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ANIMAL PRODUCTION

Influence of breeders age on initial embryonic development (from 0 to 72 hours) of Japanese quails during incubation

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ABSTRACT. The initial stages of early embryonic development were analyzed as a function of the incubation period and age of Japanese quail breeders. A total of 203 Japanese quails housed in 29 conventional laying cages with 5 females and 2 males at 31, 39, 48, and 59 weeks of age were used, and the fertile eggs from these breeders were selected and incubated. The eggs were opened, and the embryos were isolated, fixed in a glutaraldehyde solution, analyzed and classified according to the stage of development. For after laying and the incubation periods of 24, 48, and 72 hours, the embryos presented, on average, Hamburger-Hamilton stages XI, HH 6.1, HH 12.7, and HH 18.5, respectively, with no effect of breeder age. It was also observed that, between 31 and 59 weeks of age in Japanese quail breeders, the eggs become longer and wider, with greater weight, volume, and area. Therefore, it is concluded that the age of the Japanese quail mother influences the weight, length, width, volume, and area of the eggs but does not influence the embryonic development up to 72 hours.

Keywords: *coturnix japonica*; egg size; embryo; fertile eggs; HH stages.

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Introduction

In order to improve the technique of artificial incubation of fertile quail eggs and ensure the maximization of production, research was conducted on factors that influence such process. The breeder's age influences egg quality and hatching rates (Silva et al., 2016). As the breeders age, internal and external components of the egg undergo modifications, such as reduction in albumen height and shell thickness (Samiullah, Omar, Roberts, & Chousalkar, 2017). In addition, during the aging period, there is an increase in the interval between ovulations and the production of larger ovarian follicles, which leads to a reduction in the laying rate and the production of larger eggs (Zakaria, Miyaki, & Imai, 1983). Larger eggs can cause changes in embryonic development during the incubation period and affect hatchability rates since larger eggs have greater difficulty of losing heat due to the reduction of the thermal conductance of the shell, thus making it difficult to eliminate the metabolic heat generated by the embryo (French, 1997).

In general, this process of embryonic development in quails is comprised of two phases: inside and outside the breeder. Inside the body of birds, the process begins when the ovum is fertilized in the infundibulum, and then several cell divisions take place. After oviposition, the embryogenic process continues through evolution through the stages of blastula, gastrula, neurula, and organogenesis until the complete formation of the embryo (Persaud & Galef Jr., 2004). Each step of this process was described by Hamburger and Hamilton (1951) in 46 stages.

Thus, this work aims to identify the stages of early embryonic development (from 0 to 72 hours) as a function of the age of the Japanese quail breeders, as well as morphological characteristics of the eggs such as weight, volume, length, width, and area.

Materials and methods

The present research was approved by the Animal Care Committee at the State University of Maringa (protocol number 8172270619) and developed in the Animal Farm of the State University of Maringa, PR, Brazil (S 23° 21', W 52° 04', and 564 m of altitude).

Animals and management

The breeders in the experiment were housed in a conventional laying barn. A total of 203 Japanese quails were used (145 females + 58 males), which were selected by weight and laying (10.52 ± 0.72 g) and housed in a ratio of 5 females to 2 males in galvanized wire cages (25 × 39 cm) with a nipple on top and a trough-type feeder in front of the cages. The birds received food and water *ad libitum*, with the laying diet provided based on corn and soybean meal with 18.92% crude protein, 2,800 kcal kg^{-1}, 2.99% Ca, and 0.309% available P, according to the composition of ingredients and nutritional requirements for these birds in the laying phase corresponding to the Brazilian tables of poultry and swine (Rostagno et al., 2017). The lighting regime adopted was 17 hours of light (natural + artificial) to maintain the posture stimulus for the birds. The means of temperature, maximum (28.71 ± 5.20°C) and minimum (19.24 ± 3.26°C) as well as humidity, maximum (66.50 ± 10.15%) and minimum (65.02 ± 10.98%), were measured using a digital thermohygrometer daily.

