



Full Length Research Paper

Morphological and immunohistochemical studies on the dromedary camel poll gland in relation to reproductive activity

Zarroug Hassan Ibrahim^{1*}, Khalid Ali Al-kheraije¹, Eltigani Ahmed El-Tigani-Asil²

¹College of Agriculture and Veterinary Medicine, Qassim University, PO Box: 6622- Buraidah: 51452, KSA.

²Current Address: Department of pathology and Sample Management, Abu Dhabi Food Control Authority, Abu Dhabi, UAE.

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The two poll glands are modified sweat glands resting close to each other on the dorsal side of the poll(neck) region behind the ear of camels (*Camelus dromedarius*). Their function has remained largely unknown, but they secrete a watery yellowish secretion with offensive odor during breeding seasons. The present seasonal study on the poll gland morphology, and immunoreactivity of S-100 and alpha smooth muscle actin (α -SMA) proteins might shed some light on its function. The study showed seasonal morphological and immunohistochemical variations. During breeding season the gland was larger in size and darker in colour and showed increased secretion. As compared to non-breeding season, the alveoli and intra-lobular ducts in breeding season were larger and their epithelium changed from simple squamous or low cuboidal to simple cuboidal. The morphometric study recorded significantly increased alveolar diameter and epithelial height ($P < 0.05$) with consequent non-significantly increased luminal diameter ($P > 0.05$) in breeding season compared to those in non-breeding season. On the other hand, significantly decreased inter-alveolar tissue thickness was observed in breeding season in relation to that in non-breeding season. Positive S-100 immunoreactivity was observed in both the alveolar epithelial and inter-alveolar tissue, whereas α -SMA reaction was mainly observed in glandular myoepithelial cells, smooth muscles and blood vessels. S-100 and α -SMA immunoreactivity increased during breeding season compared to that in non-breeding season. These results suggest that the poll gland secretory activity is correlated with male camel sexual activity. Moreover, S-100 and α -SMA proteins could be involved in regulation of cellular and muscular functions of the gland.

Keywords: Dromedary camel, Poll gland, Morphology, Immunohistochemistry, S-100, α -smooth muscle actin.

INTRODUCTION

The dromedary camel (*Camelus dromedarius*) is

biologically and economically important animal in tropical and sub-tropical regions. In many countries in Africa and Asia the camel withstands hot and dry environment making it capable for providing meat and milk, and to help in

*Corresponding Author's Email: zarrougibrahim@hotmail.com

transportation. The dromedary camel is generally considered as seasonal breeder because its reproductive activity increases during certain months of the year (Musa and Abusineina, 1978; Tingari et al., 1984a; Deen et al., 2003; Hegazy et al., 2004; Ghazi, 2007). Therefore, a number of seasonal morphological and functional studies have been conducted on the camel reproductive organs (Osman et al., 1979; Tingari et al., 1984a,b; Zayed et al., 1995, 2013; Ibrahim and Singh, 2014; Ibrahim, 2015; Ibrahim et al., 2017).

The poll glands are symmetrical subcutaneous exocrine glands of the male dromedary camel which are located behind the ears in the neck region (Leese, 1927; Singh and Bharadwaj, 1978; Tingari and George, 1984; Ebada et al., 2012). The gland which has been considered as a tubule-alveolar modified apocrine sweat gland (Singh and Bharadwaj, 1978; Manivannan et al., 1996), is histologically similar to the mammary gland (Purohit and Singh, 1958). Functionally, the gland is known to undergo cyclic activity, yielding a yellowish watery secretion with offensive odor, especially during the breeding season (Singh and Bharadwaj, 1978; Taha and Abdalla, 1980; Yagil and Etzion, 1980). The secretion has also been described as very copious with strong, foetid smell which becomes black when exposed to the air, dribbling down the neck of the rutting camel (Skidmore, 2004). The poll gland morphology and histochemistry have earlier been described (Purohit and Singh, 1958; Singh and Bharadwaj, 1978; Tingari and George, 1984; Tingari et al., 1984b; Manivannan et al., 1996; Atoji et al., 1998). However, immunohistochemical reports on the poll gland are rather rare. Immunohistochemical studies on biologically active proteins including S-100 and α -SMA, have been performed on reproductive organs in some mammalian species including testis (Abd-Elmaksoud, 2005) and epididymis (Alkafafy and Sinowatz, 2012) of bovine, testis (Alkafafy et al., 2016) and epididymis (Alkafafy et al., 2011a) of dromedary camel and epididymis of Egyptian water buffalo (Alkafafy et al., 2011b). Seasonal immunohistochemical studies have also been conducted on camel efferent ductules (Ibrahim et al., 2015), camel testis (Alkafafy et al., 2016) and camel epididymis (Ibrahim et al., 2017).

