

*Full Length Research Paper*

# The development of ovary in quail's embryo

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The experiment was conducted to study the development of ovary in quails' embryos which were incubated for 4 to 17 days and incubated out for 1 day. The quails' embryos or gonads were cut out and HE staining was carried out. The results showed that when embryo was hatched for 4 days, lots of primordial germ cells (PGCs) clustered in the region where gonad would be formed. On the 5th day of hatching, the gonad of the embryo began to be formed and exhibited the feature of ovary or testis. On the 7th hatching day, the right ovary began to degenerate, just a few PGCs began to differentiate into oogonia. On the 10th day, there were many oogonia in the ovary, some of which were surrounded by some other cells distributed like circles. On the 11th day, there were more oogonia, the skinniness became thicker while the medulla was thinner. On the 13th day, the division between skinniness and medulla was obvious and the ovary formed the early original ovum. On the 14th day, more original ova were seen in the skinniness. On the 17th hatching day and on the 1st day of hatching out, the shape of ovary tended to be mature, also the ovum was clear and more; the medulla was full of vessels. On the 5th hatching day, gonad began to differentiate. On the 7th hatching day and later, the differentiation of gonad was obvious; the right ovary began to degenerate. On the 13th hatching day, early original ovum began to be formed in the skinniness of ovary. The results established groundwork for the research of the development of gonads of quail and other poultry.

**Key words:** Quail, embryo, gonad, ovary.

## INTRODUCTION

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 - 10<sup>th</sup> day of

hatching so that we can identify the sex of them anatomically (Mi and Zhang, 2004). Zhang et al. (2002) suggested that the incubation period of quail was 16 days or so, characterized by discoidal cleavage and gastrulae had been formed after 14 h of hatching which occupied 3.6% of the whole incubation period. After 96 h of hatching, embryo came into neurula period which took up 21% of the whole. At last, organogenesis stage occupied 75.4% (Zhang et al., 2002). Armengol et al. (2007) studied embryos of Japanese quail (*Coturnix coturnix japonica*) by light and laser confocal scanning microscopy, using the QH1 antibody as a tool to identify PGCs at all stages, results suggested that some selective sugar binding sites on the PGCs play a significant role in their migration, colonization and maturation (Armengol et al., 2007). Kannankeril and Domm (1968) reported that the germ cells in the female Japanese quail appeared in the gonadal area between two and two and a 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

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**Abbreviations:** HE, Hematoxylin-eosin; PGCs, primordial germ cells.

and five and a half days. The secondary or cortical cords were found to proliferate in the left gonad on the sixth day of hatching, while the right gonad revealed neither cortical cords nor cortex in any of the cases studied (Kannankeril and Domm, 1968).

In this experiment, quail's embryos of 4 to 17 days and the 1st day quails of hatching were used as the objects of study and then they were observed for the time of gonadal differentiation and a series of morphological and configurable variation in the process of ovary development. The results provided basis for the differentiation and development mechanisms of gonads in quails and other birds.

## MATERIALS AND METHODS

### Sample collection and preparation

Quail's hatching eggs used in the study were from Taizhou quail Egg Plant. The quails were reared intensively in the plant and they were mated randomly with each other to produce fertile eggs.

### Embryo collection

Embryo collection on the 4 - 6th day of hatching: the blunt end of the hatching egg (environmental control: temperature, 38°C; humidity, 60%) 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 Rossman's fixative labeling samples, date and stationary overnight.

### Gonads collection

Gonads collection on the 7 - 17th day of hatching and the 1st day of hatching out: the belly of the embryo was anatomized with ophthalmic forceps; the parts except gonads and kidney were removed. Then the samples were transferred into 4% formaldehyde fixative labeling samples, date and stationary, fixed overnight.

### Tissue slicing and staining

Samples above were embedded with paraffin for about 40 to 50 min at 59°C, slicing up successively for a thickness of 5 – 7 μm. Then the slices were stained with Hematoxylin-Eosin (HE). At last, they were observed under the inverted fluorescence microscope Olympus, SP70 and photographed.

