

## Light and Electron Microscopic Studies of the Quail (*Coturnix coturnix*) Harderian Gland

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**Abstract:** The aim of this study was to present the light and electron microscopic structure of the *Coturnix coturnix* harderian gland. The quail harderian gland was a single lobe of glandular tubuloalveolar profiles with columnar epithelial cells of varying heights. The secretory cells contained abundant mitochondria and well-developed rough endoplasmic reticulum and varying numbers of secretory vesicles in their cytoplasm (type I). These cells exhibited apically located microvilli. The secretion was collected by the ducts of harderian gland which were generally lined by a single layer of epithelium varying from cuboidal to columnar. The mode of secretion of the quail harderian gland was of holocrine and the secretory cells reacted positively with PAS. Myoepithelial cells were present in the basal region of secretory cells. The plasma cells were observed in the subepithelial region of the gland both among the corpus glandulae and in the connective tissue between the ducts. No difference was observed between sexes.

**Key words:** Harderian gland, quail, histology, histochemistry, electron microscopy, plasma

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### INTRODUCTION

The harderian gland is an orbital gland located medioventrally to the eyeball in a majority of terrestrial vertebrates (Djaridane *et al.*, 1999). The gland was first distinguished by the Swiss physician and anatomist Johann Jacob Harder in two deer species. However, comprehensive research on the structure and functions of the harderian gland was conducted only after the 1970's. The harderian gland has been studied in many vertebrate species, particularly in mammals and it has been reported that the gland serves important functions which may vary with species.

The gland functions lubrication of the eye and the nictitating membrane as a source of pheromones as a source of growth factors as a site of immune response, particularly in avian species as a source of salt in some turtle species in osmoregulation in some rodents in photoreception in rodents and in thermoregulation in some rodents (Chieffi *et al.*, 1996).

Electron microscopic studies of the harderian gland revealed different cell types in various species. Reptiles, *Pseudemys scripta* three cell types (type I-III) (Chieffi *et al.*, 1993), other reptile species (*Chelonia*) 4 cell types (type I-IV), hamsters, rats and mice four cell types

(Chieffi *et al.*, 1996), domestic fowl 4 types of cells (Rothwell *et al.*, 1972). The excretory duct system of the harderian gland is well developed in avian species and can be subdivided in primary, secondary and central ducts (Shirama *et al.*, 1996).

Despite histological, histochemical and fine structural studies in many avian species (Maxwell *et al.*, 1986), the light and electron microscopic structure of the harderian gland in *Coturnix coturnix* was not found in the literature. Hence, the light and electron microscopic structure of the *Coturnix coturnix* harderian gland evaluated and recorded.

### MATERIALS AND METHODS

**Animals:** The harderian glands from 10 male and 10 female healthy adult quails were harvested at Ankara University, Faculty of Agriculture, Department of Zoology, Quail Breeding Unit.

Birds were overdosed by intramuscular administration of a combination of xylazine, 6 mg kg<sup>-1</sup> (Bayer, Istanbul, Turkey) and ketamine 60 mg kg<sup>-1</sup> (Parke-Davis, Istanbul, Turkey). The guidelines of the ethical committee of Ankara University was strictly followed during the procedure.

**Light microscopy:** Part of the tissue samples taken was fixed in 10% neutral-buffered formalin for 24 h while the remaining samples were fixed in Bouin's solution for 6 h subjected to routine tissue processing for light microscopic examination, embedded in paraplast and sectioned into six-micron-thick slide. The sections were applied Mallory's modified triple staining method (Crossmon, 1937) to demonstrate the general structure. In order to demonstrate, the histochemical structure of the glandular secretion, sections cut from the blocks fixed in 10% neutral-buffered formalin were stained with Periodic Acid Schiff (PAS). Furthermore in order to determine the density of plasma cells, part of the tissue samples was fixed in alcohol-formalin solution and stained with methyl green-pyronin after being processed using the same method.

**Electron microscopic investigation:** For electron microscopic examination, the tissue samples were pre-fixed in glutaraldehyde-paraformaldehyde (pH 7.4) as described by Karnovsky (1965) and subsequently fixed in 1% osmic acid solution for 2 h. Following the second fixation, tissue samples were maintained in 1% uranyl acetate for 2 h, dehydrated through an ascending series of graded alcohols and propylene oxide and embedded in Araldite M (Fluka, Steinheim, Germany). The semi-thin 1-1 m thick sections cut from these blocks were stained with toluidine blue and following the marking of the targeted area, sections of 30-40 nm thickness were cut. These sections were contrast stained as described by Veneable and Coggeshall (1965) and were then examined under a Carl Zeiss EM 9 S-2 transmission electron microscope (Zeiss, Oberkochen, Germany).

