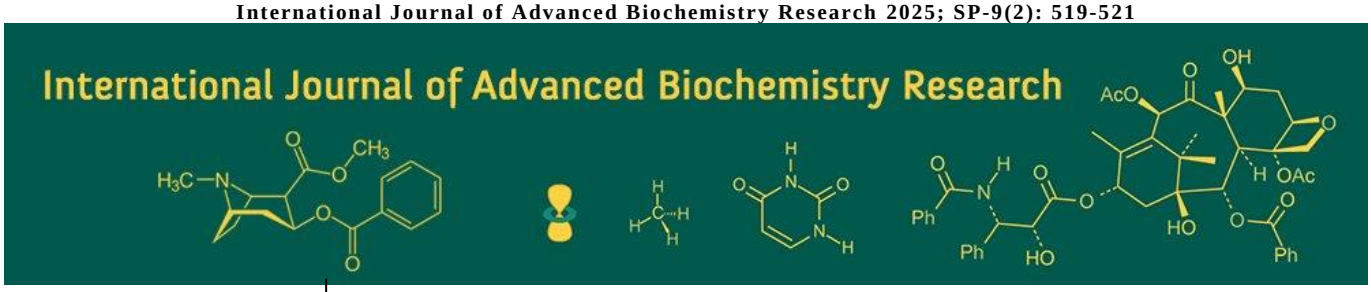

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PN Thakur
Department of Veterinary Anatomy and Histology, College of Veterinary and Animal Sciences, Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra, India

JY Waghaye
Department of Veterinary Anatomy and Histology, College of Veterinary and Animal Sciences, Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra, India

NM Karad
Department of Veterinary Anatomy and Histology, College of Veterinary and Animal Sciences, Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra, India

Corresponding Author:
PN Thakur
Department of Veterinary Anatomy and Histology, College of Veterinary and Animal Sciences, Maharashtra Animal and Fishery Sciences University, Nagpur, Maharashtra, India

Ultrastructural observation of Infundibulum in Pre-laying and laying Japanese quail (*Coturnix coturnix japonica*)

PN Thakur, JY Waghaye and NM Karad

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Abstract
This study elucidates the histomorphological characteristics of the oviduct in Japanese quail (*Coturnix coturnix japonica*) across different reproductive phases, specifically pre-laying and laying periods, in an effort to address the knowledge gap regarding the avian reproductive tract. Utilizing transmission electron microscopy (TEM), fresh tissue samples were collected from the oviductal segments of quails and processed for detailed ultrastructural examination. Findings revealed that the infundibulum's pseudostratified columnar epithelium primarily consists of ciliated, non-ciliated, and goblet cells, with ciliated cells being the most predominant. The study documented the morphology of ciliated cells, noting their oval to spherical euchromatic nuclei, which exhibited features consistent with active transcription. In contrast, non-ciliated cells displayed numerous cytoplasmic granules that increased in size and density during the 6th and 7th weeks of age, suggesting enhanced secretory activity. Glandular cuboidal cells in the ichnological layer revealed variable-sized cytoplasmic secretory granules, particularly enhanced in laying birds, aligning with previous findings in similar avian studies. These observations support the active role of both ciliated and non-ciliated epithelial cells in the physiological processes of the avian oviduct, particularly regarding secretion and egg formation, thereby contributing to a better understanding of quail reproductive biology.

Keywords: Ultrastructural, infundibulum, pre-laying, laying, Japanese quail

Introduction
Numerous studies have been conducted on the avian oviduct in various poultry species, with a significant focus on domestic chickens. At the time of egg-laying, the histological development of the oviduct reaches its peak, playing a crucial role in ovulation and egg formation (Duncan, 2019) [3]. Understanding the shape and histological structure of the reproductive tract is vital for evaluating quail economically, especially concerning factors like incubation time, growth rates, and hatching percentages (Liu *et al.*, 2020) [5]. Research into hormonally regulated differentiation and proliferation of primitive epithelial stem cells in the oviduct can provide valuable insights when using an avian oviduct model, particularly if the histomorphology of oviducts in pre-laying, laying, and non-laying quails are correlated (Huang *et al.*, 2021) [4]. Such studies could reveal how hormonal fluctuations impact the structure and function of the oviduct across different reproductive states. There remains a significant gap in the knowledge concerning the histomorphology and function of the oviduct in Japanese quail (*Coturnix coturnix japonica*). Limited information exists on ultrastructural examinations and electron microscopic observations of quail oviducts transitioning from pre-laying to laying and non-laying states (Nisbet & Smith, 2018) [6]. Consequently, attention must be directed toward morphological changes within the oviduct before and after sexual maturation in quail (Borges *et al.*, 2020) [2]. The objective of the current research was to examine the histomorphological characteristics of the oviduct in Japanese quails before and during the laying period. This aim seeks to fill the existing knowledge gap regarding the histomorphological anatomy of the female reproductive system in this bird species (Yoshimura *et al.*, 2022) [9].