## RESULTS

### Quail's genital region on the 4th day

On the 4th day of hatching, mesonephros had begun to take shape, and the primordial genital ridge had already begun to form in the inboard of mesonephros. The

primordial genital ridge was separated with mesonephros, but there were no significant boundaries between them which seemed like a substantive region (Figure 1a). A large number of PGCs collected neatly in the genital region which consisted of substantive PGCs and the incrassated epithelium coelomicus.

### Quail's gonads on the 5th day

Gonads began to differentiate in this period, serial sections showed that the development of mesonephros was obvious and could be clearly observed. Mesonephros was separated distinctively with germarium which enlarged and extruded inside the mesonephros. There were sections showed that ovarian-like germarium which seemed to be divided into cortex and medulla was shaped like strips (Figure 1b). At this stage, germarium had the morphological characteristics of ovarian development, so we could rename it as gonad.

### Quail's gonads on the 7th day

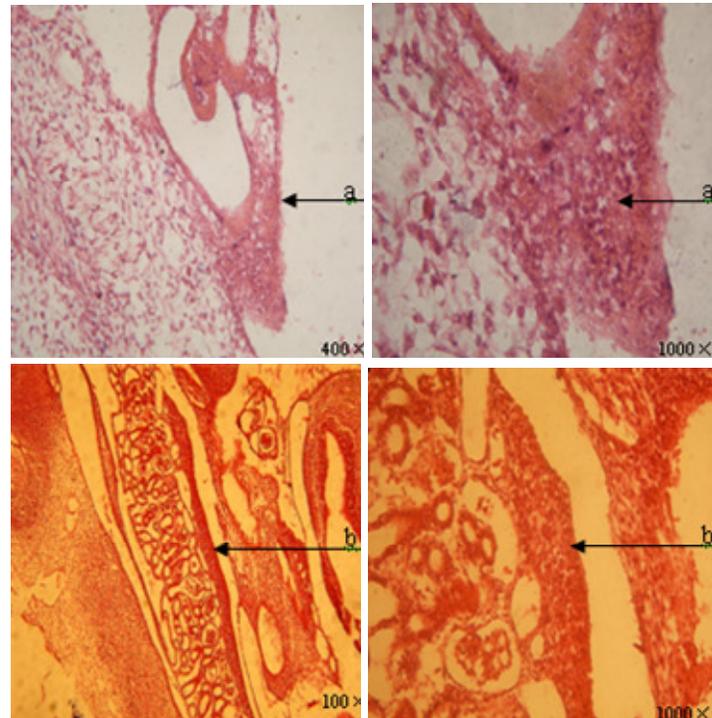
Sections of this period showed that there had been significant features of ovarian biopsy. Both the left and right ovaries was smooth like strips, but the left ovary slightly longer than the right ovary while testis was clearly not the same with it. Under the microscope observation, there were no significant differences between cortex and medulla, and oogonium characterized by large cell and nuclear began to emerge (Figure 2a and b). In this stage, mesonephros degraded slowly and metanephros began to form.

