoris* species (Kırıkçı *et al*., 2004; Tilki and Saatci, 2004; Çağlayan *et al*., 2009) under farming conditions.

Table 3: Chick weight at hatch and length of the incubation period in red-legged partridge according to the length of the storage period and pre-storage incubation[1]

Item	n[2]	Chick weight at hatch (g)	Incubation length (days)
Storage time (d)			
7	30	14.05	23.29[b]
42	30	13.84	24.17[a]
Pre-storage incubation (h)			
0	20	14.15	23.85
6	20	13.85	23.63
12	20	13.82	23.26
Storage time (d) × pre-storage incubation (h)			
7-0	10	14.23	23.44
7-6	10	14.09	23.18
7-12	10	13.82	23.26
42-0	10	14.08	24.27
42-6	10	13.61	24.08
42-12	10	-	-
SEM		0.11	0.09
P-value			
Storage time		0.225	< 0.001
Pre-storage incubation		0.289	0.382
Interaction		0.528	0.814

[1]Mean; [2]Number of replications of 7 eggs each; [a-c]Values in the same column with different superscripts are significantly different ($P < 0.05$)

Table 4: Effect of storage length and pre-storage incubation on embryonic mortality of red-legged partridge eggs

Item	n[2]	Embryonic mortality[1] (% of the fertile eggs)					
		FND	PD	EA	LA	P	Total
Storage time (d)							
7	30	0.83	1.67[b]	0.83[b]	11.67[b]	0.56	15.56[b]
42	30	1.11	21.22[a]	12.44[a]	33.83[a]	0.00	68.61[a]
Pre-storage incubation (h)							
0	20	0.00	3.50[b]	6.17	27.58	0.00	37.25[b]
6	20	1.25	3.75[b]	7.08	23.58	0.83	36.50[b]
12	20	1.67	27.08[a]	6.67	17.08	0.00	52.50[a]
Storage time (d) × pre-storage incubation (h)							
7-0	10	0.00	2.50[b]	0.00	22.50	0.00	25.00[c]
7-6	10	2.50	2.50[b]	2.50	7.50	1.67	16.67[c]
7-12	10	0.00	0.00[b]	0.00	5.00	0.00	5.00[c]
42-0	10	0.00	4.50[b]	12.33	32.67	0.00	49.50[b]
42-6	10	0.00	5.00[b]	11.67	39.67	0.00	56.33[b]
42-12	10	3.33	54.17[a]	13.33	29.17	0.00	100.00[a]
SEM		0.69	3.20	1.80	3.50	0.28	4.58
P-value							
Storage time		0.842	< 0.001	0.001	0.001	0.322	< 0.001
Pre-storage incubation		0.597	< 0.001	0.976	0.413	0.375	0.005
Interaction		0.237	< 0.001	0.874	0.377	0.375	< 0.001

[1]FND: fertile, no development; PD: positive development; EA: early abortion; LA: late abortion; P: Pipped but not out of shell; [2]Number of replications of 7 eggs each; [a-c]Values in the same column with different superscripts are significantly different ($P < 0.05$)

We found that both storage length and PRESI increased significantly ($P < 0.001$ and $P = 0.001$, respectively) the percentage of weight loss in eggs during the storage period. This is consistent with previous research on quail (Imai *et al*., 1986), broiler breeder (Fasenko *et al*., 2001b; Reijrink *et al*., 2010) and partridge (Tilki and Saatci, 2004; González-Redondo, 2010) eggs which state that both storage and PRESI favor the conditions for loss of water vapor from the eggs. So, as storage or PRESI is lengthened, water loss and, therefore, eggs weight loss during storage increases. As a result, the increase in the egg weight loss that occurs during storage progressively deteriorates the internal quality of the eggs (Tilki and Saatci, 2004; Çağlayan *et al*., 2009) impairing the hatching rate. In concordance with previous studies on broiler breeder eggs (Fasenko *et al*., 2001b), we found PRESI × storage length interaction for the percentage of eggs weight loss during the storage period ($P = 0.005$). As can be expected, this variable increased linearly with storage and PRESI length. Fasenko *et al*. (2001b) already showed that the reason behind this is that longer PRESI and storage treatments lead to conditions favoring loss of water vapor from the eggs.

The lack of differences among treatments in the percentage of fertile eggs weight loss at 21 d of incubation, agrees with the fact that, after storage, all batches were incubated together in the same incubator under the same conditions. This pattern matches the results of other studies for broiler eggs (Fasenko *et al*., 2001b; Silva *et al*., 2008;

Reijrink *et al*., 2009).

The total percentage of fertile eggs weight loss was highly influenced by the storage length, but not by the PRESI treatment, probably because the great influence of storage on shells permeability and total water loss masks that of the PRESI treatment. The absence of interaction PRESI × storage length found in this study for the total eggs weight loss concurs with previous results in broiler breeder eggs (Fasenko *et al*., 2001b; Reijrink *et al*., 2009, 2010).