Materials and Methods
To study the ultrastructural cellular details, fresh tissue samples of oviductal segments from

each group were collected for transmission electron microscopy. The samples were collected in 4% glutaraldehyde and stored at 4 °C. Subsequently, the tissue was washed in cold sodium cacodylate buffer solution (pH 7.4) with three changes of 30 minutes each. The tissue was then fixed in 1% osmium tetroxide for two hours at 4 °C. After fixation, the tissue was rehydrated in ascending grades of alcohol (50%, 70%, 80%, 90%, 95%, and absolute ethyl alcohol) and embedded in an Epon-araldite mixture. Ultrathin sections were obtained using a microtome, then mounted and stained with a saturated solution of uranyl acetate and lead citrate. The sections were examined and photographed using a transmission electron microscope for ultrastructural cellular details at RUSKA Labs, College of Veterinary Sciences, Rajendranagar, Hyderabad.

Results and Discussion

The tissues of the infundibulum subjected to transmission electron microscopy (TEM) confirmed the structural characteristics observed under light microscopy. In all age groups of the birds, TEM studies revealed that the pseudostratified columnar epithelium of the neck of the infundibulum consisted of ciliated, non-ciliated, and goblet cells (Figures 1, 2, and 3). The ciliated cells were the predominant type observed in the lining pseudostratified columnar epithelium. The tufts of cilia attached to the basal bodies were prominent at the apical surface of the ciliated cells. The oval to spherical-shaped euchromatic nuclei contained scattered irregular heterochromatin particles throughout the nucleoplasm, often positioned apically or centrally (Figures 1, 2, 3, 4, 5, and 6).

In the current study, the non-ciliated cells displayed basally placed oval to spherical euchromatic nuclei with scattered heterochromatic clumps. These cells exhibited numerous apical cytoplasmic granules of variable sizes and densities. Notably, non-ciliated cells showed an increase in the number of apical cytoplasmic granules around the 6th and 7th weeks of the birds' age (Figures 1 and 2). Mucosal glands in the neck region of the infundibulum were lined by cuboidal cells that contained spherical nuclei and secretory granules of varying sizes. The glandular cells of laying birds demonstrated an increase in cytoplasmic secretory granules, indicative of heightened secretory activity.

The TEM observations of the current study are consistent with findings by Saber *et al.*, (2009) [7], who noted the presence of both ciliated and non-ciliated cells in the lining epithelium of the infundibulum of ostriches, with ciliated cells predominating. Aligning with these observations, Bansal *et al.*, (2011) [1] also reported the presence of ciliated and non-ciliated cells in the infundibular surface epithelium of chickens, noting that columnar ciliated cells had centrally placed oval to round nuclei with associated cilia on basal bodies. Similarly, Sukhadeve *et al.*, (2018) [8] reported findings in Punjab white quail, observing a lining epithelium in the infundibulum comprised of both ciliated and non-ciliated cells, with ciliated cells having apically located ovoid euchromatic nuclei and distinct nucleoli alongside patches of heterochromatin. The non-ciliated cell nuclei were described as elongated and basally placed.

The observations from the present TEM study regarding euchromatic nuclei in ciliated and non-ciliated cells suggest active transcription processes within these cells. The increment in cytoplasmic granules in non-ciliated cells as age progresses indicates a greater involvement in the secretory function, correlating with increased secretory activity as the birds mature.

