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Original article

EARLY EMBRYONIC DEVELOPMENT OF THE HARDERIAN GLAND OF THE COMMON QUAIL (*COTURNIX COTURNIX)*

E. Y. SALAH EL-DIN[1] & A. I. DAKRORY[1, 2]

[1]Zoology Department, Faculty of Science, Cairo University, Egypt;
[2]Biology Department, Faculty of Science, Taif University, Egypt

Summary

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The present research aimed at giving a special focus on the early development of the Harderian gland – the principal orbital gland in birds. The development of the Harderian gland was studied in the common quail (*Coturnix coturnix*) at 7, 8, 9 and 10 days of incubation. The Harderian gland started its development as a long tube with a narrow lumen proximally and undifferentiated lumen distally at the seventh day of incubation, while at the eighth day of incubation, the studied gland differentiated into: the upper group formed from five opened tubules but the lower one consisted of three relatively closed tubules; the Harderian gland attained a strap-like appearance with hour-glass major borders, an increase in number of acini which share in formation of gland and its corresponding closed duct at the ninth day of incubation. More additional acini shared in formation of the Harderian gland. The tubular wide opened Harderian gland duct has appeared. Innervation of the Harderian gland was carried by the inferior ramus of oculomotor nerve at tenth day of incubation.

Key words: *Coturnix coturnix,* development, Harderian gland

INTRODUCTION

In birds, the Harderian gland is the dominant orbital gland (Walls, 1942) and besides a lubricating and cleaning function, it plays an important role in the local immunity of the eye and upper respiratory tract (Burns, 1992; Scott *et al.*, 1993; Schmidt *et al.*, 2003). Histological, histochemical and ultrastructural studies of the Harderian gland have been made in domestic fowl (Wight *et al.*, 1971a,b; Rothwell *et al.*, 1972; Kittner *et al.*, 1978; Niedorf & Wolters, 1978; Walcott *et al.*, 1989), in ducks (Ballantyne & Fourman, 1967; Fourman & Ballantyne, 1967; Brobby, 1972; Kühnel & Beier, 1973; Wight & Mackenzie, 1974), turkeys (Maxwell *et al.*, 1986), native chickens (Mobini, 2012) and laying hens (Bejdić *et al.*, 2014). Harderian gland histology was investigated in ospreys (Kozlu *et al.*, 2010), whereas Ask (1913) reported on the morphology of the penguin Harderian gland and its development in the embryo. The Harderian gland of the guinea fowl was studied at embryonic and post embryonic stages (Onyeanusi *et al.*, 1993). Morphological aspects of the Harderian, paraorbital gland were investigated in domestic geese *(Anser anser domesticus)* (Boydak & Aydin, 2009). Morphological studies on this gland during the embryonic and post-natal period were carried out in the ostrich (*Struthio camelus domesticus*) (Klećkowska-Nawrot *et al.*, 2014). Dimitrov (1997; 1999; 2001; 2009) recorded several researches about avian infraorbital glands. Recently, histometrical parameters of the third eyelid (Harderian) gland of the common pheasant were reported (Dimitrov, 2014). A comparative survey on Harderian gland organogenesis was carried within vertebrates (Baccari, 1996).

Most studies on Harderian gland focus on morphological and histological studies using different methodologies. Studies on the Harderian gland in the quail (*Coturnix coturnix*) were carried out using light and electron microscopy (Kozlu & Altunay, 2011). The sex-related features of gland's weight, length and circumference were reported in two Japanese quail breeds by Dimitrov & Genchev (2011). The development of the Harderian gland in *Coturnix coturnix* was not so far described in the literature. The present study aimed at giving a sight on the early embryonic development of the common quail Harderian gland; being the principal orbital gland in birds. Hence, the development of Harderian gland in quails was evaluated and reported.

MATERIALS AND METHODS

The species chosen for this study was the common quail, *Coturnix coturni*x *Linn* (Order: Galliformes; Family: Phasianidae) according to Tharwat (1997). In Egypt, the quail inhabits the Nile Valley and Delta as well as El-Fayoum governorate (100 km, south of Cairo). It feeds on vegetation and small invertebrates. The nests are built on holes in the ground among the vegetation.

The fertilised eggs of *Coturnix coturni*x were provided from the Quail Unit at the Faculty of Agriculture, University of Cairo. The embryos were daily extracted from the incubated eggs. Extracted embryos were classified into different developmental stages corresponding to days of incubation. Also, the total body length (T.B.L.) and the head length (H.L.) of each stage were measured. We started from the first appearance of the anlage of Harderian gland at the seventh day of incubation and we traced the early development of the gland till the tenth day of incubation. Days of incubation, the total body length (T.B.L.) and the head length (H.L.) of each stage are listed in Table 1.