Experimental design and egg collection

The treatments consisted of four breeder ages (31, 39, 48, and 59 weeks). Every 60 days, eggs from each treatment were collected immediately after laying (between 2h00 and 4h00 pm), weighed (mean weight ± 5%), and selected (eggs with shell problems were discarded). With the aid of a digital caliper (Digimess, with a precision of 0.02 mm), the length and width of the eggs were measured. Through these data, the variables volume (V) and surface area (SA) of the egg were determined and calculated by the equations proposed by Narushin (2005):

$$V = (0.6057 - 0.0018)\, L \times W^2$$

where: V = volume (cm^3); L = Egg Length (mm) and W = Egg Width (mm).

$$SA = (3.155 - 0.0136 \times L + 0.0115 \times W) \times W \times L$$

where: SA = Egg Surface Area in (cm^2); L = Egg Length (mm); W = Egg Width (mm).

Then, 15 eggs were opened immediately after laying and 45 eggs were incubated for 24, 48, and 72 hours in a vertical incubator (Petersime®, Labo 13 model) at 60% humidity and 37.4°C, with automatic turning.

Embryo collection and analysis

After laying, 24, 48, and 72 hours of incubation, 15 eggs of each treatment were removed from the trays, opened, identified, and the embryos were isolated, washed in phosphate buffer solution (PBS), and fixed in 2.5% glutaraldehyde solution pH 7.4 PBS 0.1M according to the methodology described by Gupta and Bakst (1993). After collection, embryos were transferred to the laboratory and analyzed under a stereomicroscope with a digital camera and digital analysis software (Motic®). For classification of the embryos obtained immediately after spawning, we used the morphological criteria described in the tables of Eyal-Giladi and Kochav (1976), which classify embryos in Roman numbers from I to XIV (the number was converted to a corresponding Arabic number for statistical analysis), and for the groups 24, 48, and 72 hours of incubation, we used the table of Hamburger and Hamilton (1951), which classifies embryos in 46 stages.

Statistical analysis

For statistical analysis, each embryo and each egg were considered experimental units. The data obtained were analyzed using PROC GLM of SAS (Statistical Analyses System [SAS], 2008) at a 5% level of significance to describe the influence of the age of the matrices and incubation hours, and in cases of significant differences, the means were compared using Tukey's test ($p < 0.05$).

Results and discussion

The means of the eggs from the breeders under study were 10.65 g weight, 31.10 mm length, 24.47 mm width, 10.50 cm^3 volume, and 2.10 cm^2 total area. The variables weight, length, width, volume, and area were influenced ($p < 0.05$) by the age of the breeder (Table 1). The 59-week-old breeder produced eggs with the highest weight (10.97 g). Breeders with 31 and 39 weeks of age presented lower weights, which are statistically equal (10.57 and 10.48 g respectively), and the 39-week-old breeder presented a weight of 10.67 g. This same effect was evidenced by Nasri, Van den Brand, Najar, and Bouzouaia (2020) in their work with heavy layers of different ages (31, 42, and 66 weeks). This author found that young breeders aged 31 weeks produced lighter eggs when compared to older breeders aged 66 weeks. In Japanese quails, Nowaczewski, Kontecka, Rosiński, Koberling, and Koronowski (2010) described that younger breeders at 9 weeks of age produce lighter eggs when compared to breeders at 31 weeks of age.

Regarding the length variable, the 59-week-old produced the longest eggs (31.66 mm). Breeders at 31 and 39 weeks of age presented means of 30.98 and 31.28 mm, respectively, and the eggs from 48-week-old birds had the shortest length (30.61 mm). Regarding the width, eggs from 59-week-old hens had the greatest width value, followed by the width values of eggs from 39, 48, and 31 weeks old, which averaged 24.68, 24.34, and 24.20 mm, respectively. According to Uyanga, Onagbesan, Oke, Abiona, and Egbeyale (2020), there is an increase in length, width, and weight of eggs ($p < 0.05$) as a function of the age of broiler breeders from 43 to 65 weeks of age. In a study with Pharaoh quails, Wilkanowska and Kokoszyński (2012) found that there is an increase in the length and width of the egg as a function of the breeder's age. The increase in egg length and width in older hens can be attributed to the increase in egg weight with hen age, as reported by Iqbal, Khan, Mukhtar, Ahmed, and Pasha (2016).