Mean chick weight at hatch (13.95 g) found in this study matched with mean values recorded for *A. rufa* (Pérez y Pérez, 1981) and *A. graeca* (Kırıkçı *et al*., 2004) in captivity. The lack of influence of storage length and PRESI treatments on chick weight at hatch we have found concurs with previous studies in broiler breeder eggs which found that day-old chick weights are positively correlated with the egg weights at setting, irrespective of the storage time (Tona *et al*., 2003). However it is well known that egg storage length shows strong influence on chick quality, hence on its relative growth (Becker, 1960; Tona *et al*., 2003). These effects of storage may be explained by the deterioration of the egg internal quality, especially albumen height during storage (Hurnik *et al*., 1978; Lapão *et al*., 1999; Tona *et al*., 2002), which influences on the potential performance of day-old chick that is dependent on the quality of the albumen in the incubating egg at this stage (Deeming, 1989; Tona *et al*., 2003). So, further studies are needed to establish the effect of storage and PRESI treatments on the embryo development and growth performance of *A. rufa* chicks.

Mean incubation length found in our study for eggs stored for 7 d (23.29 d) was within the mean values found in literature for the species studied under farming conditions (Flores, 1979; Torres and Garcés, 1995; González-Redondo *et al*., 2012). The increase we found in incubation length with the storage length (24.17 d of incubation for 42-d storage period) is in agreement with previous studies in broiler showing that longer storage periods lead to longer incubations as a result from the deterioration of internal egg quality which impairs embryo performance and development (Elibol *et al*., 2002; Ruiz and Lunam, 2002; Tona *et al*., 2003).

Due to the low fertility registered, the average hatchability of the total eggs set (30.95%) was below the values found for *A. rufa* eggs (ranging from 53.4% to 84.1%; Mori *et al*., 1985; Paci *et al*., 1992; González-Redondo, 2006, 2010).

Mean value found for hatchability of the fertile eggs (57.5%) was also below the values described for this species in the literature (ranging from 72.6% to 91.6%; Bagliacca *et al*., 1988; Paci *et al*., 1992; González-Redondo, 2006, 2010), due, basically, to the high difference found between hatchability of 7-d and 42-d stored eggs (83.61 and 31.39%, respectively). Thus, in agreement with results reported by other researchers in several poultry species, we found that embryo mortality during incubation increased significantly with the storage length (Proudfoot, 1969; Meijerhof, 1992; Christensen, 2001; Fasenko *et al*., 2002; González-Redondo *et al*., *in press*), particularly the rates of mortality at positive development stage, early abortions and late abortions (Woodard and Morzenti, 1975; Nahm, 2001; Fasenko, 2007).

The reason is that during storage, when eggs are held at temperatures below their physiological zero, there is no discernible embryonic development; however, the storage period influences on the survival rate of the original cells and the rate of replacement by the new cells (Fasenko *et al*., 1991). In turn, this influences the number of cells contained by the blastoderm before incubation, which determines embryonic viability (Mayes and Takeballi, 1984; Meijerhof, 1992; Narushin and Romanov, 2002). As occurs in other poultry species, this could be produced, on the one hand, by a deterioration of embryo performance caused by aging (Woodard and Morzenti, 1975; Nahm, 2001; Fasenko, 2007) and, on the other hand, it could be also linked to the egg weight loss (Hassan *et al*., 2005; Romao *et al*., 2008). In fact, egg weight loss observed in this study for 42-d stored eggs was higher than for 7-d stored eggs.

In another vein, matching with previous studies on broiler and turkey eggs stored up to 8 d (Fasenko *et al*., 2001a,b; Reijrink *et al*., 2009), we found in this study nor a detrimental or beneficial influence of PRESI on hatchability of eggs stored during 7 d at 15ºC and 80% RH. Other researches on broiler, quail and turkey eggs have shown that warming eggs prior to a 12 to 15-d storage period improves hatchability, as it helps the embryo to develop to the so-called hypoblast stage: a stage of embryonic development that is able to survive storage better (Kosin, 1956; Fasenko *et al*., 2001a, b; Petek and Dikmen, 2004; Silva *et al*., 2008; Lotfi *et al*., 2011). However, we did not found any influence of 6 h of PRESI on hatchability of 42-d stored eggs, and 12 h of PRESI deteriorated even more viability of 42-d stored eggs. This was probably because even though PRESI helps the embryo to develop to a stage that is able to survive long storage better (Kosin, 1956; Fasenko *et al*., 2001a, b; Petek and Dikmen, 2004; Silva *et al*., 2008; Lotfi *et al*., 2011), in case of too long storage (42 d) PRESI leads to greater water losses during storage and therefore contributes to a greater deterioration of the embryo and hence, a higher embryo mortality during incubation.

It can be concluded that, under the experimental conditions tested, PRESI does not produce any beneficial effect on hatchability of *A. rufa* eggs. Even though, 12 h of PRESI previous to 42 d of storage severely deteriorates the hatching rate of *A. rufa* eggs. However, literature on broiler and turkey eggs shows that 6 or 8 h of PRESI of eggs before long storage periods (11-15 d) result in higher proportions of live chicks, with lower rate of embryonic mortality, in comparison to unheated eggs. In view of this evidence, further research is needed to determine whether PRESI improves hatchability of red-legged partridge eggs in case of long storage periods, although shorter than 42 d (i.e., 14 to 28 d).

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