As soon as embryos were extracted from the eggs, they were quickly fixed in aqueous Bouin's solution for 24 hours. Large embryos were treated with EDTA solution for decalcification of their bones. After that, the embryos were washed several times with 70% ethyl alcohol.

Table 1. Day of incubation, total body length and head length of *Coturnix coturnix*

Days of incubation	7^{th}	8^{th}	9^{th}	10^{th}
Total body length	4.1 cm	4.5 cm	5.3 cm	5.6 cm
Head length	2.0 cm	2.4 cm	3.1 cm	3.0 cm

The embryos were treated in ascending series of ethyl alcohol and then cleared with xylene. The specimens were transferred into a melted paraffin wax. After changing the paraffin wax twice (one hour for each), specimens were transferred outside the oven and oriented in paraffin wax for blocking. This was followed by sectioning of the embryos transversely at 8 micrometer thickness using Reichert microtome.

The sections of each specimen were mounted serially on microscopic slides and prepared for staining. The mounted sections were stained with haematoxylin (Ehrlich) and counterstained by eosin to obtain permanent histological preparations.

Several sections were chosen for photography using Zeiss photomicroscope supplied by Canon digital camera to demonstrate the early developmental stages of the Harderian gland of the common quail.

RESULTS

On the seventh day of incubation, the Harderian gland started its origin from an ectodermal conjunctiva of the nictitating membrane at its junction with the cornea in the anterior angle of the eye. The primordial Harderian gland is directed posteriomedial to the eye (Fig. 1). It was surrounded by a fibrous connective tissue sheath. It appeared as a long tube with a narrow lumen and a wall of cuboidal cells proximally (Fig. 2) and undifferentiated lumen distally (Fig. 3).

On the eighth day of incubation, the Harderian gland appeared more advanced and consisted of a small number of tubules (acini). They were divided into two groups; the upper group consisted of about five opened tubules (Fig. 4) while the lower one consisted of three relatively closed tubules (Fig. 5). The gland was elongated anteroposteriorly. It extended from in front of the eye, in the region of the posterior end of the olfactory organ to the posterior and the ventral side of the eye.

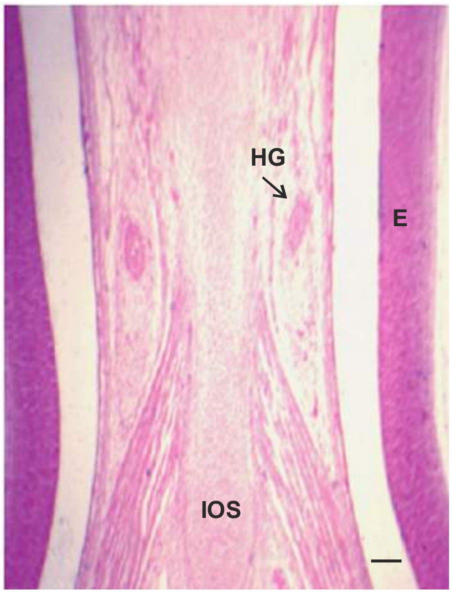

Fig. 1. A photomicrograph of a transverse section through the head region on the 7th day of incubation, showing the eye (E), the Harderian gland primordium (HG), the interorbital septum (IOS). H & E; bar=151 µm.

On the ninth day of incubation stage the Harderian gland was more developed compared to the preceding stage; it appeared strap-like with hour-glass major borders, was located posteromedially and ventrally to the eye. The gland was surrounded by a loose fibrous connective tissue sheath. Generally, it consisted of numerous tubules (acini). Also, they were divided into two groups; the upper group consisted of numerous tiny acini (Fig. 6) which aggregated forming the closed Harderian gland duct (Fig. 7) while the lower one consisted of a relatively small

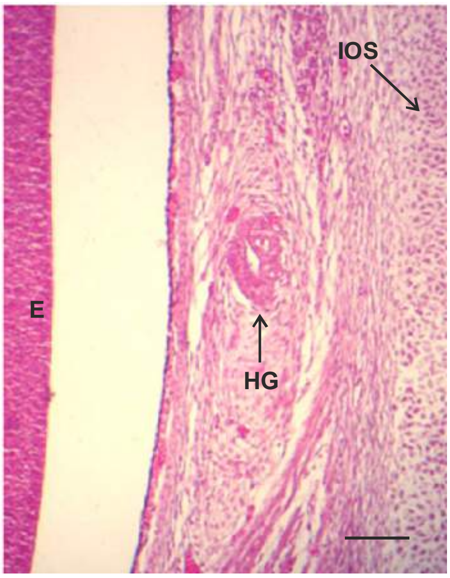

Fig. 2. A photomicrograph of a transverse section through the head region, 7th day of incubation, showing the Harderian gland with a narrow lumen. H & E; bar=50 µm.