## RESULTS AND DISCUSSION

**Light microscopy:** The quail, harderian gland was located ventromedially to the eyeball and had a close yet loose connection with the periorbital connective tissue. Therefore, the harderian gland was able to be easily isolated. The isolated harderian gland resembled a teardrop in shape and was light pink in colour.

The harderian gland of the quail was surrounded by a connective tissue capsule whose trabeculae penetrated the single lobe gland separating into lobules. These interlobular trabeculae made of connective tissue were thinner compared to the capsule surrounding the whole lobe. The gland lumen is lined by columnar epithelial cells of varying height (Fig. 1).

The excretory ducts were observed in the gland, named as primary and secondary ducts. The wall of the first part of the excretory ducts, namely, the primary ducts

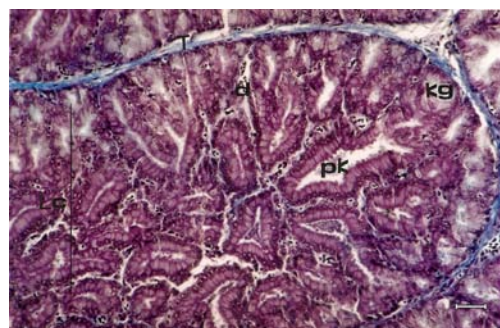


Fig. 1: Light micrograph quail harderian gland. Lc: Lobulus, T: Trabeculae, kg: corpus glandulae, pk: primary duct, d: capillar, triple stain. Bar: 5  $\mu$ m

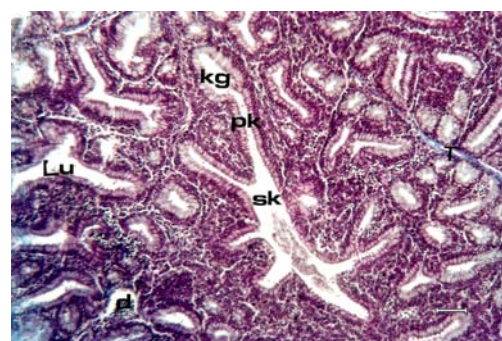


Fig. 2: Primary and secondary ducts of harderian gland. pk: primary duct, sk: secondary duct, Lu: Lumen, T: Trabeculae, d: vessel, triple stain. Bar: 5  $\mu$ m

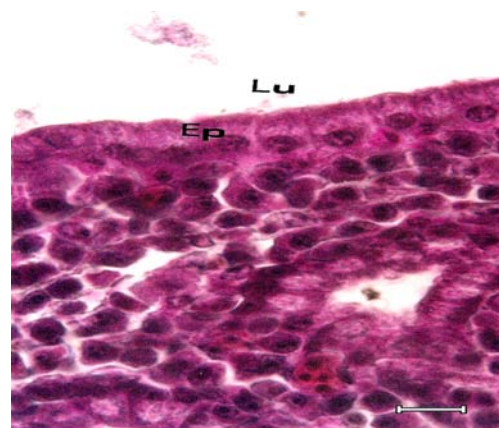


Fig. 3: The wall of the secondary duct. Ep: Epithelial cells, Lu: Lumen, triple stain. Bar: 25  $\mu$ m

was lined by a single layer of columnar cells which had single basally located spherical or oval euchromatic nuclei (Fig. 2). Different from primary ducts, the wall of the secondary ducts was lined by cuboidal cells (Fig. 3). The findings obtained with the application of Mallory's triple

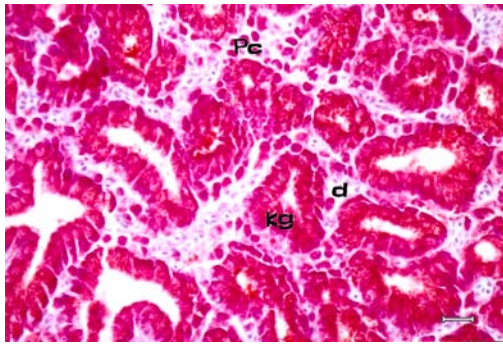


Fig. 4: An appearance of rarely plasma cell's region. Pc: Plasma cells, kg: corpus glandulae, d: vessel, methyl green-pyronin stain. Bar: 9.5  $\mu$ m

