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Original article

HISTOLOGICAL AND HISTOCHEMICAL STUDY ON THE UROPYGIAL GLAND OF THE GOOSE (*ANSER ANSER*)

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Summary

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A study on the microscopic anatomy of the uropygial gland was conducted in 10 female and 10 male one-year-old healthy geese (*Anser Anser*). The tissue samples were stained by haematoxylin eosin and special techniques: Van Giesson, Verhoeff's, Gomori's, Alcian blue, Periodic acid- Schiff, Oil red O, and Sudan black B. The goose uropygial gland was enclosed by a capsule of connective tissue which contained Herbst corpuscles, smooth muscles, fat cells, blood vessels, nerves, elastic, reticular and collagenous fibres. All the connective tissue fibres and lymphatic aggregations were found in the intertubular interstitium of gland. The gland's parenchyma was composed of many simple secretory tubules that opened central main ducts. The tubular epithelial cells are classified into germinative, intermediate, secretory and degenerative layers. Each lobe was divided into two different zones, an outer sebaceous and an inner glycogen zones. In the Alcian blue staining, positive reaction was observed in all surface epithelial cells. Neutral mucosubstances (weakly acid mucopolysaccharides such as hyaluronic acid and sialomucins) were seen in the glands. Not only neutral lipids, but also sudanophilic lipids have been observed in both sexes. No significant sex-based differences were found.

Key words: goose (*Anser anser*), histochemistry, histology, sex, uropygial gland

INTRODUCTION

The uropygial gland, also known as the oil gland or the preen gland, is the only organised tegumentary structure of birds external secretion, typical for birds (Montalti & Salinian, 2000). The gland, surrounded by a capsule of dense connective tissue is located at the base of the tail (Hayder, 2005). It has been reported that the uropygial gland is especially bigger in waterbirds than land-birds, and is present in most bird species while absent or vestigial in the adult ostrich and emu (Johnston, 1988), some pigeons, the majority of parrots and the swan (Johnston, 1988; Gezici, 2002). Each lobe, which is separated by an interlobular septum, has a central cavity that collects the secretion from tubules arranged radially around the cavity (Aughey & Frye, 2001). The gland secretion contains fatty acids antibacterial agents and vitamin D precursors, preserves feather structure by keeping keratin

flexible, and also maintains feather waterproofing (Bandyopadhyay & Bhattacharyya, 1999; Shawkey *et al.,* 2003; Harem *et al.,* 2005).

The histology and histochemistry of the gland have been examined in a reduced number of species, such as the broiler and native chickens (Mobini & Zyaii, 2011), Japanese quails (Suzuki & Kusuhara, 1996), Moorhen (Sawad, 2006), Pekin duck (Kamiya *et al.,* 1986), white stork (Kozlu *et al.,* 2011) and European Starling (Sadoon, 2011). It was reported that the tubular pithelial cells are classified into germinative, intermediate, secretory and degenerative layers (Montalti *et al.,* 2001; Mobini & Zyaii, 2011; Sadoon, 2011).

The present study was conducted to provide information on the microscopic structures of uropygial gland in geese.

MATERIALS AND METHODS

Twenty clinically healthy geese (*Anser Anser*), weighing 3180–3350 g of both sexes were used to determine the histological structures of the uropygial gland. The birds, which were reared in a floor-pen house from hatch to one year of age, received feed and water *ad libitum.* The birds were deeply anaesthetised by excess ether inhalation. The guidelines of the ethical committee of Shahrekord Azad University were strictly followed during the procedure. The uropygial glands were removed from the subjects and immediately fixed in 10% buffered neutral formalin solution for 20 hours. Then specimens were submitted to dehydration in a series of ascending grades of ethanol (70–96%), cleared in several changes of xylene and embedded in paraffin. Tissue samples were stained by haematoxylin eosin for general observations and special techniques: Van Giesson, Verhoeff's, Gomori's method for reticulum, Alcian blue (AB) (pH 2.5), Periodic acid- Schiff (PAS), Oil red O, and Sudan black B (Kiernan, 2008). Sections were observed under light Olympus microscope (model BX50). After removal of glands, the birds were used for teaching purpose in the Department of Anatomy, College of Veteri-