### Quail's ovary on the 10th day

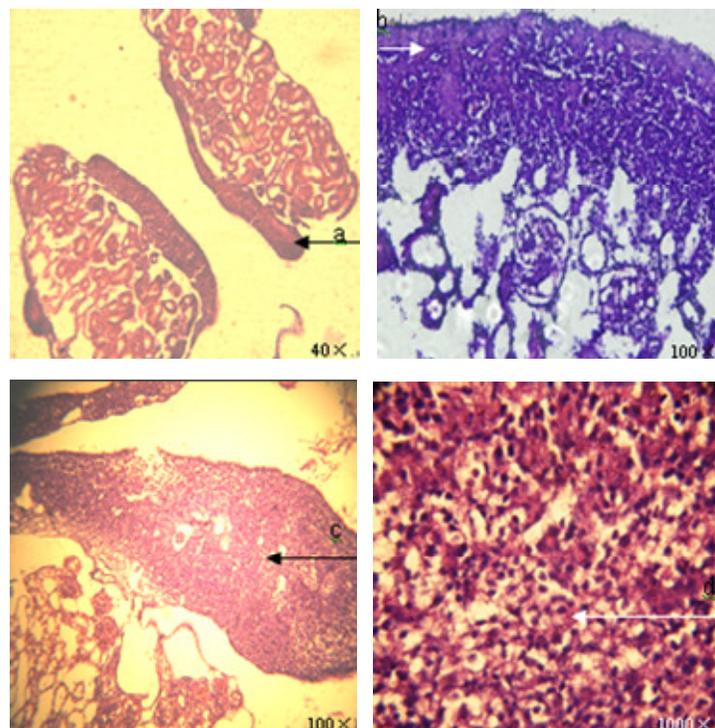
This period, the ovary that seemed like a locust pupae could be seen in the medial part of metanephros, and the left was much larger and wider than the right side. The edge of the ovary was not smooth. Serial sections showed that ovary was divided into cortex and medulla. The cortex or the outer layer was more dense than the inter layer, and there were many oogonia characterized by large cell, large nuclear and clear cytoplasm within the region. Some oogonia in the outer part of cortex were surrounded like a nest by some irregular shaped cells, showing the distribution of cord-like cells (Figure 2c and d).

### Quail's ovary on the 11th day

The distinction between cortex and medulla was more visible in the stage. The cortex was loose, within which there were many cable-like cells that were connected by



**Figure 1.** Quail's embryo sections of dyeing HE on the fourth and fifth day of hatching. **a)** Region of gonad development on the 4th day; **b)** ovary on the 5th day.



**Figure 2.** Ovary section in quail's embryo of dyeing HE on the seventh and tenth day of hatching. **a)** Right ovary on the 7th day; **b)** ovary on the 7th day; **c)** ovary on the 10th day and, **d)** ooconium which was surrounded by abnormal cells.

irregular cells to form a syncytium. The medulla was dense and was composed of blood vessels and dense connective tissues (Figure 3a).

#### **Quail's ovary on the 12th day**

Cortex and medulla of the ovary were more obvious (Figure 3b). And a large number of cord-like cells could be seen in the cortex. A small amount of honeycomb structures near the metanephros could be observed in the sections, which was similar to the situation of the 11th day.

#### **Quail's ovary on the 13th day**

The outer part of the ovary was not smooth. Cortex in this period was wider than before, while medulla with vascular distribution was relatively narrow. Primordial follicles began to appear in the cortex part that was distinctively divided observed in the figure. And they appeared in the outer layer, within which there were about 2 oocytes each. There were also a small amount of blood vessels and disordered spindle stroma cells in the outer layer where germinal epithelium would develop. The inter layer of cortex was composed of many stroma cells, small blood vessels and abundant primordial follicles (Figure 3c).

#### **Quail's ovary on the 14th day**

The edge of the 14th day ovary was not smooth either. The cortex was wider obviously, rich in small blood vessels and increasing in the number of primordial follicles, while the medulla was narrower, rich in large blood vessels and dipping into medulla from the metanephros (Figure 3d).

#### **Quail's ovary on the 15th day**

Germinal epithelium and the cortex of the ovary separated clearly and the majority part was cortex where primordial follicles some of which had 2 follicular oocytes were obvious. The closer the follicles were to the outer layer, the bigger they were. The cortex also had a wealth of spindle cells. Germinal epithelium was filled with primordial follicles which were demonstrated as many circles of different sizes in the sections because of rinsing. Some follicles were like cavity follicles, characterized by the appearance of cumulus, but there was only a one layer of follicle cells. At last, the narrower medulla consisted of greater blood vessels and other cells (Figure 3e).

#### **Quail's ovary on the 16th day**

There were many hollow circles in the germinal epithelium, which was similar to the statement of the 15th day.