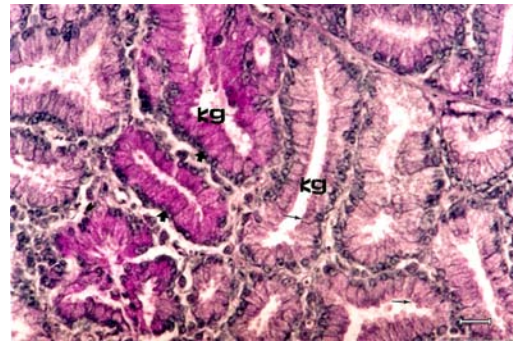


Fig. 7: An appearance of strong and weak PAS (+) corpus glandulae. kg: corpus glandulae, thick arrow: strong PAS (+) corpus glandulae, thin arrow: weak PAS (+) corpus glandulae, Periodic Acid Schiff (PAS) stain. Bar: 5  $\mu$ m

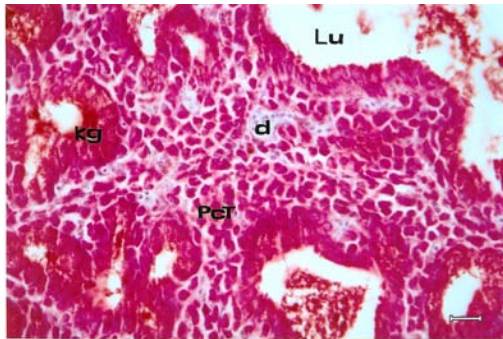


Fig. 5: An appearance of abundant plasma cell's region. PcT: Plasma cells group, Lu: Lumen, kg: corpus glandulae, d: vessel, methyl green-pyronin stain. Bar: 6  $\mu$ m

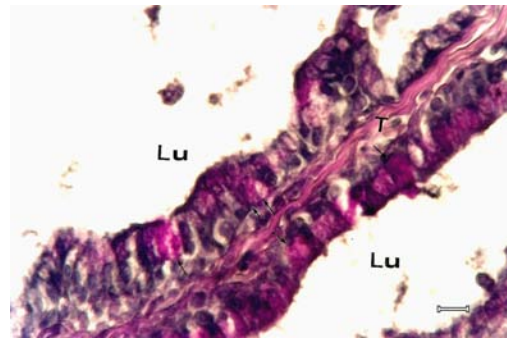


Fig. 8: An appearance of PAS (+) duct epithelial cells. Lu: Lumen, T: Trabeculae, arrow: PAS (+) duct epithelial cells, Periodic Acid Schiff (PAS) stain. Bar: 15  $\mu$ m

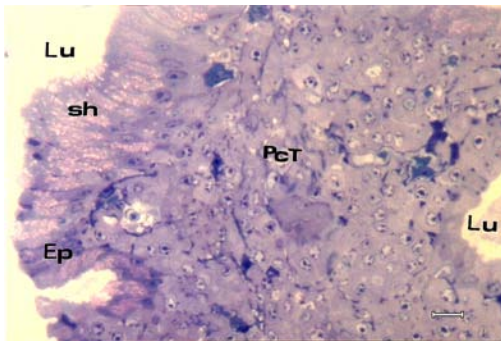


Fig. 6: An appearance of plasma cells in semi-thin sections. PcT: Plasma cells group, Lu: Lumen, Ep: Epithelial cells, sh: secretory epithelial cells, toluidin blue stain. Bar: 9.5  $\mu$ m

staining and toluidin blue staining to semi-thin sections with an aim to demonstrate the structure of stroma, ducts and acini showed that no difference existed between males and females at light microscopic level. The methyl

green-pyronin staining method applied to demonstrate the presence of plasma cells showed that plasma cells were abundant in the thin and thick interlobular trabeculae. However when examined for density, it was observed that these cells did not display a uniform distribution throughout the gland (Fig. 4 and 5). The abundance of plasma cells among the corpus glandulae and in the connective tissue in-between the ducts was also observed in the semi-thin sections (Fig. 6).