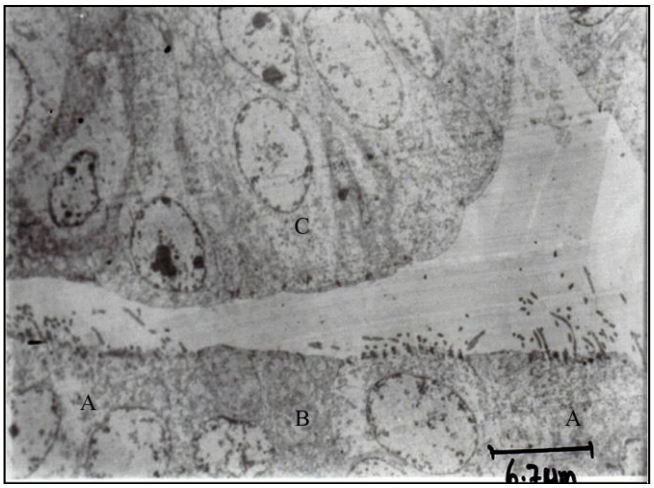

Fig 1: Transmission electron photomicrograph of the infundibulum at 4th week old bird showing-
A. Ciliated cell B. Non-ciliated cells C. Goblet cells (Printing magnification 2895 X)

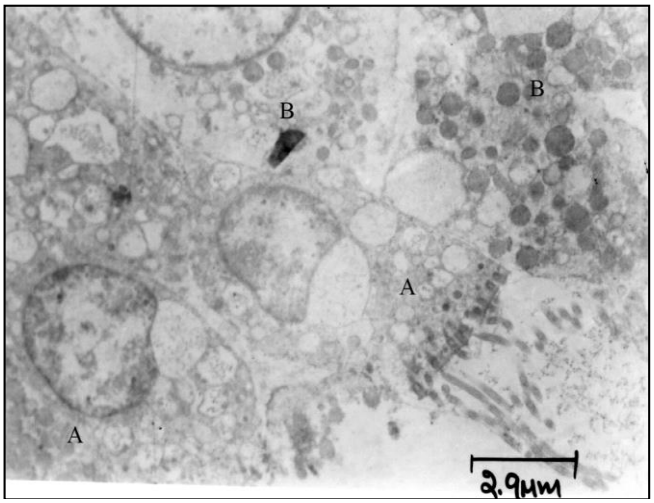

Fig 2: Transmission electron photomicrograph of the infundibulum at 5th week old bird showing-
A. Ciliated cell B. Non-ciliated cells with electron dense granules (Printing magnification 6755 X)

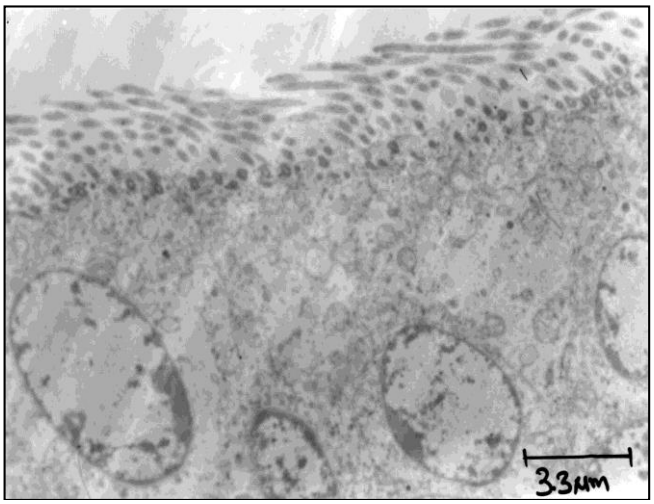

Fig 3: Transmission electron photomicrograph of the infundibulum at 7th week old bird showing-Ciliated cell in the long apical cilia and euchromatic nucleus (Printing magnification 5790 X)