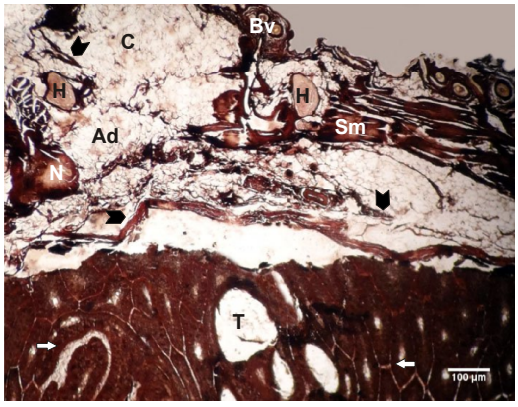

Fig. l. Thick capsule (C) of the geese uropygial gland consisted of Herbst corpuscles (H), adipose tissue (Ad), blood vessels (Bv), smooth muscles (Sm), nerve bundles (N) and elastic fibers (arrowheads), intertubular interstitial septa (arrows) separated the peripherally situated secretory tubules (T). Verhoeff's stain.

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RESULTS

Light microscopic examination revealed that the bi-lobed uropygial gland of the goose was enclosed by a moderate thick capsule consisting of dense connective tissue of irregular elastic (Fig. 1), reticular Fig. 2) and collagenous fibres (Fig. 3), Herbst corpuscles, smooth muscles, adipose tissue, blood vessels and nerves (Fig. 1).

This capsule sends intertubular interstitial septa into the gland (Fig. 4) which contained all types of connective tissue

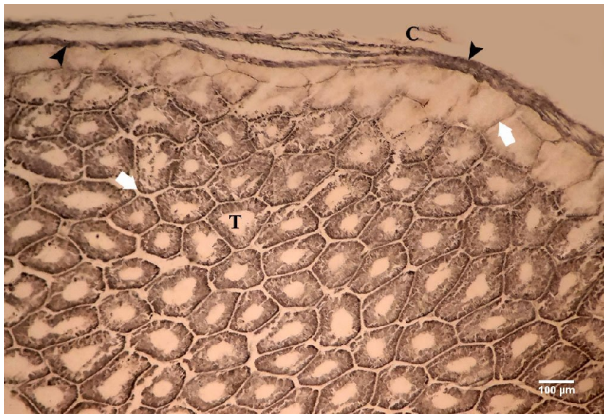

Fig. 2. Reticular fibres (arrowheads) in the capsule (C), interstitial septa (arrows) and among secretory tubules (T) of the geese uropygial gland, Gomori's staining for reticulum.

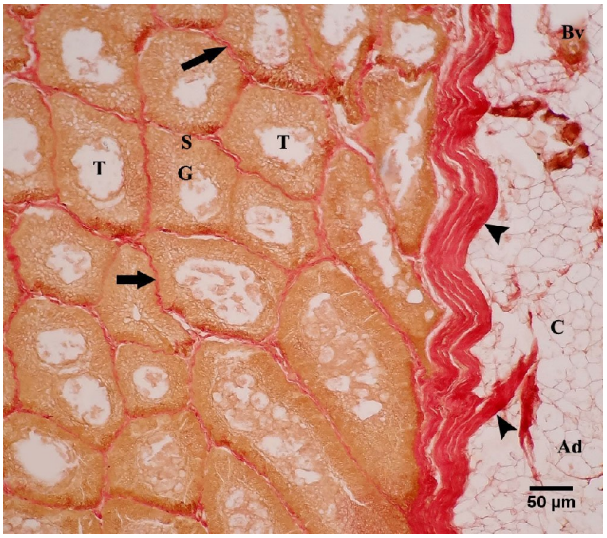

Fig. 3. Collagenous fibres (arrowheads) in the capsule (C), and intertubular interstitial septa (arrows) of the geese uropygial gland, Adipose tissue (Ad), blood vessels (Bv). Each tubule (T) divided into outer sebaceous (S) and inner glycogen zones (G), Van Giesson staining.