Nonetheless, the density of plasma cells was observed to be almost the same in female and male quails. PAS staining applied to determine the histochemical character of the glandular secretion revealed that neutral mucosubstances gave a PAS (+) reaction. Interestingly in the harderian gland of both female and male quails while some corpus glandulae produced a strong PAS (+) reaction, adjacent corpus glandulae gave a weak PAS (+) reaction (Fig. 7). Furthermore, PAS (+) reaction was observed in some cells of the duct epithelium (Fig. 8).



**Electron microscopic findings:** It was found that the harderian gland of the quail was of the tubuloalveolar type. The gland lumen is lined by columnar epithelial cells of varying height. The apical surface of the cells which faced the lumen was covered with microvilli (Fig. 9). The investigation conducted for existing cell types revealed that the harderian gland of the quail was composed of a single type of secretory epithelial cell (type I) (Fig. 9). However, the cytoplasm of these cells was of either dark or light colour, depending on the phase of secretion that they were in Fig. 10. Furthermore, the cytoplasm of these cells contained both large and small secretory vesicles of varying number (Fig. 9 and 10). The nucleus of these cells was observed to be situated basally.

When observed for organelles, it was determined that secretory cells contained abundant mitochondria (Fig. 10 and 11) and well-developed rough endoplasmic reticulum (Fig. 10) in their cytoplasm. The study of the general structure of the gland showed the presence of epithelial cells which composed the parenchyma and the presence of interlobular trabeculae which composed the stroma of the gland (Fig. 9). The interlobular trabeculae contained fibroblasts, blood vessels and nerve fibres. Also, these interlobular trabeculae contained abundant plasma cells which were rich in rough endoplasmic reticulum and had eccentrically located nuclei (Fig. 12).

Myoepithelial cells are present at the basal site of the epithelial cells. Oval shaped myoepithelial cells with light coloured nuclei were present between the secretory cells and their basal membrane (Fig. 11-13). The cells composing the secretory epithelium of the quail harderian gland were observed to liberate the contents of the secretory vesicles in their cytoplasm into the lumen of the gland by means of holocrine secretion (Fig. 14).

The present study demonstrated that the harderian gland of the quail was located ventromedially to the eyeball, similar to other avian species and that it had a tubuloalveolar structure, similar to the majority of vertebrate species which have been reported previously (Olcese and Wesche, 1989; Altunay and Kozlu, 2004; Burns, 1992).

In quails, the capsule composing the stroma of the harderian gland, enclosed the whole gland as in other species and the connective tissue capsule was continuous with the interlobular trabeculae which contained in addition to fibroblasts and collagen fibres, blood vessels, nerve fibres and homogeneous material as described by other researchers (Rothwell *et al.*, 1972). Furthermore, Rothwell *et al.* (1972) reported that the capsule which was thin similar to that of the domestic chicken, sent extensions into the gland and divided it into lobules of varying size. In quails, differ from that of those previously reported, these interlobular trabeculae which

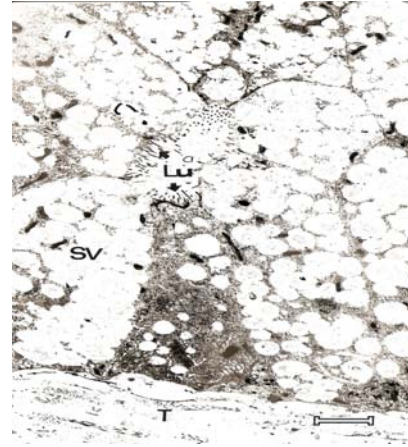


Fig. 9: TEM micrograph of the harderian gland in the quail showing microvilli on the surface of the columnar epithelium. Lu: Lumen, SV: Secretory Vesicles, T: Trabeculae, arrowheads: microvilli. Bar: 5.5  $\mu$ m



Fig. 10: TEM micrograph of the harderian gland in the quail showing secretory cells which have dark and light colour cytoplasm. As: secretory cells which have light colour cytoplasm, ks: secretory cells which have dark colour cytoplasm, c: nucleus, arrow: mitochondria. Bar: 4.5  $\mu$ m

enclosed the lobules and filled in the spaces in the harderian gland of quails were thinner inside of the gland and relatively thicker in the periphery of the gland. The classification suggested by Burns (1992) in avian species contributed greatly to the present study. Accordingly, the harderian gland of avian species is classified under 3 groups, namely, type I-III with respect to the lobulation and epithelium of the gland. Altunay and Kozlu (2004) reported the presence of 2 different types of cells which

