I J R B A T, Issue (X) Vol (III) Sept 2022: 188-196 e-ISSN 2347 – 517X
A Double-Blind Peer Reviewed & Refereed Journal OPEN ACCESS Original Article

INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOGY



# Anatomy of Reproductive System in Japanese Quail, *Coturnix coturnix japonica*, Embryo

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Communicated :28.05.2022 Revision: 31.05.2022 & 30.06.2022 Accepted: 06.08.2022 Published: 30.09.2022

**ABSTRACT:**
Researchers developed special interest to study sexual dimorphism in Birds. Visibility of external gonads can determine the sex of Bird. The gonads, Testis in Males and Ovary in Female are different in morphology and locations are also different. The testis in Mammals is situated in the scrotum. However, in birds, the testes are found within the abdominal cavity, situated on either side of the backbone adjacent to the cranial pole of the left and right kidney. In female bird, the ovaries are present within the abdominal cavity. There are two ovaries in mammals, but in birds, there is only one functional ovary, which is always located on the left side, positioned similarly as the left testis. To locate the gonads of birds, one must therefore enter the abdominal cavity surgically. The male bird's reproductive tract consists of the paired testes, epididymis, and ductus deferens. Accessory sex glands are absent in birds. The female bird's reproductive tract consists of the left ovary and the left oviduct. The left and right ovary and oviduct develop embryonically as paired structures, but after hatching, the right ovary and oviduct degenerate.

**Keywords: -** *Anatomy, Reproductive Organs, Japanese Quail Embryo.*

## INTRODUCTION:

The Japanese quail, *Coturnix japonica*, is a migratory, gallineous, ground dwelling game bird native to East Asia and Japan. The Quail is a small type of bird that belongs to the Pheasant family. They were first domesticated in Japan in 1595. There are two species of quail in India; the black-breasted quail found in jungle *(Coturnix coromandelica*) and the brown-coloured Japanese Quail (*Coturnix Coturnix Japonica)* which is bred for meat or the one used for commercial Quail production. Like chickens, these birds are being used for food purpose in India. There are 45 species of quail. Although the Japanese quail is the largest species, it is much smaller then pigeon. The Indian quail weighs up to 100 gm and lays 100 eggs a year, the Japanese quail weighs up to 200 gm and lays 280 eggs a year. Quails have been classified as game or hunting birds. They cannot be compared with chicken because their needs vary. Though Government of India is encouraging entrepreneurs to start quail farm, a government license is required to sell commercial Japanese quail considering the safe guard of jungle variety of the bird, which is a protected specie.

Embryology is the study of the growth and differentiation undergone by an organism in the course of its development from a single fertilized egg cell into a highly complex and independent living being like its parents. Avian embryos are important experimental models. They become useful in investigating embryonic development and organogenesis. Mainly important is egg which provides all the components necessary for development. Most research on avian embryos investigates the development of the embryo while the extra-embryonic and the non-embryonic components within the egg have attached less attention, even though they are essential for embryonic development. The extra embryonic components (e.g. yolk sac, allantois and amnion) are temporary structures participating in fundamental metabolic process such as

respiration, nutrition and excretion. The non-embryonic components of the egg (egg yolk, albumen and shell) provide nutrients and also physical and microbial protection for the growing embryo. Reproductive Organs are Testis and Ovary. Avian species utilize a variety of reproductive strategies that allow them to reproduce under a diversity of conditions and environments.

**MATERIALS AND METHODS**

In this study the developmental stages of domesticated quail was studied with special reference to the development of endocrine glands. Since, domesticated quails do not have brooding tendency, the eggs must be incubated under a brooder hen or by artificial incubation. For successful hatching, quail eggs should be fertile, fresh and clean. The fertilized eggs of Japanese quail were purchased from quail farm i.e. Venkateshwara Hatchery, situated at Pune, Maharashtra (Fig.1.) Fertile eggs are alive and since, these eggs need at least 2 days (after collection) to bring in the laboratory, care was taken during transportation. The eggs were carried with all necessary care to avoid any physical damage to them.

