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Comprehensive Study of Organogenesis During Embryonic Development of Japanese Quail, *Coturnix coturnix japonica*.

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ABSTRACT

The avian embryo is unique for studying morphogenesis in birds. Today widely used model for experimentation is chick and the focus on the chick embryo has somewhat overshadowed the other avian species which can also prove to be of much importance. One avian species which is equally powerful is the Japanese Quail, *Coturnix coturnix japonica*. The Japanese Quail, due to its easy maintenance, early sexual maturity, shorter generation interval and high rate of egg production has become a pilot animal in the field of research.

In the present study, normal stages of chick embryo development were used as a basis for studying the development of quail embryo. Japanese Quail embryo takes approximately 17 days to hatch, a primitive streak were seen on Day 1 while Day 2 shows prominent somites. Eye pigmentation, beak region, various joints were seen on Day 4 and Day 5 respectively. On Day 9 and onwards there was prominent pigmentation were observed. Thus, the major objective of the present research work was to study the embryonic development of the Japanese Quail and to identify the period of incubation where the Japanese Quail embryo gives the faster ontogeny than chick embryo.

KEYWORDS

Japanese Quail, morphogenesis, development, embryo.

INTRODUCTION

The research work in developmental biology till date has used the avian eggs for the morphological study and majority of researchers focused on chick embryo (*Gallus domesticus)* and proved it as a role model. The focus on the chick embryo led Hamburger and Hamilton (1951) to produce their definitive stage series nearly 60 years ago and this series is still a basic study for all the developmental biology works. Since the noted work by Hamburger and Hamilton (1951) on normal chick embryos appeared, many workers have studied embryos of various species of birds. Rampel and Eastlick (1957) showed constant differences between embryos of the White Silkie Bantam and standard varieties of the domestic chicken. Koeck (1958) described the embryonic stages of the domestic duck.

Mun and Kosin (1960) correlated the development of broad breasted bronze turkey embryos with the chick so this focus on the chick embryo has skipped the importance of another avian species, that proved to be of equal importance for developmental studies, the Japanese quail, *Coturnix coturnix japonica.* The discovery by Le Douarin in the late (1960) that chimeras could be made using chick and quail embryos revolutionized the approach taken to study the development and has helped to unlock some of the mysteries underlying various embryonic processes including the cellular and molecular interactions that occur during morphogenesis (Le Douarin, 2008).

The Japanese quail is a member of the Pheasant family and is considered to be a separate species from the common quail. Japanese quail eggs are small; 30mm in length and approximately 10 gms in weight and their incubation period is approximately 16.5 to 17 days. Japanese quail were first reported as a useful research model by Padgett and Ivey (1960) and since then have become a common laboratory bird for many investigations such as developmental, behavioural and environmental studies.

Padgett and Ivey (1960) were among the first to describe the development of *Coturnix coturnix japonica* in detail. Zacchei (1961) staged the quail embryo and compare the time with a specific Hamerger Hamilton stage of chick development. The fact that so many research groups are using the quail embryo in a number of new ways and the availability of literature suggests that the quail embryo is a primary animal model in developmental biology. In view of this we felt that the production of definitive stages with some internal organ development for Japanese quail is necessary so the primary objective of this research is to study the embryogenesis of quail which slightly differs from the chick embryology, and gives faster ontogeny.

MATERIALS AND METHODS

About 200 eggs of Japanese quail were obtained from Venkateshwara Hatchery, Pune. The *Coturnix* egg weighs approximately 10 gms., an estimated 8 percent of the female body weight. The basic shell colour is white or buff with patches of brown, black or blue. Eggs were of 2 days old when obtained from Pune. Eggs were cleaned with sand paper and fumigated with Potassium Permagnate and were systematically numbered (n=200), weighed and set to incubate in BOD incubator at 35^0C temp and 60% relative humidity. Turning of eggs was done manually after every 4 hours for 24 hours time period daily. From Day 3 regularly the eggs were broken and the embryo is fixed in Bouin's fixative after weighing for studying the various developmental stages. The embryos were staged every 24 Hrs. from 3 days until hatching. The embryos collected were photographed using digital camera.

RESULTS AND DISCUSSION

Nearly 200 eggs were used in the development of the Japanese quail using BOD incubator for variations in temperature (Zacchei 1961; Graham and Meier, 1975; Lilja et.al. 2001; Grenier et.al., 2009).

Table 1: Showing different developmental features of developing quail embryo.