S-100 and alpha smooth muscle actin (α -SMA) have been suggested to affect the cellular absorption, secretion and muscular contractile activities (Alkafafy et al., 2011b). S-100 is a calcium-binding protein which has many intracellular and extra cellular activities including modulation of enzymatic activity, motility and energy metabolism, cellular secretion, chemotaxis and neurite extension (Heizman et al., 2002). S-100 protein has earlier been considered specific to the nervous system as it was initially detected in brain glial cells and Schwann's cells of peripheral nerves (Bock, 1978). However, recent studies indicated its localization in many other organs, especially in the mammalian testis (Haimoto et al., 1987; Amselgruber

et al., 1992, 1994; Cruzana, 2003; Alkafafy et al., 2016), epididymis (Haimoto et al., 1987; Alkafafy, 2009; Alkafafy et al., 2011a,b; Ibrahim et al., 2017) and efferent ductules (Ibrahim, 2015). α -SMA is classified as a contractile protein which is mainly found in cells performing contractile functions; it has also been used as a means for differentiation of normal and pathological smooth muscle cells (Skalli et al., 1989). In the camel epididymis α -SMA was mainly seen in the epididymal peri-tubular muscles and connective tissue as well as in the smooth muscles of epididymal blood vessels (Ab-Elmaksoud, 2009; Ibrahim et al., 2017). According to Ibrahim et al. (2017), S-100 and α -SMA show distinct variations in relation to reproductive activity in dromedary camel epididymis and they might change the structural and physiological states of this organ.

To the best of our knowledge, Ebada et al. (2012) have presented the only report on the immunohistochemistry of the camel poll gland during rutting season. However, detailed seasonal immunohistochemical studies on the camel poll gland during both the rutting and non-rutting seasons are apparently lacking from the literature reviewed. This study, therefore, aims to investigate the immunoreactivities of S-100 and α -SMA in the dromedary poll gland during breeding and non-breeding seasons. Complementary anatomical, histological and morphometric investigations are also included.

MATERIALS AND METHODS

Animals and tissue processing

Twenty four adult (aged 5-10 years) and healthy dromedary Camels (*Camelus dromedarius*) slaughtered at Buraidah Central Slaughterhouse, AL-Qassim, Saudi Arabia, were used in this study. Specimens from 12 animals in breeding season (between December and February) and from 12 animals in non-breeding season (between May to July), as stated by Tingari et al (1984), were quickly collected after animal slaughter.

Histology and morphometry

Histological samples from each animal group were fixed in 10% buffered formalin, dehydrated in ascending series of ethyl alcohol, cleared in xylene and embedded in paraffin wax, following the procedure of Culling (1974). Paraffin sections (5 μ m thick) from each animal group were cut using a rotary microtome; the sections were stained with haematoxylin and eosin (H&E) stains (Culling, 1974) for both general histological and morphometric analyses.

For morphometric study paraffin sections stained with haematoxylin and eosin were examined. Twelve animals from each season of the annual reproductive cycle were



Figure 1: Dark brown secretion of the paired camel poll gland exuding through the skin of the rutting camel (Arrows).

studied. For each animal ten sections from each poll gland were randomly chosen for measurement of alveolar diameter, epithelial height, luminal diameter and inter-alveolar connective tissue thickness. The measurements were carried out using Leica DMD108-Germany (provided with Leica microscope, digital camera and measuring software). The study only included transverse alveolar sections. The obtained morphometric results (expressed as means \pm standard deviation), were analyzed using Students T-test. $P < 0.05$ was considered as statistically significant.