The eggs were stored at room temperature away from sunlight. It should be within 3 days after collecting from the farm. First the eggs were fumigated by using potassium permanganate and formalin (40%). Potassium permanganate is placed in earthenware dish and formalin is added at last. The egg box is removed after 20 min. of fumigation. Then these eggs were incubated in BOD incubator with environmental control-temperature is 38°C and Relative Humidity 60% (Fig. 2). Fertile eggs are alive and each egg contains living cells that develops into an embryo and finally into a quail chick (Randall, 2008). Till the incubator is set properly the eggs were stored at room temperature. Since, the relative humidity (relative humidity is the water vapour in the air expressed as a percentage of the greatest amount of water vapour possible at that temperature) plays a very important role during incubation and hence, while storing eggs the relative humidity in the storage room was kept between 60 and 70% and the room temperature/storage temperature before incubation was 55˚ F (13˚C). Appropriate temperature, humidity, turning and ventilation are the most important factors in incubation (Northwest Game Birds, 2009). Temperature is one of the physical factors that determine the success of incubation. Therefore, it was essential to determine and use a temperature that promotes the highest hatchability (Swann and Brake, 1990) and the best hatchling quality (Wilson, 1991, Decuypere and Mitchels, 1992). The optimum incubation temperature of wild fowl eggs is within a wide range of values, varying from 33°C to 39°C, whereas a narrower range (37°C to 38°C) is considered as optimum for domestic poultry (Visschedijk 1991). Since, the temperature can highly influence the relative humidity and can contribute to water loss during incubation; both the parameters were carefully monitored during incubation (Romao *et al.*, 2009). In this study the temperature of the incubator was set at 38˚C and relative humidity was maintained at 70% (Ainsworth, 2010). In this study, stages at 24-hours intervals were chosen for description, so embryos were prepared every 24-hr from 3 to 17 days of incubation. The external developmental stages of the embryos used were roughly estimated according to Zacchei (1961). Regularly after 24 hrs of incubation one egg i.e. egg no. 1 was removed and it was weighed. The egg was gently knocked out and opened at the wide part with forceps (Rong, 2010). Subsequently, the shell and shell membrane is removed. Embryo sac and outer membrane surrounding the embryo was cut off. The embryo was transferred into Petri dish filled with saline water with the help of brush and blunt forceps and after

washing the embryo was weighed i.e. Embryo weight. Embryo was fixed Bouin's fixative (aqueous). The embryo were dehydrated and embedded in paraffin wax for blocks. Paraffin sections were taken of 5 micron in thickness and stained with Haematoxylene Eosin and were examined with Light Microscopy (Ahmed and Soliman, 2013). The process was repeated after every 24 hours and each day total five embryo (eggs) were taken out and fixed in the fixative. The eggs were broken and the observations were recorded with the aid of Digital Camera (Digital Sony Cyber Shot Camera) and the photographs and videos were stored. Furthermore, the embryos were matched using the Hamburger and Hamilton (HH) system for chicks (Hamburger and Hamilton, 1951) applied to quail (Le Douarin, 1996).

**RESULT AND DISCUSSION**

Both female and male hormones are produced by the different gonad. Oestrogenic hormones are secreted by the interstitial cells of the medulla from about day 4, whereas testosterone is produced by the cord cells. The pituitary-gonadal axis is established from about day 13 (see discussion by Freeman and Vince, 1974). Luteinizing hormone, (LH) is present in the blood early in development but does not reach a high enough level to stimulate steroidogenesis in the gonads until 13.5 -14.5 days (Woods, 1987). Nevertheless, steroidogenic factor-1, which, which regulates steroidogenic enzyme expression, was detected by in-situ hybridization in the undifferentiated genital ridge of both sexes as early as stage 21-22 (3.5 days). By stages 30-35 it had become higher in the ovaries than in the testes and eventually was highest of all in the left (functional) ovary (C.A. Smith *et. al*, 1999). There was a reduced response in the right ovary as it regressed, and a comparable reduction was noted by Pedernera *et. al.* (1999), in gonads treated with follicle –stimulating hormone (FSH). Both the left and right ovaries of 8-day embryos responded to FSH by secreting steroids but in embryos older than 13 days the right ovary failed to respond.