For the variable volume, the lowest means were obtained in eggs from 39, 31, and 48-week-old breeders, which were represented by values of 10.73, 10.26, and 10.21 cm^3, respectively. Older birds (59 weeks old) had the highest mean, represented by 11.07 cm^3. In terms of the surface area of the eggs, the 59-week-old breeders produced eggs with the highest average area represented by 2.18 cm^2. On the other hand, breeders 39 and 31 weeks of age produced eggs with smaller averages of 2.13 and 2.08 cm^2, respectively. On the other hand, hens 48 weeks old produced eggs with an area of 2.06 cm. Crosara et al. (2019) verified that with the advancing age of heavy broiler breeders, there is an increase in the production of eggs with greater volume and surface area, and that these two variables are directly related to egg weight.

Regarding embryonic development, there was no effect of the age of the breeder ($p > 0.05$) on the incubation hours for the stages of embryonic development (Table 2). In this study, seventeen of the embryos collected immediately after laying were in stage X, twenty-eight in stage XI, twelve in stage XII, and three in stage XIII. Embryos in stage X show the completed formation of the zone pellucida and a demarcated area between the zone pellucida and the opaque area. At this stage, the posterior-most region of the zone pellucida remains a transparent, sickle-shaped band. The embryos from stages XI (Figure 1) to XIII, which are part of period C, were described by Eyal-Giladi and Kochav (1976). In this work, seventeen are in stage XI (Figure 1), twelve in stage XII, and four in stage XIII.

In stage XI (Figure 1), on the upper surface of the blastoderm, there is a thin, smooth layer where deeper cells can be seen. In front of the posterior section of the opaque area (ao) is a transparent belt-shaped area whose anterior edge is demarcated by a relatively narrow concentration of horseshoe-shaped cell clusters called Koller's sickle (ks) which, from a morphological point of view, comprises the beginning of the hypoblast.

Table 1. Mean characteristics of eggs from 31, 39, 48, and 59-weeks-old in Japanese quail breeders.

Age	Number of eggs	Weight (g)	Length (mm)	Width (mm)	Volume (cm^3)	Surface Area (cm^2)
31	145	10.57 b	30.98 bc	24.20 b	10.26 bc	2.08 bc
39	77	10.67 ab	31.28 ab	24.68 ab	10.73 ab	2.13 ab
48	58	10.48 b	30.61 c	24.34 b	10.21 c	2.06 c
59	58	10.97 a	31.66 a	24.96 a	11.07 a	2.18 a
	Mean	10.65	31.10	24.47	10.50	2.10
	SEM	0.044	0.083	0.065	0.069	0.008
	P-value	0.004	0.001	0.0002	< 0.0001	< 0.0001

Legend: SEM: Standard error of mean; age of the breeders in weeks. Equal letters do not present differences in the same column, different letters differ, by Tukey test.

Table 2. Mean classification of embryos from Japanese quail breeders at 31, 39, 48, and 59 weeks of age After laying and eggs incubated for 72 hours.

Age (weeks)	Number of Embryos	After Laying	Hours of Incubation		
			24	48	72
31	15	XI	HH 6.26	HH 12.76	HH 18.5
39	15	XI	HH 6.20	HH 12.09	HH 18.2
48	15	XI	HH 6.00	HH 13.11	HH 18.6
59	15	XI	HH 6.00	HH 13.10	HH 18.7
	Mean	XI	HH 6.11	HH 12.74	HH 18.5
	SEM	0.108	0.194	0.188	0.156
	P-value	0.798	0.948	0.322	0.738

Legend: SEM: Standard error of mean.

In stage XII embryos, the posterior transparent anterior belt is observed, where the newly formed lower layer, the hypoblast, already coats half of the lower surface of the zone pellucida. The hypoblast at this time is not completely continuous and appears to be formed by the fusion of separate cell masses. In stage XIII, the posterior margin of the hypoblast is very pronounced on the ventral side and can also be seen on the dorsal side through the transparent epiblast, giving the appearance of a continuous upper surface.