Immunohistochemistry

Paraffin sections from each animal group were de-waxed using two changes of xylene and rehydrated in a decreasing concentrations of ethyl alcohol. The sections were then washed in distilled water and phosphate buffer saline (PBS). Antigen unmasking was performed using microwave heating (750 W) in 0.01 M sodium citrate buffer (pH 6.0) for 20 min. Immunohistochemical staining was carried out using Mouse and Rabbit Specific HRP/DAB (ABC) Detection IHC Kit (ab64264;Abcam, Cambridge, UK), as described by the manufacturer's instructions. Sections from both animal groups were incubated with

rabbit polyclonal anti-S100 primary antibody (ab868;Abcam, Cambridge, UK), for overnight at 4 °C and with rabbit polyclonal anti- α -SMA primary antibody (ab5694;Abcam, Cambridge, UK), for 3hr at room temperature. The dilutions for both anti-S100 (1:100) and anti- α -SMA (1:5000) primary antibodies were performed using a universal diluent (ab79995;Abcam, Cambridge, UK).

Immunohistochemical negative control immunostaining was carried out by omitting the primary antibody; this produced negative staining. On the other hand, positive control immunoreactivity was performed according to the manufacturer's instruction for each primary antibody kit.

RESULTS

Gross anatomy

There were two poll glands in the male dromedary camel resting dorsally on either side of the neck region, about 7 cm behind the occipital crest (Fig. 1). A watery yellowish secretion with offensive odor exuded through the skin covering the gland, especially during the rutting (breeding) season. The secretion colour changed to dark brown or

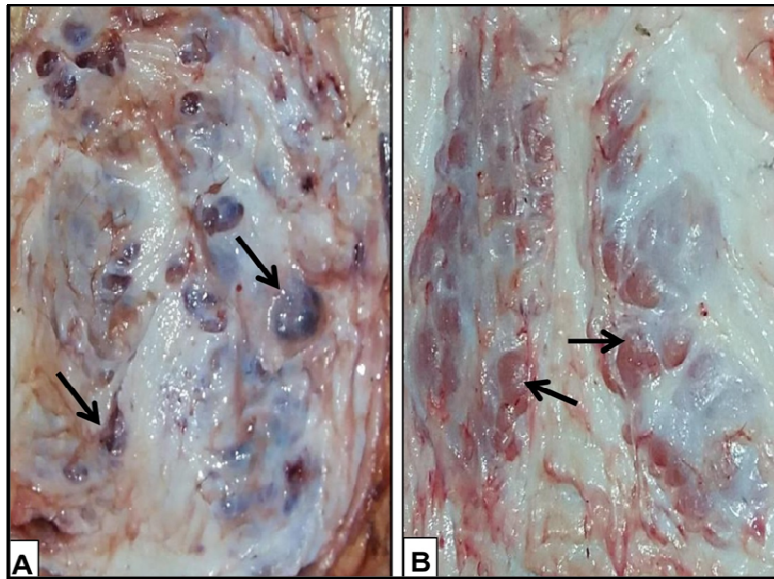


Figure 2: Fresh camel paired poll gland. The gland is large and the lobules (Arrows) are filled with glandular secretion dark during breeding season (A). In the non-breeding season the gland is smaller and the lobules look red in colour and empty (B).

black after being exposed to air. The gland was formed of many lobules of different shapes and sizes that occupied an elongated or irregularly triangular area; the area had a broad cranial basal part and a narrower caudal one (Fig. 2). During the reproductive season the lobes and lobules were markedly larger and many of them were dark in colour as they were filled with the glandular secretion. The length of the glandular area ranged between 8-10 cm during rutting season and 5-8 cm during non-rutting season.

Histology

The histological structure of the camel poll gland during breeding and non-breeding seasons is shown in Fig. 3. In both seasons the gland was surrounded by a connective tissue capsule that sent connective tissue septa. These septa which divided the gland into lobes and lobules consisted mainly of collagenous fibres, blood vessels and interlobular ducts (Fig. 3A).

During breeding season (Fig. 3A, B), the glandular lobules were occupied by large alveoli which were characterized by tall simple cuboidal epithelium and narrow lumina; the epithelium exhibited apical protruding blebs and was surrounded by myoepithelial cells with dark nuclei followed by scanty inter-alveolar connective tissue. Intra-lobular ducts with tall simple cuboidal or columnar epithelium were also found in the lobules.

During non-breeding season the alveoli and intra-lobular ducts appeared smaller and their epithelium changed to simple squamous or low cuboidal, which resulted in increased luminal diameter (Fig. 3C, D); the interlobular and inter-alveolar connective tissues were increased in comparison to those in breeding season.