**Testis**

Testis of Japanese quail is developed in the embryo. The 5$^{th}$ day embryo of Japanese Quail showed the immature testis with one layer of tunica vaginalis and tunica albuginea. The serial section of day 5$^{th}$ to day 7$^{th}$ embryo during incubation shows the development of testis in Japanese Quail (Fig 3). The Seminiferous tubules of quail testis lined by spermatogenic cells and sertoli cells. The sertoli cells are irregular, tall columnar cells located adjacent to spermatogenic cells. At the end of day 7 of incubation the sex cords (primary sex cords), which will differentiated into the seminiferous tubules continue to branch and proliferate. The primary sex cord becomes canalized after hatching only. The primordial germ cells begin to divide and differentiate about day 14 to day 15 into spermatogonia. The sertoli cells are assumed to be developed and derived from germinal epithelium. The mesonephric tubule become the vasa deferens and subsequently carries sperm to the cloaca.

Microscopically, the testis consists of tubular structure known as seminiferous tubules contain two cells spermatogonia cells and sertoli cells. There are some specified cells called interstitial cells or Leydig's cells in the spaces between seminiferous tubules (Fig. 4). Leydig's cell produces Testosterone hormone. Testosterone is responsible for many secondary sex characters such as song behavior, feather formation, color, development of a comb and wattles in some species. Testosterone helps maintaining spermatogenesis under the influence of pituitary gland. Histologically, testis is cover by two layers of germinal epithelium one is double layered tunica vaginalis and another single layered tunica albuginea. Testis has dual functions an exocrine and endocrine function.

The exocrine function is the production of spermatozoa. The endocrine function is the production of the male sex hormone Testosterone.

**Ovary**

The ovary with ova in various stages of development is an unpaired structure in the female birds (while there are two ovaries in the embryo, the right does not develop). The left ovary lies in the dorsal part of the abdominal cavity opposite the last two ribs. It weighs about 150 to 200gm. The oviduct varies in appearance according to its functional state. As said earlier, the right ovary and oviduct develops embryonically but after hatching, the right ovary and oviduct degenerates. If the left ovary is removed from a bird before 30 days of age, the remnants of the right ovary will develop into an ovitestis which may be capable of producing sperm. The Japanese quail embryo of 7$^{th}$ day of incubation and 8$^{th}$ day of incubation shows specific characteristics of ovarian development (Fig.5) The left ovary is found in the body cavity cranial to the left kidney adjacent to the adrenal glands. It is attached to the body wall by a thin ligamentous structure called the mesovarium. The ovary consists of two parts the medulla and the cortex. Cortex develops into primary oocyte after hatching. The avian primary oocyte is the largest cell in the animal kingdom. The primary oocyte (egg) of Japanese quail weighs upto 10gm to 12 gm. Normal development of the left and right ovaries was observed. The left ovary had a distinct cortex and medulla with cords of primordial germ cells in the cortex. The medulla was composed of distended cords and cavities with a few isolated primordial germ cells. The Histological study of the sections showed the smooth strips of left and right ovaries. But the ovary does not have any difference between medulla and cortex. The germ cells in the female Japanese quail appeared in the gonadal area between two and two and half days incubation with a preferential distribution favoring the left gonad. Proliferation of the primary or medullary cords from the germinal epithelium was observed between four and five and a half days.(Fig.6) Only the left ovary is functional in birds. The expansion of primary sex cords by the day 6 of incubation gives rise to the secondary sex cords from the epithelium. The primary sex cords become the medulla of the ovary and the secondary sex cords become the cortex. The right gonads fail to form a cortex and remain rudimentary. There is no communication between ovary and mesonephros like testis. It is usually considered that the gonad is incapable of becoming a true ovary without the cortex.