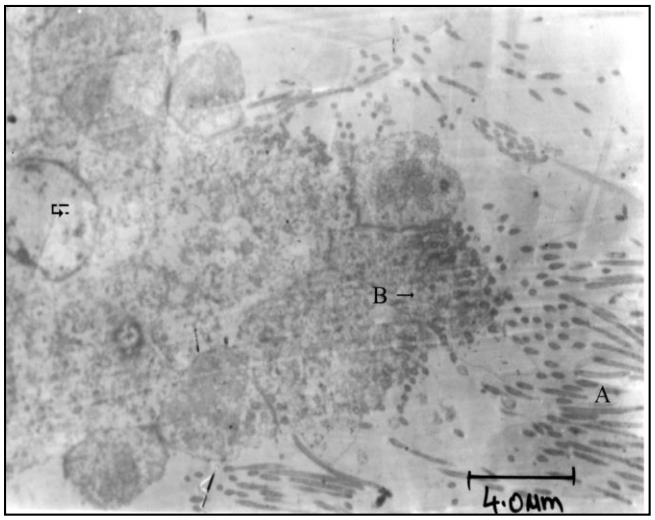

Fig 4: Transmission electron photomicrograph of the infundibulum at 4th week old bird showing-Ciliated cell
A. Cilia B. Basal bodies C. Nucleus (Printing magnification 4825 X

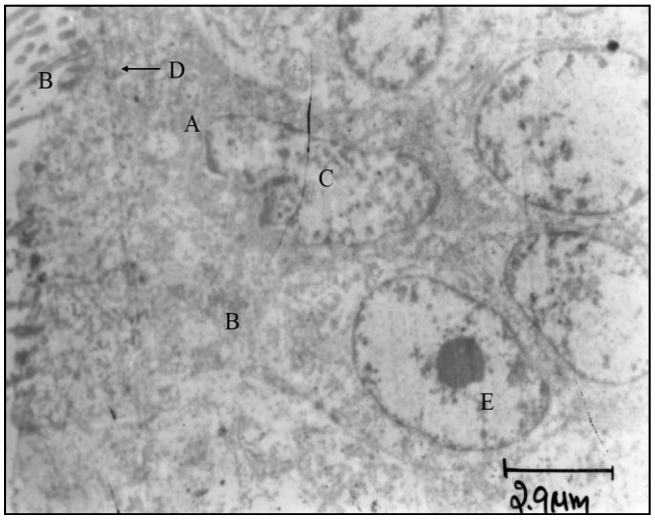

Fig 5: Transmission electron photomicrograph of the infundibulum at 6th week old bird showing-
A. Ciliated cells B. Cilia C. Non-ciliated cells D. Basal bodies E Nuclei (Printing magnification 6750 X)

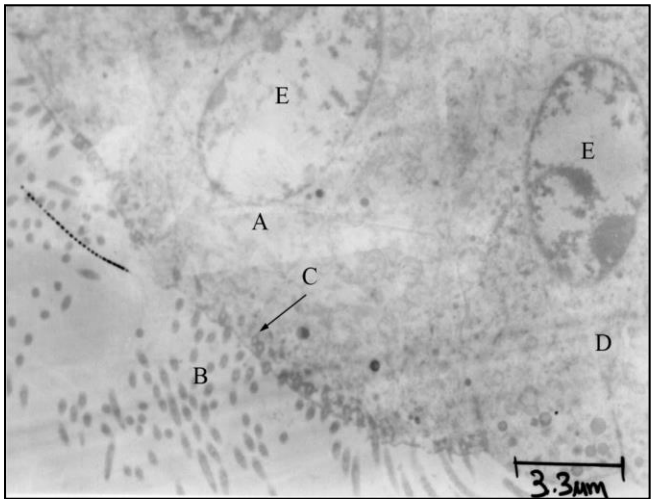

Fig 6: Transmission electron photomicrograph of the infundibulum at 7th week old bird showing –
A Ciliated cells B. Cilia C. Basal bodies D. Non-ciliated cells E. Nuclei (Printing magnification 5790 X)

Conclusions

The funnel part of the infundibulum was lined by simple columnar ciliated epithelium with non-ciliated and a few secretory goblet cells in pre-laying birds, which changed to pseudostratified columnar ciliated epithelium with non-ciliated and secretory cells in laying birds. The neck part of the infundibulum was lined by pseudostratified columnar ciliated epithelium with non-ciliated and secretory cells in birds of all ages. These observations contribute to a more comprehensive understanding of avian reproductive anatomy, providing essential knowledge for the fields of poultry science and veterinary medicine.

Acknowledgments

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Conflict of Interest

The authors declare that they have no conflicts of interest with any institutions or researchers involved in similar studies.

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