Sr. No.	Days of Incubation	Identifying features
01	Day 01	Primitive streak is seen. Head fold is seen.
02	Day 02	Somites are evident, embryo bents towards right, optic stalk is seen.
03	Day 03	Leg bud is visible, eyes remain unpigmented. Maxillary process becomes distinct.
04	Day 04	Eye pigmentation distinct. Limb buds are prominent.
05	Day 05	Beak region is identified, knee and elbow joints in limb buds are distinct.
06	Day 06	Wing elbow is seen bend. One or two sclera papillae is evident. Egg tooth is present.
07	Day 07	Six to eight scleral papillae is seen. Toes length increased.
08	Day 08	Eyelids begin to grow over the surface of eyeball.
09	Day 09	Black and brown pigmentation is seen in the lumbosacral region.
10	Day 10	Black pigmentation is seen in the skull region.
11	Day 11	Pigmentation expands in the wing region and around intertarsal joints. Beak length increases.
12	Day 12	Pigmentation visible on toes.
13	Day 13	Third toe and beak length increases. Feathers are longer and glossy.
14	Day 14	Embryo increases in size.
15	Day 15	Beak length increases and toes get folded.
16	Day 16	Pre Hatching stage. Yolk completely utilised.
17	Day 17	Quail chick Hatched.

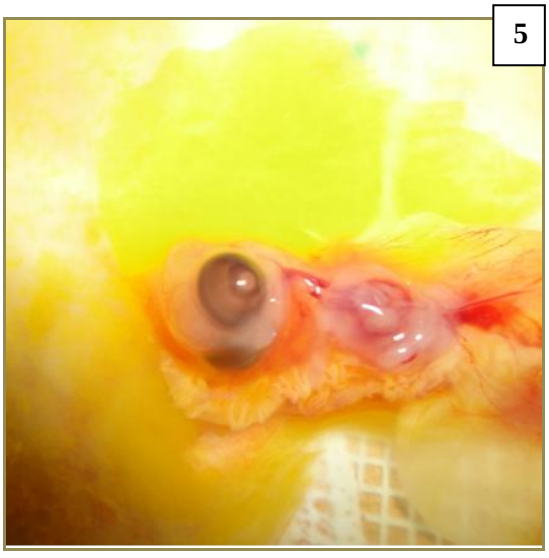

PLATE - 1:	
Fig. 1 : Incubator **Fig. 2 :** Egg Incubation **Fig. 3 :** Day 1, Allantois is first apparent. Amnion is closed. **Fig. 4 :** Day 2, Primitive streak and Head fold is seen.	**Fig. 5:** Day 3,One to two scleral papillae are visible. Egg tooth is present. Six scleral papillae evident. **Fig. 6 :** Day 4, Eye pigmentation distinct, limb buds are equal in width and length. Elbow and Knee joints distinct.

PLATE 2:

Fig. 7 : Day 5, Eyelids begin to overgrow surface of eyeball. 13-14 Scleral Papillae are seen.

Fig. 8 : Day 6, Feather formation starts

Fig. 9: Day 7, Black and brown pigment is apparent in feather buds. Black pigmentation is present either side of the spine (Tapering off in the region of the pelvis). There is a single line of black feathers in the region overlying the coracoids. Golden brown pigmentation is present in the region of the tail.

Fig. 10: Day 8, Pigmentation is visible on the head with black pigmentation present on the forehead and crown. A single distinct line of black feathers in the region of the coracoids is now prominent; Golden brown pigmentation increases near leg, arms and tail region. No pigment is yet present on the feet.

PLATE-3

Fig. 11 Day 12, Prominent beak length is seen.

Fig. 12 Day 13, Pigmentation on the feet is increasingly prominent and for the first time apparent along the ventral surface of the toes. There is an increased density of feather germs and each feather is considerable longer and produce a glossy look.

Fig. 13 Day 14, The overall length of the bird is variable and therefore the beak and third toe length should be used for accurate staging

Fig. 14 Day 15, Fully grown bird (quail)

Fig. 15 Day 17, Hatching of quail

The fixed Day 1 embryo shows distinct primitive streak and head fold was seen. There was occurrence of somites and optic stalk on Day 2 also occurrence of eye pigments and prominent limb buds on Day 4. On Day 8, eyelids were developed over the surface of eyeball. Black and brown pigmentation were observed on lumbosacral region of Day 9 embryo. The developing embryo from Day 9 onwards shows increase in size of various organs and decrease in yolk content. The various identifying developmental features are summarized in Table 1. The morphological details of eggs from Day 3 onwards till hatching (Day 17) are presented in Plates 1-3.

The Japanese quail remains one of the favoured animal models in developmental biology and is being used to investigate a variety of developmental systems. Quail are most notably used as part of the chick-quail chimeric methodology and more recently as part of other chimeric approaches (Le Douarin, 2008; Lwigale and Schneider, 2008). As the popularity of this animal is growing we have studied the developmental stages. Padgett and Ivey (1960) had reported the variations in developmental timings and physical factors associated with the incubation. The appearance of various developmental features after specific periods of incubation (Day wise) make possible to attribute quail development.

CONCLUSSIONS

The present work concludes by studying the specific developmental features of quail embryos and hope that this study will act as a key laboratory aid for those using the Japanese quail in their developmental investigations. One specific finding in Japanese quail embryo is the appearance of pigmentation patterns within the feather germs. This demonstration of pigmentation patterns may be helpful to those investigating the feather mechanisms and morphogenesis.

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