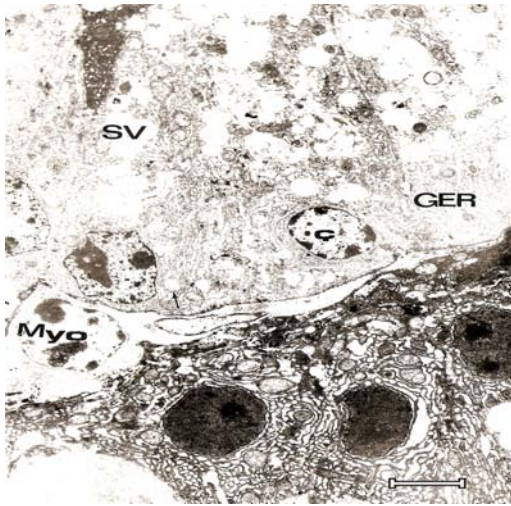


Fig. 11: TEM micrograph of the harderian gland in the quail showing organelles of secretory cells and showing myoepithelial cells. SV: Secretory Vesicles, GER: rough endoplasmic reticulum, c: nucleus, arrow: mitochondria, Myo: Myoepithelial cell. Bar: 5  $\mu$ m

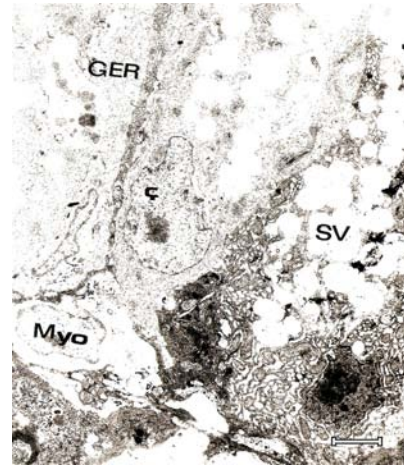


Fig. 13: TEM micrograph of the harderian gland in the quail showing myoepithelial cells in the subepithelial region. SV: Secretory Vesicles, GER: rough endoplasmic reticulum, c: nucleus, Myo: Myoepithelial cell. Bar: 2.5  $\mu$ m

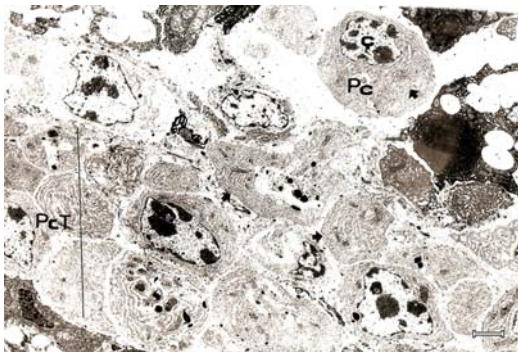


Fig. 12: TEM micrograph of the harderian gland in the quail showing plasma cells in the interlobular trabeculae. Pc: Plasma cell c: nucleus, arrow: rough endoplasmic reticulum, PcT: Plasma cells group. Bar: 5.5  $\mu$ m

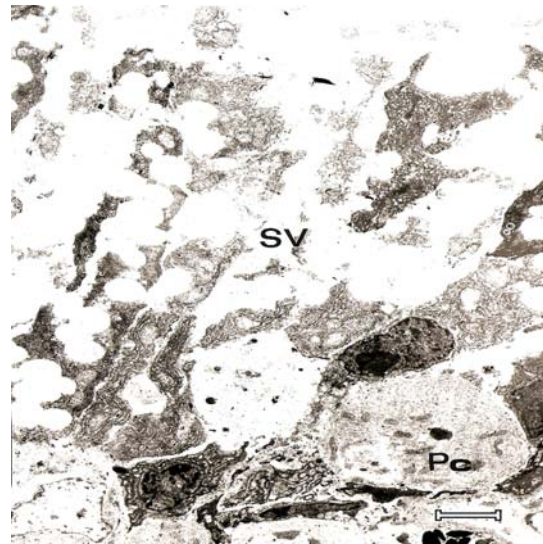


Fig. 14: TEM micrograph of the harderian gland in the quail showing holocrine secretion. SV: Secretory Vesicles, Pc: Plasma cell. Bar: 4  $\mu$ m

