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Development of gonads in *Coturnix coturnix japonica* (Japanese quail) embryo

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Abstract
The experiment was conducted to study the development of Gonads in quail embryos. The eggs of Japanese Quail are incubated in BOD incubator at 35 ºC and 60% Relative Humidity. The embryos were cut out, fixed, paraffin sectioned and stained with Heamatoxylene-Eosin. The result shows that the formation of ovaries starts from 4th day of incubation. It matures till 17th day of incubation. The testis starts developing by showing the epididymal linings on 5th day. These results established the basic groundwork for the research of the development of other endocrine glands of quail as well as other poultry.

Keywords: Japanese quail, embryology, gonads, endocrinology

Introduction
Embryology is the science of development of the individual during the embryonic stage and subsequent stages of the life cycle. Chick embryo proceeds from egg lying to having a functional heart within 36 hours. Obtaining embryos does not require the death of the mother as in the case of mice and rats.

Endocrinology is the study of endocrine system and its role in the physiology of the body. Avian endocrine system includes Pituitary gland, Gonads, Thyroid gland, Parathyroid gland, Adrenal gland, Pineal bodies and Thymus gland. The Gonads are under the control of Anterior Pituitary gland which is considered to be the master gland. The sex organs of males and females are called the Gonads. These organs produce hormones namely Oestrogen, Testosterone and Progesterone. Both male and female need all three hormones but in different amounts.

Quail, *Coturnix coturnix*, belongs to Aves, *Galliformes*, *Phasianidae* and *Coturnix bonnaterre*. It has been widely studied in the fields of developmental and reproductive biology as a usual kind of experimental animal model. Now domestic studies about raising quails scientifically have been reported in detail (Hu, 1990), while reports on development of quail's embryo and gonad are few. Differentiation and development processes of poultry gonads are similar to other vertebrates. Both are differentiated into testis and ovary from gonads in which there are no morphological differences. Gonadal differentiation generally occurs on the 5 - 7th day of hatching and gonads differentiate completely on the 8th - 10th day of hatching so that we can identify the sex of them anatomically.

In this experiment, quail's embryos of 4 to 17 days and the day one of quail hatching were used as the objects of study and then they were observed for the time of differentiation of endocrine glands and a series of morphological development. The results provided basis for the differentiation and development mechanisms of endocrine glands in quails and other birds.

Materials and Methods
Sample collection
Quail's hatching eggs used in the study were from Venkateshwara Hatchery, Pune. The eggs were incubated in BOD incubator (environmental control: temperature, 38 °C; humidity, 60%) after fumigation.

Embryo collection
Embryo collection on the 4 - 6th day of hatching: the blunt end of the incubated egg was gently knocked out with ophthalmic forceps, the shell and shell membrane were removed; the embryo was transferred into Petri dish filled with warm (38 – 39 °C) 0.75% saline with forceps; embryo sac and the outer membrane surrounding the embryo were cut off and then the embryo was rinsed with clean warm 0.75% saline gently for 2 -3 times. Finally, the embryo was soaked in Bouine's fixative labeling samples, date and stationery overnight.

Results and Discussion
Gonads
Both female and male hormones are produced by the indifferent 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) [1] 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) [2]. 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 teses and eventually was highest of all in the left (functional) ovary (C.A. Smith *et. al*, 1999) [3]. There was a reduced response

in the right ovary as it regressed, and a comparable reduction was noted by Pedernera *et al*. (1999) [4], 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

A pair of testis located in anterior aspect of the abdominal cavity just above the kidneys. The testes are either oblong or cylindrical in shape, smooth on the surface and creamy-white in color. The testis are very small and usually a vascular and enlarge during the breeding season.

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. 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 [5].

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. Histologically, testis is cover by two layers of germinal epithelium one is double layered tunica vaginalis and another single layered tunica albuginea [6].

Testis of Japanese quail is developed in the embryo. The 5th day embryo of Japanese Quail showed the immature testis with one layer of tunica vaginalis and tunica albuginea. The serial section of day 5th to day 7th embryo during incubation shows the development of testis in Japanese Quail. 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.

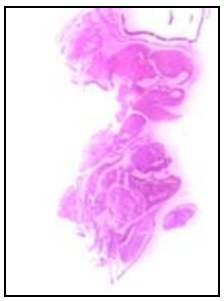

Fig 1: Embryo showing location of Gonads

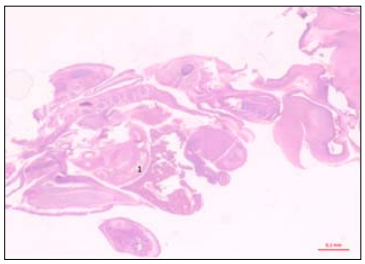

Fig 2: Photomicrograph of T.S. Day -7 Embryo showing testis

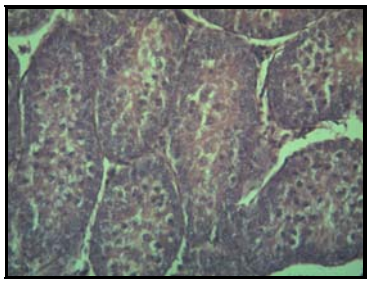

Fig 3: Photomicrograph of T.S. Testis 40X

Ovary

Avian species utilize a variety of reproductive strategies that allow them to reproduce under a diversity of conditions and environments. Endocrine and behavioral components of reproduction are directed by the hypothalamus in response to environmental triggers like photopheriod. The ovary of the reproductive female is remarkable in that hen ovulates almost daily, with some commercial layer strains of chicken layer over 300 eggs per year. In galliformes, the smallest follicles visible on the surface of the ovary (about 0.2- 1.0 cm in diameter) are called as small white follicles which increase in diameter. Now the yellow yolk is visible. The primary follicles secretes estradiol hormone and the large follicle produces progesterone. The shell formation of egg is under the control of progesterone [7].