Quinn *et al.*, (2008) studied the effects of a one time embryonic exposure to p,p′-DDE (dichlorodiphenyldichloroethylene; DDE) on the reproductive development and function in Japanese quail, wherein, embryos were exposed at day one of incubation to either 20 or 40 µg DDE or a sesame oil vehicle control (injection volume = 20 µl). Based on their study of quails Rong *et al.*, (2011) have reported that when embryo was hatched for 4 days, lots of primordial germ cells clustered in the region where gonad would be formed, which is congruent to our observations. On the 5$^{th}$ day of hatching, the gonad of the embryo began to be formed and exhibited the feature of ovary or testis. Thus, our results establish the groundwork for the research of the development of gonads of quail and other poultry. Moreover, since, the reproductive system is important for the sustainability of the life cycle of the quails, it needs more emphasis in the future studies as Bruggeman *et al.*, (2002) have stated that the

heterogametic sex in avian species is the female and the presence of estrogens and their receptors plays a crucial role in female sexual differentiation, which shows that the development of gonads in birds is very sensitive to changes in the embryonic hormonal environment, sometimes resulting in changes of postnatal reproduction and even growth. Grzegorzewska *et al.*, (2009).

**CONCLUSION:**

Though several inter breeding subspecies are recognized, the more important being the European quail, *Coturnix coturnix coturnix*, and the Asiatic or Japanese quail, *Coturnix coturnix japonica*. Furthermore, apart from its economic advantages, the Japanese quail remains one of the favored animal models in developmental biology and is being used as part of the quail chick chimeric methodology and more recently as part of chimeric approaches (Le Douarin, 2008; Lwingale and Schneider, 2008). As the popularity of this animal is growing it was necessary to study the developmental stages of the bird. This is especially important as this knowledge will help in boosting the production as well as maintaining the livestock. Padgett and Ivey (1960) had reported the variation in developmental timings and physical factors associated with the incubation. The appearance of various developmental features after specific periods of incubation possible to attribute quail development. Ovary formation starts from the 5$^{th}$ day embryo and appears. Testis was observed from the 4$^{th}$ to 7$^{th}$ day embryo. A pair of testis is located in the anterior portion of the abdominal cavity just above the kidney. Spermatogenesis takes place and secretes testosterone –Male Hormone. Before fertilization the sperms are transported through a rudimentary epididymal region into the ductus deference.The Ovary of reproductive female is remarkable as Galliformes ovulates almost daily. At the time of egg production, the ovarian layer Theca externa, theca interna and the germinal epithelium are the primary sources of the steroids. The developed follicle produces greater amount of estradiol and progesterone. Embryologically, Ovary formation starts from the 5$^{th}$ day embryo. Testis was observed from the 4$^{th}$ to 7$^{th}$ day embryo.

**CONFLICT OF INTEREST**

Author declares no conflict of interest about the present study.

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**Fig. 1. Japanese Quail Egg**