Morphometry

The analysis of morphometric parameters of the camel poll gland during breeding and non-breeding seasons is shown in table 1.

In the breeding season, the alveolar diameter and epithelial height of the camel poll gland were significantly increased in comparison with non-breeding season. On the other hand, significantly decreased inter-alveolar tissue thickness was observed during breeding season as compared to non-breeding season. However, the luminal diameter did not show any significant change during seasonal reproductive cycle.

Immunohistochemistry

Seasonal variations were exhibited in the immunoreactivity of S-100 and α -SMA during reproductive and non-reproductive seasons (Table 2).

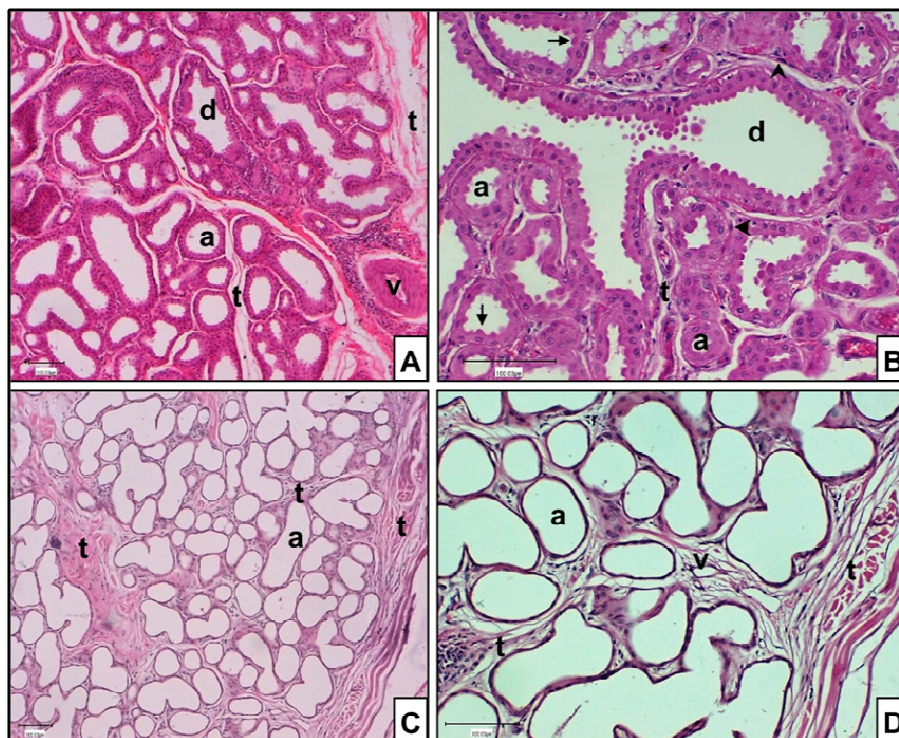


Figure 3: H & E-stained sections of camel poll gland (A-D). The glandular alveoli (a) in Breeding season (A, B) shows apical blebs (Arrows) and myoepithelial cells (Arrowheads); the epithelium is higher, the lumina are narrower and the inter-alveolar and inter-lobular connective tissue (t) is thinner, than those in non-breeding season (C, D). Note the intra- and inter-lobular ducts (d) and blood vessels (v). Scale bars: 100 μm.

Table 1: Seasonal morphometric measurements (μm) of the camel poll gland

Parameter	Breeding season	Non-breeding season
Alveolar diameter	64.45±4.04 _a	52.34±6.83 _b
Epithelial height	14.63±0.93 _a	9.50±1.55 _b
Luminal diameter	44.75±1.87 _a	38.46±2.81 _a
Inter-alveolar tissue thickness	13.80±1.58 _a	32.37±2.78 _b

Values (mean ± SD, N = 12).

Means within the same row with different subscripts are significantly different (P<0.05)

Table 2: Seasonal immunohistochemical reactions of S-100 and α-SMA in the different camel poll gland camel.