#### **Quail's ovary on the 17th day**

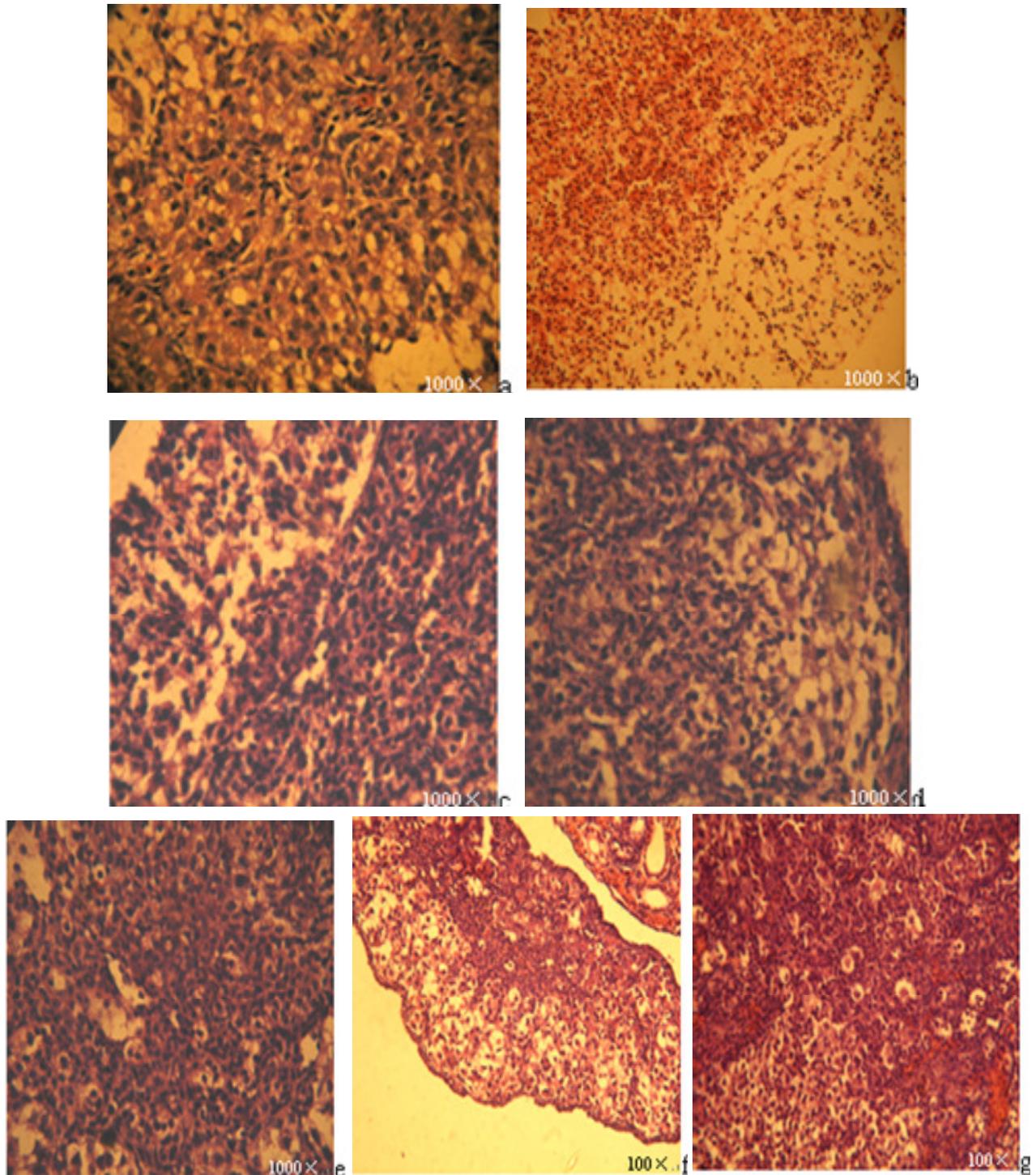
A large number of diverse follicles in the germinal epithelium and stroma cells were rich also. Under low power microscope, germinal epithelium was composed of different sizes of round or oval-shaped rings. A large number of primary oocytes which were surrounded by a layer of follicles were observed in the cortex and then formed into primary follicles. The medulla was filled with blood vessels and connective tissues. Ovarian stalk could be seen clearly.