Fig. 3. A photomicrograph of a transverse section through the head region, 7th day of incubation, showing the Harderian gland with undifferentiated lumen. H & E; bar=50 µm.

number of larger closed acini (Fig. 8) compared to the upper group. Also, the lower group aggregated forming the rest of the closed duct (Fig. 9). So, the Harderian gland ducts crossed the Harderian gland from its posterior to its anterior end.

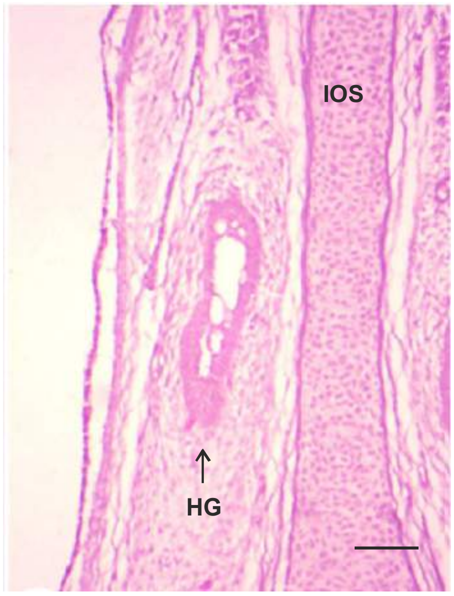

Fig. 4. A photomicrograph of a transverse section through the head region on the 8th day of incubation, showing about five opened tubules forming the Harderian gland. IOS: interorbital septum, H & E; bar=100 µm.

On the tenth day of incubation the Harderian gland was more developed compared to the preceding stage; additional acini in the upper group of the gland appeared opened and aggregated with already present acini to form the Harderian gland and its duct which was lined with a cuboidal epithelium (Fig. 10). The latter appeared more tubular in its shape and acquired a wide cavity. The inferior ramus of the oculomotor nerve appeared to innervate the Harderian gland (Fig. 11). In the lower group, the additional opened acini also aggregated to

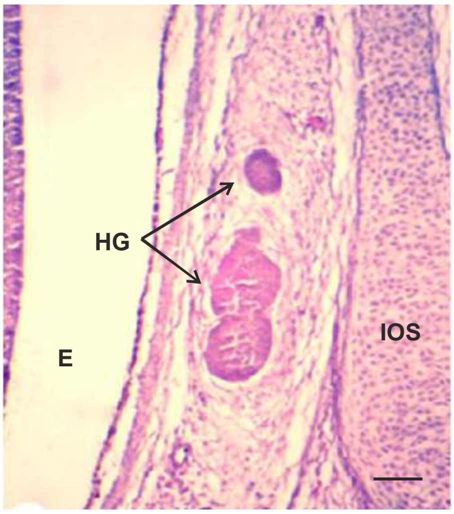

Fig. 5. A photomicrograph of a transverse section through the head region, 8th day of incubation, showing three relatively closed tubules forming the Harderian gland. H & E; bar=100 µm.

Fig. 7. A photomicrograph of a transverse section through the head region, 9th day of incubation, showing closed Harderian gland duct (HGD). IOS: interorbital septum, H & E; bar=50 µm.

Fig. 6. A photomicrograph of a transverse section through the head region, 9th day of incubation, showing tiny numerous acini (ACI) of the Harderian gland. H & E; bar=50 µm.

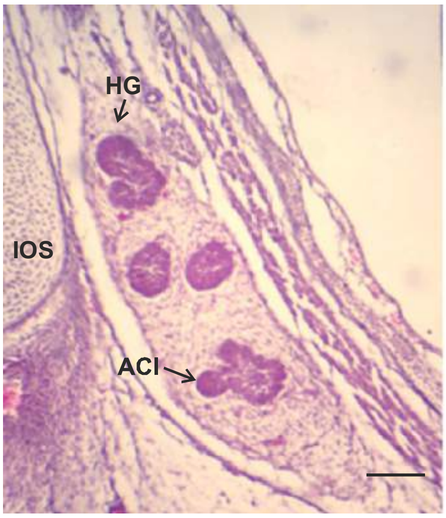

Fig. 8. A photomicrograph of a transverse section through the head region, 9th day of incubation, showing small number of larger closed acini (ACI) of lower part of Harderian gland (HG). IOS: interorbital septum, H & E; bar=50 µm.