Protein	Season	AE	DE	MECs	IASM	CT	VE	VSM
S-100	Breeding	+++	+++	++	+	+	+++	++
	Non-breeding	++	++	+	+/-	+/-	++	+/-
α-SMA	Breeding	-	-	++++	++	++	+++	+++
	Non-breeding	-	-	++	+	-	+	+

Alveolar Epithelium (AE); Ductal Epithelium (DE); Myoepithelial Cells (MECs); Inter-alveolar Smooth Muscles (IASM); Connective Tissue (CT); Vascular Endothelium (VE); Vascular Smooth Muscles (VSM). Very strong (++++); strong (+++), moderate (++); weak to moderate (+/+); weak (+), weak to negative (+/-) and Negative (-) reaction.

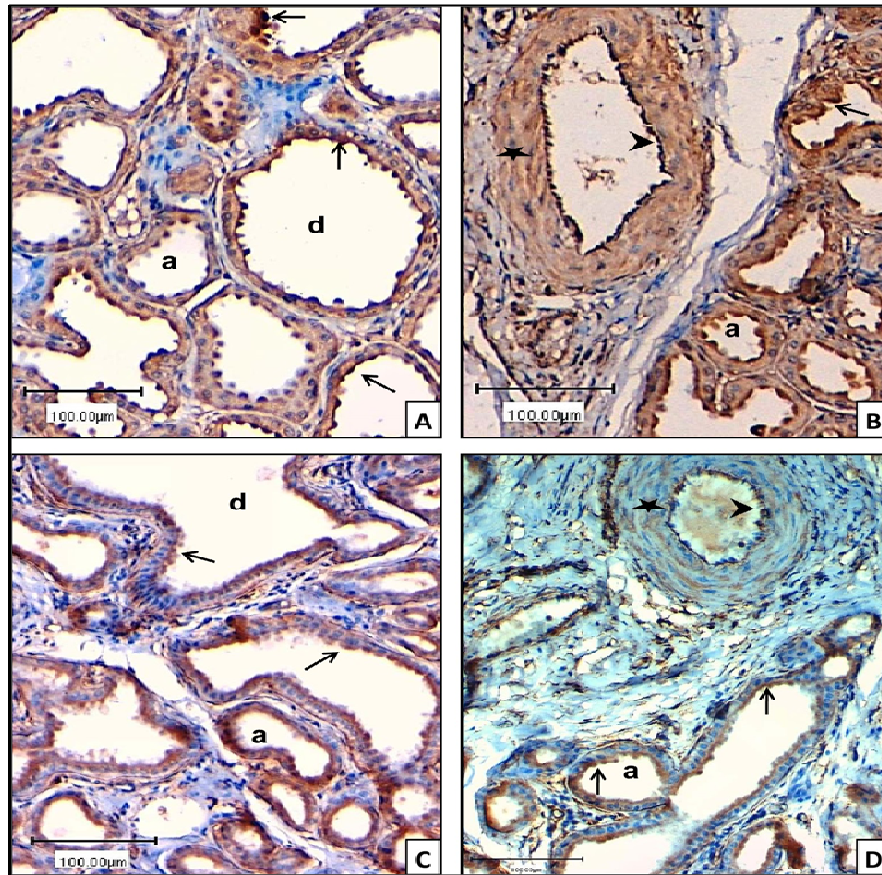


Figure 4: S-100 immuno-stained sections of camel poll gland (A-D). In breeding season (A, B) the alveoli (a) and ducts (d) apical epithelium (Black arrows) and blood vessels endothelium (Arrowheads) show stronger S-100 immuno-reaction than those in non-breeding seasons (C, D). The blood vessels tunica media (Stars) show moderate S-100 immuno-reaction in breeding season, whereas it is weak to negative in non-breeding seasons. Scale bars: 100 μ m.

S-100

In the breeding months glandular alveoli and ducts exhibited numerous epithelial cells with intense S-100 immunostaining, especially in their supra-nuclear region and apical protruding blebs (Fig. 4A–C). Intense immunoreaction was also observed in the blood vessels endothelium. On the other hand, while a moderate reaction was shown in the myoepithelial cells and tunica media of blood vessels, the inter-alveolar and interlobular connective tissue appeared with weak S-100 reaction.

During non-reproductive season, decreased intensity of S-100 reaction was observed in the glandular and ductal epithelial cells, and blood vessel endothelium; in the myoepithelial cells, glandular stroma and blood vessel tunica media the reaction intensity was negative to weak (Fig. 4A–C).