#### **Quail's ovary on the 1st day of hatching out**

Figure 3g showed that the medulla of the ovary was filled with blood vessels and connective tissues and the cortex was wider. These were the same as the end of the incubation period. And the distribution of many different sizes of follicles was also the same. The formation of secondary follicles could be seen in the cortex, characterized by oocytes was surrounded by 2 - 3 layers of cells. And there were many stromal cells between the follicles.

### **DISCUSSION**

The study showed that on the 4th day of hatching, PGCs were still in the process of migration, but some of them had been gathered in the gonad formation region in succession. However, in some sections a large number of PGCs were clustered in the gonadal formation region, because the process of embryonic development was different between individuals. The gonadal differentiation had not begun on the 4th day. On the 5th day of hatching, the gonads began to differentiate and had an initial emergence of the characteristics of the testis or ovary and on the 7 - 10th day; the sex difference was very obvious that the right ovary degraded gradually while the left developed naturally. According to Li et al. (2005) on the gonadal development of chicken embryo research, the gonadal differentiation of the chicken embryo began on the 6th day of the incubation period and this kind of differentiation became more significant on the 7th day (Li et al., 2005). This may be associated with quail's shorter incubation period, in other words, quail's embryonic development was slightly faster than chicken's, so the gonadal differentiation also began earlier than chicken. In addition, the incubation temperature setting mainly referred to the hatching process of chicken. And under



**Figure 3.** Ovary section of dyeing HE from the 11 to 17th day of hatching and the 1st day of hatching out. **a – e)** Ovary from the 11 to 15th day of hatching; **f)** ovary on the 17th day of hatching and, **g)** ovary on the 1st day of hatching out.

the same volume, the number of quail eggs was higher, so the actual incubation temperature is relatively high, which to some extent, also promoted the development of quail embryos. Although there were morphological diffe-

rences between the PGCs of the quail and chicken and the detection methods were also not the same, but the time difference of the early gonadal development was not significant, with a difference of only a day or so. Previous

studies in the chick embryo suggested that on the 8th day of hatching, the PGCs in the ovary began to differentiate into oogonia (Kingston and Bumstead, 1995). In the experiment, oogonia were found on the 7th day sections, which demonstrated that the differentiation of quail's PGCs was earlier than the chicken's that led to the time difference of the early gonadal development.

During the development of the ovary of poultry embryo, boundaries between cortex and medulla were obvious. Oogonia located in the middle of the germinal epithelium and distributed dispersedly, which is different with mammals. In addition to the differences on the mechanism of the gonadal development, there were still differentiation in time between quail and mammal. Testis differentiation was earlier than the differentiation in the ovary of mammal. MIS and androgen of the testis were essential for differentiation. However, the female non-mammals developed earlier than male and estrogen played an important part in sex determination and differentiation (Morohashi, 2002). In this study, female gonadal development was observed earlier than the male. And on the 13th day, the primordial follicles began to appear. According to Li et al. (2001) on the research of gonadal development of chicken embryo, on the 14th day only a few primordial follicles emerged and different sizes of follicles formed in the cortex on the 18th day (Li et al., 2001). After the differentiation of PGCs (the 7th day), quail's later embryo development was observed faster than chicken, which showed that the differences between the incubation periods were associated with the speed of embryo development. Quail and chicken all belongs to Phasianidae and they can produce interspecific hybrid or chimera because of the similar chromosome number, the above which demonstrated that there are resemblances in the genetic basis between them. The study also found that the two were similar in the process of ovary generation and development, but slightly different in time. Practice in the production, most of quail hatching was also made reference to the conditions of the chicken, which also explained one aspect of the case that they can form a hybrid or chimera. As for the mechanisms of the gonadal generation and development of quails, chickens and other poultry, they could be analyzed through the generation and migration rules of PGCs in the embryo period to reveal the genuine processes of gonadal generation and development, and provide a new basis for the poultry developmental biology.

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