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 [8].

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 ovary has two functions to produce the ovum and the female hormones oestrogen and progesteron. In addition, some testosterone is also produced by the ovary. 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.

Ovary secretes oestrogen and progesterone and some amount of testosterone. These hormones regulate the ovarian functions. Progesterone is most important ovarian hormone essential for ovulation and for post ovulatory follicles. Oestrogen is involved in many sex characteristics such as development of an incubation patch, plumage colour, the development of oviduct, nest building behavior, egg shell production. Oestrogen and Progesteron combines and promotes incubation behavior [9].

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 [10].

The Japanese quail embryo of 7th day of incubation and 8th day of incubation shows specific characteristics of ovarian development. 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. This observation is supported by the gonadal development of chicken embryo research. The gonadal differentiation of the chicken embryo began on the 6th day of incubation period and its kind of differentiation became more significant on the 7th day of incubation [11].

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 [12].

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.

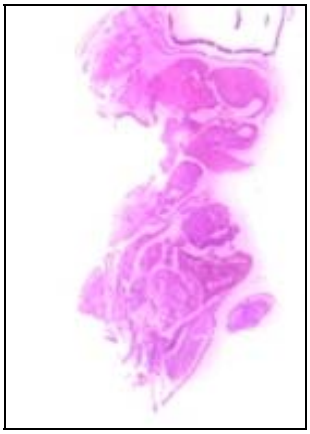

Fig 4: Embryo showing location of Gonads

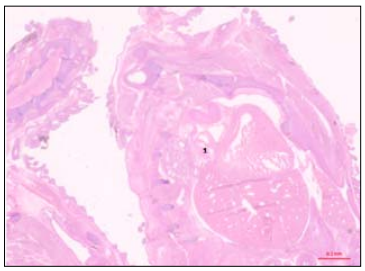

Fig 5: Photomicrograph of T.S. Day-8 Embryo showing ovary

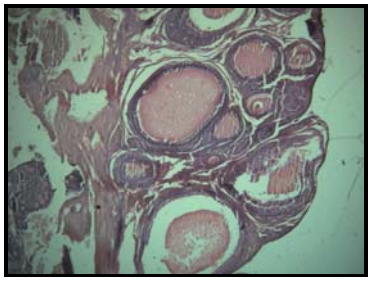

Fig 6: Photomicrograph of T.S. Ovary 40X

Based on the gonadal histological analysis on pure triploid White Leghorn chicken embryos Bonaminio and Fechheimer (1993) [13] reported that the morphology of the testes of the ZZZ triploids resembled that of normal ZZ males. The right and left ovaries of the ZWW triploid embryo were similar histologically to those of normal diploid ZW females. Though the scope of our study did not go beyond the emergence or development of the endocrine glands, the assessment of triploidy in Japanese quails would form an interesting subject.

Quinn *et al*., (2008) [14] studied the effects of a onetime 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). Authors report that gonadal morphology and sperm motility appeared to be unaffected and the results from this study provide evidence that the neuroendocrine system may be more sensitive and less resilient to embryonic exposure to contaminants than traditional measures of reproductive success following contaminant exposure in adults.

Based on their study of quails Rong *et al*., (2011) [15] 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 5th 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) [16] 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. Furthermore, the role of synthesis of sex steroids by embryonic gonads, which is regulated by luteinizing hormone and follicle stimulating hormone is also an important aspect of the developmental studies of the quails Grzegorzewska *et al*., (2009) [17]. Hormonal regulation of embryonic development is crucial in all vertebrate classes and Japanese quails are no exception. Although gonadal hormones are known to affect organogenesis in avian embryos, Haldar *et al*., (2003) [18] on the basis of their study reported that gonadal steroids (testosterone and estradiol) have effect on the morphogenesis and cell differentiation of the avian pineal gland clearly suggesting active roles of gonadal steroid hormones on embryonic pineal morphogenesis and cell differentiation and its physiological activity as they do in adult animals.

Conclusions

The above results conclude the mechanisms of the endocrine development of quails, chickens and other poultry, these could be analyzed through the migration in the embryo period to study the genuine processes of development of endocrine gland, and provide a new basis for the poultry developmental biology. The endocrinological study have provided us with a wealth of information on the differentiation and maturation of endocrine glands, the anatomical and functional development, ontogenic changes in the responsiveness and activity of the glands and their target organs, and the interactions of different regulatory systems to coordinate developmental processes such as hatching. The Endocrine system consists of various glands and nodes which secrete hormones. The importance of these hormones (which are chemical messengers) lies in the fact that they travel in the blood to activate target cells. These target cells have special receptors, into which only certain hormones can fit. For example, testosterone acts on the male gonads, but not the adrenal glands. In view of the importance of endocrine glands, their (endocrine gland's) development in Japanese quail was assessed on the basis histological studies. The slides were prepared from the sections of embryo of each day. Serial sections were studied. The specific findings with respect to the development of gonads in Japanese quail is as follows-

- **Ovary:** Ovary formation appeared on 5th day embryo.
- **Testis:** Testis formation appeared on 4th day embryo.

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