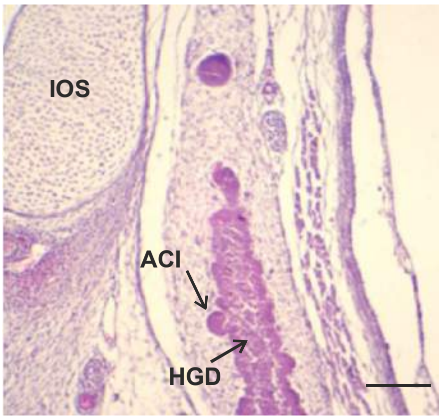

Fig. 9. A photomicrograph of a transverse section through the head region, 9th day of incubation, showing the rest of the closed Harderian gland duct (HGD). H & E; bar=50 µm.

Fig. 10. A photomicrograph of a transverse section through the head region, 10th day of incubation, showing the Harderian gland (HG) and its duct (HGD). H & E; bar=60 µm.

form the rest of the Harderian gland and its corresponding duct (Fig. 12).

DISCUSSION

The findings observed at the seventh day of incubation of the common quail embryo are in accordance with those reported by Ibrahim (2008) in 36 10 day-old chick embryos. On the other hand, in one of the embryos of the chick which seems to be in a more advanced stage but in 10 day-old embryos, the gland consists only of few number of tubules which open together in a common duct (Ibrahim, 2008).

Fig. 11. A photomicrograph of a transverse section through the head region, 10th day of incubation, showing innervation of Harderian gland (HG) with inferior ramus of oculomotor nerve (R.IN). H & E; bar=60 µm.

The posteromedial and ventral location of the gland with respect to the eye agrees with the data from the comparative survey on the Harderian gland by Baccari (1996) who stated that in birds the gland originated from the conjunctival epithelium at a late embryonic stage. In the English sparrow, *Passer domesticus* (incubation period of about 13 days), the Harderian gland appeared between the seventh and the 8th day of incubation.

In the chick embryo (incubation period of about 21 days) it originates between the eleventh and twelfth days. In the common quail (incubation period of about 19 days) the gland appeared at seventh day of incubation. Also, the previous studies of Niedorf & Woltersw (1978) and Onyeanusi *et*

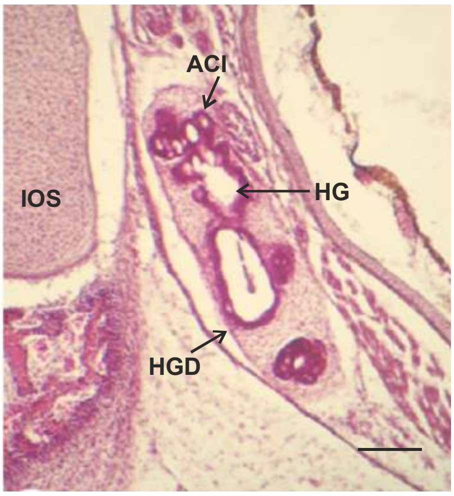

Fig. 12. A photomicrograph of a transverse section through the head region, 10th day of incubation, showing aggregation of acini forming the rest of the Harderian gland and its duct H & E; bar=60 µm.

al. (1993) confirmed that the inception point of the Harderian gland development was about midway through embryonic development in birds, reaching maturity in the post-hatching stages. Dimitrov (2009; 2012) described the Harderian gland in the family Phasianidae as a compound tubuloacinar glandular structure which was confirmed by our research. Macroscopically, avian Harderian gland varies in shape athough the most usual shape is that of the chicken (Ibrahim, 2008), the domestic fowl (Burns, 1974; Survashe, 1976). The gland's description as strap-like with hour-glass major border (Wight *et al.*, 1971a; Ibrahim, 2008) agrees with our research. It may also be roughly triangular as in the rock hopper penguin (Burns, 1978).

The position of the Harderian gland in birds varies slightly (Chieffi *et al.*, 1993). Some glands might be more anterior than others, but all are ventromedial to the eyeball (Burns, 1992). In chickens (Ibrahim, 2008), ospreys (Kozlu *et al.*, 2010), and in many terrestrial vertebrates, the gland occupies a position ventromedial to the eyeball (Payne, 1994; Djaridare *et al.*, 1999), findings which are confirmed by our results. Others however reported that the Harderian gland was generally located medially to the eyeball in all species (Olcese & Wesche, 1989). Slonaker (1918) described the gland in the sparrow and its position differed a little from that in the fowl (Wight *et al.*, 1971a) and in over 80 birds (Burns, 1974; Survashe, 1976).

The finding that the Harderian gland was innervated by inferior branch of oculomotor nerve in present results was also in concordance with data reported by other researchers (Slonaker, 1918; Burns, 1974; Payne, 1994).

In conclusion, the results of this study showed that the development of the Harderian gland in the common quail was associated to its adaptation to environmental conditions. We believe that the information will guide researchers who will conduct studies in this field in the future.

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Correspondence:

Eman Youssri Salah EL-Din
Zoology Department,
Faculty of Science,
Cairo University, Egypt
e-mail: e_youssri@yahoo.com