they referred to as type I and II in the harderian gland of the ostrich. In the present study, it was demonstrated that the lobules united to form a single lobe in the harderian gland of the quail and differ from that those of reported, it was observed that the gland was composed of a single type of epithelial cell. But based on these findings according to the classification of Burns (1992), the harderian gland of the quail can be classified as type I. Furthermore, depending on the secretion phase the cells were in a darkening was observed in the colour of the

cytoplasm. In quails, the secretory cells which contained a varying number of secretory vesicles were rich in organelles. In particular, the rough endoplasmic reticulum and mitochondria were well-developed. The nuclei were basally located. The cells exhibited apically located microvilli. Chieffi *et al.* (1996) reported that the distribution of secretory vesicles and organelles in the cytoplasm varied among species. In studies, carried out in avian species in particular in chicken, researchers have

classified the excretory ducts under 3 groups, namely the central, primary and secondary ducts (Wight *et al.*, 1971; Olah *et al.*, 1992). In the present study which was carried out in quails, similarly, two types of ducts, namely primary and secondary ducts were found to be present. In quails, the epithelium surrounding the lumen of the excretory ducts of the harderian gland was lined by a single layer of epithelium, the cells of which varied from cuboidal to columnar epithelium. The first part of the excretory ducts, namely the primary ducts, the epithelial cells were columnar in shape whereas, the secondary ducts were lined by cuboidal epithelial cells. Another common property observed in harderian gland of avian species is the presence of myoepithelial cells.

These cells with light coloured nuclei were also observed in harderian gland of quail between the epithelial cell and the basal membrane. The most important type of cell observed in the subepithelial region of the harderian gland in quails was the plasma cell. While the plasma cells were very abundant in some interlobular trabeculae such that they covered the entire area. In some other trabeculae, they were fewer in number. Furthermore, no difference existed between males and females. In a study conducted on the lymphoid cells of several avian species, Schramm (1980) also reported on plasma cells and indicated that these cells were more frequently encountered than other lymphoid cells. Nevertheless, he did not report that plasma cells were distributed in very high numbers. Furthermore, Scott *et al.* (1993) reported that plasma cells were observed in great numbers in the harderian gland of chickens same as Altunay and Kozlu (2004) reported in ostrich.

Altunay and Kozlu (2004) in a study on the secretory mechanism of the harderian gland in the ostrich reported that secretory vesicles were liberated into the lumen by means of a holocrine mode. Similarly, the harderian gland was determined to have a holocrine secretory mechanism in the quail. Results obtained with PAS staining performed to determine the histochemical character of the glandular secretion which demonstrated that some corpus glandulae stained darker while some other stained lighter suggested that all corpus glandulae contained glycosaminoglycans yet secretion did not occur simultaneously. Also, PAS (+) reaction was observed in some cells of the duct epithelium. In studies, conducted in the Turkey, chicken and duck, the duct epithelium was determined to be PAS (+) and PAS/AB (+) (Burns and Maxwell, 1979; Maxwell and Burns, 1979).

Shirama *et al.* (1996) concluded that the participation of plasma cells in the mechanism of local immune system, especially with regard to the course of the immunoresponse after antigen application or eye

inflammation has been well documented in birds. Attempts to demonstrate a similar immunological role for the harderian gland of mammals have been unsuccessful. It is still unclear why an immunological mechanism is found only in birds and a chemical mechanism exists only in mammals. The suggestion by Burns (1975) and Aitken and Survashe (1977) that habitat may determine the gland types and immune system is an important clue to the eventual understanding of harderian gland function. Having gone through literature, we did not come across with any histological study previously conducted in *Coturnix coturnix*. Further, observations will be needed to reach definite conclusions.

## CONCLUSION

In this study, researchers concluded that the participation of plasma cells in the mechanism of local immune system, especially with regard to the course of the immunoresponse after antigen application or eye inflammation has been well documented in birds. Attempts to demonstrate, a similar immunological role for the harderian gland of mammals have been unsuccessful. It is still unclear why an immunological mechanism is found only in birds and a chemical mechanism exists only in mammals. Having gone through literature, we did not come across with any histological study previously conducted in *Coturnix coturnix*.

## ACKNOWLEDGEMENT

The present study was summerised from PhD thesis.

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