At 24 hours of incubation, two embryos were at stage HH3, four at stage HH4, eighteen at stage HH5, fifteen at stage HH6 (Figure 2), seven at stage HH7, ten at stage HH8, and four at stage HH9, most of which were found at stage HH5. When the embryo reaches stage HH3, the primitive line extends from the posterior margin to approximately the center of the zone pellucida. When the HH4 stage is reached, the primitive line reaches its maximum length, there is the presence of the primitive groove, the primitive fossa, the Hensen's node, and the zone pellucida takes on a pear shape. At stage HH5, the notochord, also called the ‘head process’, is visible and extends forward from the anterior border of the node of Hensen. In stage HH6 (Figure 2), which is considered a transitional stage, there are no somites, but the development of the first fold of the embryo head occurs. When stage HH7 is reached, the second pair of somites becomes visible, and neural folds are also visible in the head region. At stage HH8, the embryos have four pairs of somites, with neural pairs at the level of the mesencephalon. At stage HH9, the optic vesicles are present, and the paired primordia of the heart begin to fuse.

At 48 hours of incubation, the embryos were between stages HH8 to HH14, with most of them found at stage HH12 (Figure 3) and HH13. Two embryos were found at stage HH8, nine at stage HH12 (Figure 3), twenty-four at stage HH13, and fourteen at stage HH14 (Figure 3). Embryos at stage HH12 (Figure 3) presented sixteen pairs of somites, a left lateral-facing head, a closed anterior neuropore, an indicated telencephalon, primary optic vesicles, and a well-established optic rod. When stage HH13 is reached, they have nineteen pairs of somites, the head becomes partially turned to the left, the cranial and cervical flexures form wide curves, and enlargement of the telencephalon occurs. Stage HH14 (Figure 3) is characterized by the presence of 22 pairs of somites; the axes of the prosencephalon and rhombencephalon form a right angle, and the broad cervical flexure forms a curve.

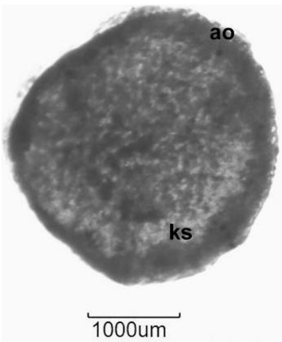

Figure 1. Japanese quail embryo collected at laying and classified in stage XI. Note the area opaca (ao) around the embryo and the Koller´s sickle (ks).

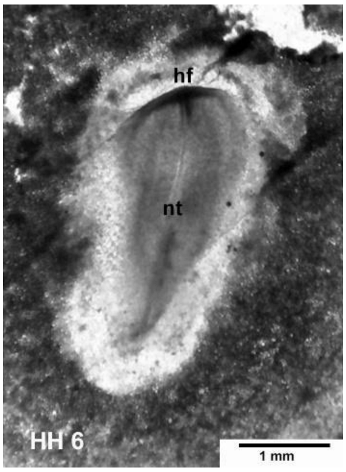

Figure 2. Japanese quail embryo incubated for 24 hours in an artificial incubator at 37.4°C and classified at stage HH 6. Note the head fold (hf) to form the cranial part of the embryo and the neural tube (nt) in the central area.