fibres (Fig. 1–3), lymphatic aggregations, blood vessels (Fig. 5), smooth muscle cells and fibroblasts (Fig. 4). The gland's parenchyma was composed of many secretory tubules and ducts. In each lobe, the tubules which were simple and arranged radially around the central cavity, divided into two different zones, an outer sebaceous and an inner glycogen zones (Fig. 3–4, 6). The wall of the secretory tubules, which were thicker in the outer sebaceous zone as compared to the inner glycogen zone, was comprised of four well defined layers; the basal or germinative, intermediate, secretory and degenerative layers (Fig. 4).

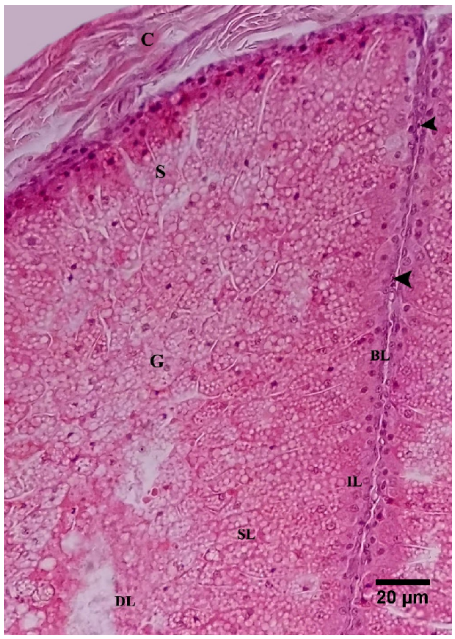

Fig. 4. PAS-positive material (arrowheads) in the all surface of secretory epithelial cells of tubules (T), lymphatic aggregations (L), blood vessels (arrow), intertubular interstitial septa (Se), PAS.

The basal or germinative layer which consisted of one row of flat-shaped cells lied on the basement membrane. The intermediate layer was composed of two rows of polygonal cells lied on the germinative layer. The secretory layer formed of 4–5 rows of pyriform or polygonal cells contained lipid droplets and secretory granules. The degenerative layer, which was adjacent to the lumen of each tubule, consisted of a few cells with pyknotic nuclei (Fig. 5).

All tubular epithelial cells of both the outer sebaceous and inner glycogen zones

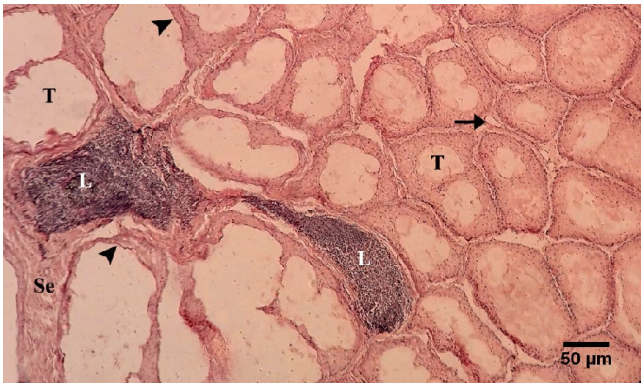

Fig. 5. Outer sebaceous (S) and inner glycogen zones (G) of each secretory tubule which comprised of the basal (BL), intermediate (IL), secretory (SL), and degenerative layers (DL). Capsule (C), fibroblasts in intertubular interstitial septa (arrowheads). Haematoxylin eosin staining.