**Fig. 2. Eggs kept for incubation in a tray**

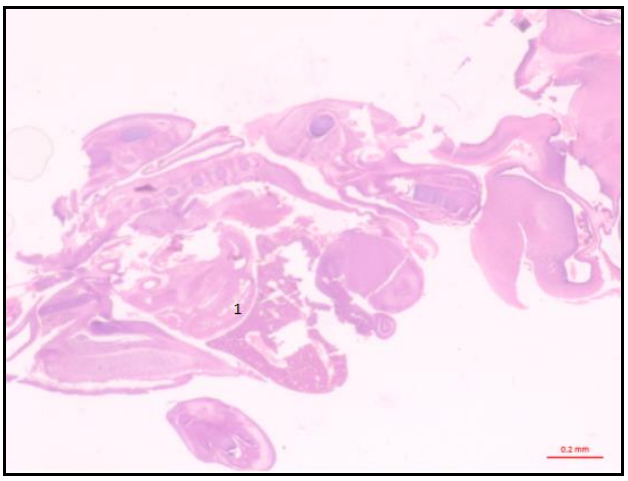


**Fig. 3. Photomicrograph of T.S. Day -7 Embryo showing testis**

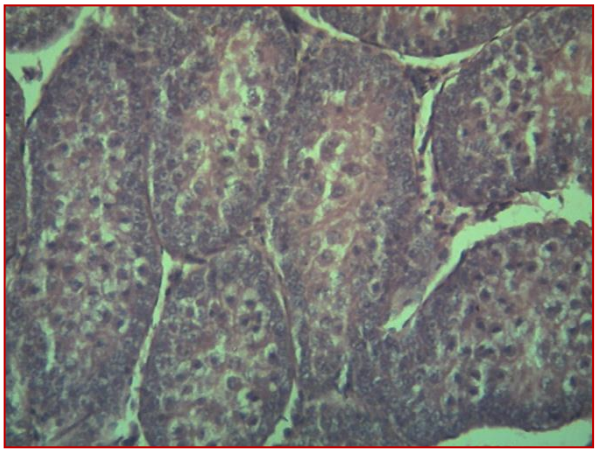

**Fig.4. Photomicrograph of T.S. Testis 40X**

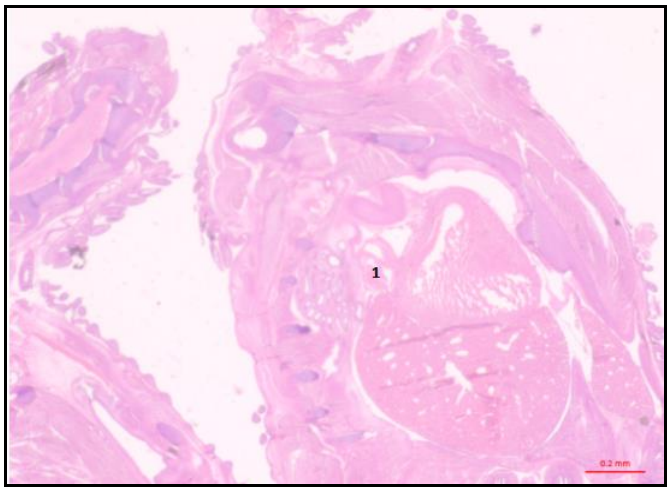


**Fig. 5. Photomicrograph of T.S. Day-8 Embryo showing ovary**

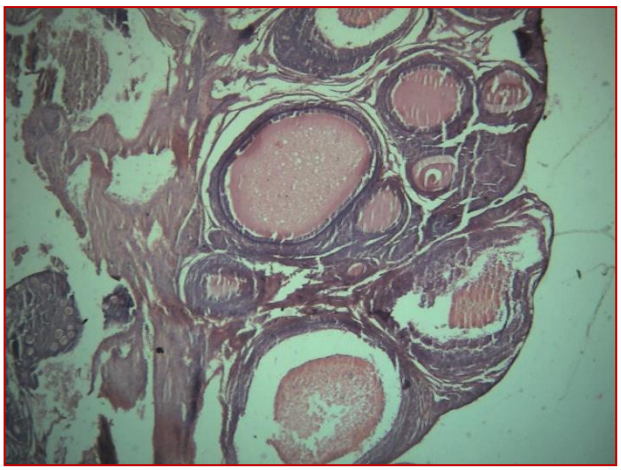

**Fig.6. Photomicrograph of T.S. Ovary 40X**