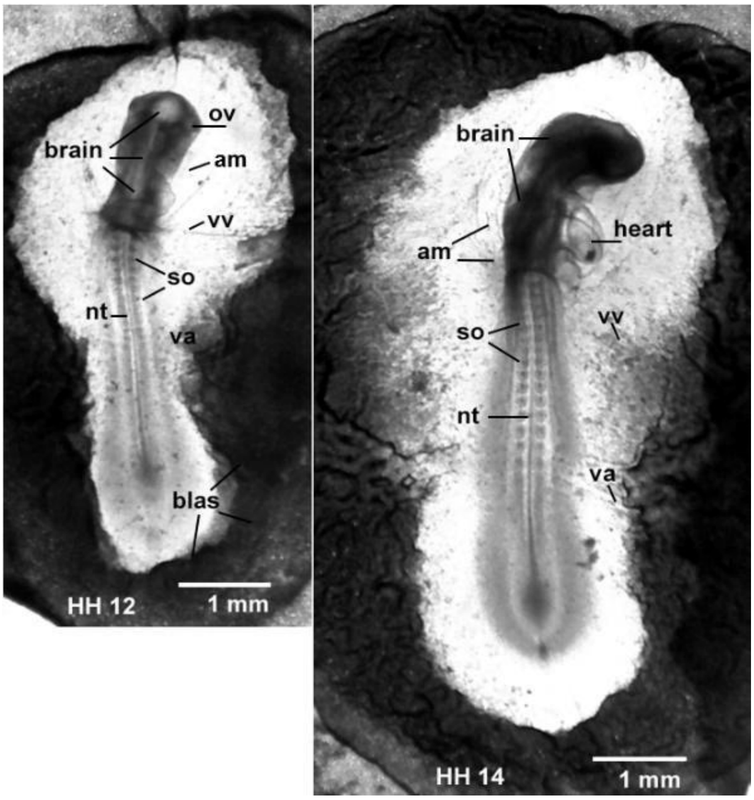

Figure 3. Japanese quail embryos classified in HH stages between 12 and 14 with 48 hours of artificial incubation at 37.4°C. Optic vesicle (ov), somites (so), neural tube (nt), vitelline vein (vv), vitelline artery (va), and blastodisc (blast).

In embryos with 72 hours of incubation, stages observed were HH17 to HH20, with six in stage HH17, twenty-one in stage HH18 (Figure 4), nineteen in stage HH19, and thirteen in stage HH20. When the embryo takes shape at stage HH17, it reaches the number of 29 to 32 pairs of somites, the leg buds become visible, and there is an increase in the wing bud. The cervical flexion becomes more pronounced than in the previous stages. Stage HH18 (Figure 4) is characterized by the existence of 30 to 34 pairs of somites, trunk flexion changes to the lumbar region, a visible allantoid, and a generally closed amnion.

At stage HH19 the embryos have both wing and leg buds, with the leg buds being slightly larger than the wing buds. They have 37 to 40 pairs of somites that extend to the tail. Embryos at stage HH20 have 40 to 43 pairs of somites, nearly symmetrical wing buds, and asymmetrical leg buds. In this stage, the rotation process is complete, and the eyes begin to show a light grayish pigmentation.

In general, the embryonic development during the incubation process of fertile eggs of Japanese quails without storage occurs in 46 stages of morphological changes in the embryo during the 17.5 day incubation period. According to Sellier, Brillard, Dupuy, and Bakst (2006), quail embryos from eggs collected at the time of egg-laying (0 hours) are at stage XI. For the 24 hours incubation period, Sellier et al. (2006) and Ainsworth, Stanley, and Evans (2010) reported that stage HH6 is the embryonic developmental stage evident for Japanese quails in this period. In contrast, Ainsworth et al. (2010) provide evidence that for the 48 hour incubation period, Japanese quail embryos reach the HH12 or HH13 stage. For the 72 hour incubation period, the Japanese quail embryo reaches the HH18 stage.

It has been reported that the age of the matrix only exerts influence on embryonic development in embryos from broiler and chicken matrices. According to Reijrink, Meijerhof, Kemp, Graat, and van den Brand (2009), at the time of egg-laying, embryos from younger dams at 28 weeks of age reach the EGK stage (Elyal-Giladi & Kochav, 1975) at 9.22, while embryos from older dams at 61 weeks of age reach the EGK stage at 11.67. Furthermore, Damaziak, Pawęska, Gozdowski, and Niemiec (2018) recorded that at 48 hours of incubation, embryos 70 weeks old reached the HH13 stage, and embryos from younger matrices at 49 weeks of age reached the HH12 stage. According to Fasenko, Robinson, Hardin, and Wilson (1992), this happens because older chickens tend to produce eggs in which the embryo is more developed at the time of laying.

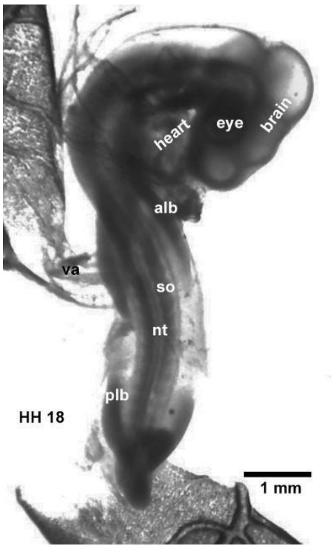

Figure 4. Japanese quail embryo classified at stage HH18 of development with 72 hours of artificial incubation at 37.4°C. Anterior limb bud (alb), somites (so), neural tube (nt), posterior limb bud (plb).

Conclusion

The egg variables in Japanese quails are affected by the age of Japanese breeders without having an effect on embryonic development between 0 and 72 hours of incubation. The weight, length, width, volume, and area of the eggs increase with age.

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