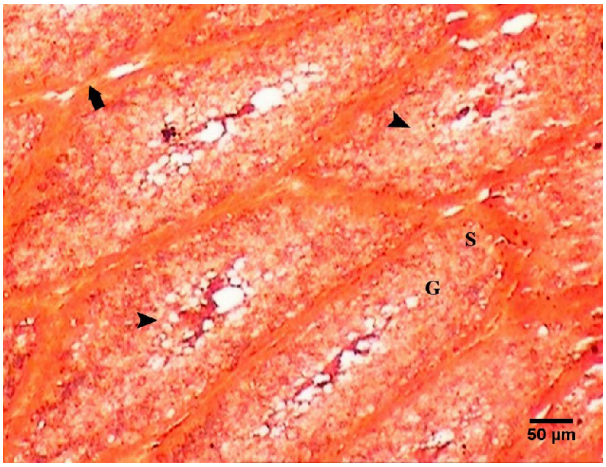

Fig. 6. All the outer sebaceous (S) and inner glycogen (G) tubular epithelial cells reacted positively to neutral lipids (arrowheads). Intertubular septa (arrow), Oil red O staining.

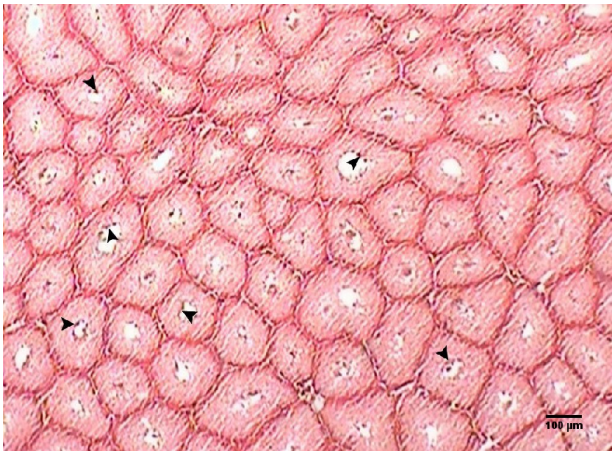

Fig. 7. Sudanophilic lipids in all surface epithelial cells (arrowheads) of the geese uropygial gland, Sudan black B staining.

reacted positively to neutral and sudanophilic lipids (Figs. 6, 7). AB-positive cells were observed in both secretory tubules and the main duct with Alcian blue (AB) staining at pH 2.5 (Fig. 8). Moderate PAS reaction was observed in all surface epithelial cells of secretory tubules (Fig. 4).

No evident difference between the male and female geese was observed in the histology and histochemistry of either gland or their duct.

DISCUSSION

The uropygial gland of the goose was covered with a capsule in agreement with the findings of Mobini & Ziaii (2011) in broiler and native chickens, Sawad (2006) in

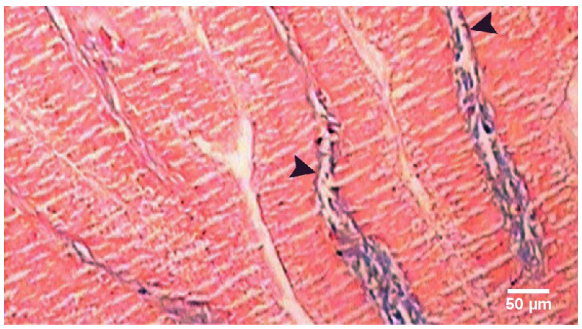

Fig. 8. AB (+) luminal epithelial cells in the geese uropygial gland (arrowheads). Alcian blue staining.

moorhen, Harem *et al.* (2005) in wild and domestic ducks, Hayder (2005) in indigenous geese and Kozlu *et al.* (2011) in white storks.

The capsule was made up of dense connective tissue composed of irregular elastic, reticular and collagenous fibres, Herbst corpuscles, smooth muscles, adipose tissue, blood vessels and nerves which concords with the findings of Mobini & Ziaii (2011) in chickens.

Harem *et al.* (2005) reported only Herbst corpuscles in the capsule and septa of the uropygial gland in wild and domestic ducks and Hayder (2005) reported blood vessels and nerves in dense connective tissue of capsule in indigenous geese. Also, Sadoon (2011) reported only irregular collagen fibres in dense connective tissue of Starling capsule and Daaj (2009) reported dense collagen and elastic fibres in capsule of local turkey. The smooth muscle fibres were absent in the uropygial capsule of moorhen (Sawad, 2006) and European Starling birds (Sadoon, 2011), whereas these fibres in the uropygial capsule of local turkey were observed (Daaj, 2009). The stroma of the uropygial gland in white stork lacked reticular fibres (Kozlu *et al.,* 2011).

In this study, intertubular interstitial septa from the connective tissue capsule penetrated into the gland which is similar to previous findings (Aughey & Frye, 2001; Harem *et al.,* 2005; Hayder, 2005; Sawad, 2006; Salibian & Montalti, 2009; Kozlu *et al.,* 2011; Mobini & Ziaii, 2011).

In the present study, the intertubular septa were composed of elastic, reticular and collagenous fibres, lymphatic aggregations, smooth muscle cells, fibroblasts and blood vessels which again concords with the findings of Mobini & Ziaii (2011) in chickens. The septa of stork uropygial gland consisted of smooth muscle cells, fibroblasts and blood vessels (Kozlu *et al.,* 2011). Harem *et al.* (2005) reported only lymphocytic infiltration in the intertubular interstitial septa of the uropygial gland in wild and domestic ducks and Sawad (2006) reported smooth fibres in septa of moorhen uropygial gland.

The glandular parenchyma was composed of many secretory tubules that opened in a central main duct. In each lobe, the secretory tubules which were simple and arranged radially around the central cavity, divided into two different zones, an outer sebaceous and an inner glycogen zones. Similar results were also reported by Suzuki & Kusuhara (1996) in Japanese quails, Sawad (2006) in moorhen, Kozlu *et al.* (2011) in white stork,

Mobini & Ziaii (2011) in chickens and Sadoon (2011) in Starling birds.

Previous studies in many avian species reported that the wall of the secretory tubules contains basal, intermediate, secretory and degenerative cell layers (Montalti *et al.,* 2001; Sawad, 2006; Kozlu *et al.,* 2011; Mobini & Ziaii, 2011; Sadoon, 2011). The four defined cell layers were also observed in this study.

The basal layer was observed to be composed of flat cells. This finding mirrors the results of Montalti *et al.,* (2001) for rock dove, Sawad (2006) for moorhens, Mobini & Ziaii (2011) for chickens, and Sadoon (2011) for starling birds. The intermediate layer was composed of two rows of polygonal cells in agreement with Sawad (2006), Mobini & Ziaii (2011), and Sadoon (2011), but it was thinner than that reported by Montalti *et al.* (2001).

In this study, the secretory layer was composed of 4–5 rows of pyriform or polygonal cells and a degenerative layer formed of few cells with pyknotic nuclei which again concords with previous findings (Montalti *et al.,* 2001; Sawad, 2006; Mobini & Ziaii, 2011; Sadoon, 2011).

All surface epithelial cells reacted positively to periodic acid Schiff. Similar results were also reported by Kamiya *et al.* (1986) in Pekin ducks, Sunanda *et al.* (2001) in domestic ducks, Harem *et al.* (2005) in wild and domestic ducks, Sari *et al.* (2009) in geese and Mobini & Ziaii (2011) in broiler and native chickens.

Lipid-positive reactions of all tubular epithelial cells of both the sebaceous and glycogen zones in goose uropygial gland mirror the findings of Sunanda *et al.* (2001) in domestic ducks, Harem *et al.* (2005) in wild and domestic ducks, Sari *et al.* (2009) in geese and Mobini & Ziaii (2011) in native chickens.

The mucosal histochemical reactions of the goose uropygial gland were similar to data of Kamiya *et al.* (1986), but Mobini & Ziaii (2011) reported no AB-reactions in broiler and native chickens.

In the present study, the histology and histochemistry of the uropygial gland showed no significant differences according to the sex which is in agreement with the results reported by Salibian & Montalti (2009), whereas Mobini & Ziaii (2011) reported sex differences in the frequency of the capsular adipose tissue and blood vessels between male and female chickens. Also, Reneerkens *et al.* (2002) reported a sex effect on the chemical composition of the secretion in sandpipers.

In summary, the histological and histochemical properties of the goose uropygial gland were generally similar to those of native chickens and some other species except for the AB-reaction. There were no significant effects of sex on histology and histochemistry of the anserine uropygial gland.

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