α -SMA

During both seasons there was positive α -SMA immunoreactivity in the myoepithelial cells, peri-alveolar smooth muscles and tunica media of inter-alveolar and interlobular blood vessels. The reaction was stronger in breeding season, especially in the myoepithelial cells, than that in non-breeding season. However, epithelial cells as well as the inter-alveolar and inter-lobular connective tissue were α -SMA negative throughout the entire reproductive cycle of the camel (Fig. 5A–C).

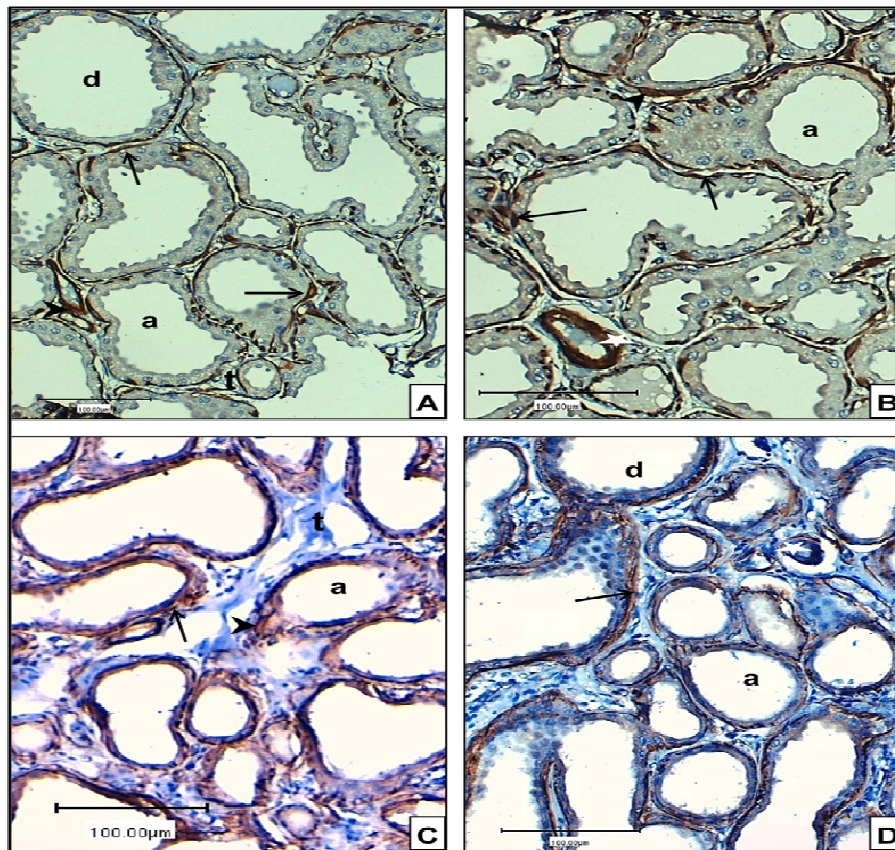


Figure 5: α -SMA immuno-stained sections of camel poll gland (A-D). The glandular myoepithelial cells (Arrows), inter-alveolar smooth muscles (Arrowheads) and tunica media of blood vessels (Stars) in breeding season (A, B) show remarkably stronger α -SMA immuno-reaction than those in non-breeding seasons (C, D). The connective tissue (t), as well as the epithelium of alveoli (a) and ducts (d) show negative α -SMA immuno-reaction. Scale bars: 100 μ m.

DISCUSSION

The present study showed that the paired poll gland was located dorsal to the poll (neck) region of the male dromedary camel where a yellowish watery secretion with offensive odor was observed during the breeding season. This is in line with earlier findings of a number of authors (Leese, 1927; Singh and Bharadwaj, 1978; Taha and Abdalla, 1980; Yagi and Etzion, 1980; Tingari et al., 1984b; Ebada et al., 2012). Yagil and Etzion (1980) have proved the presence of androgens in the secretions of the poll glands of the rutting dromedary camel. The glandular secretion has also been suggested to contain some types of pheromones that might be used to border a mating area for the male in the herd (Skidmore, 2004). The gross anatomical change of poll gland in this study showed darker glandular colour and increased glandular size in the breeding season. According to Singh and Bharadwaj (1978) the seasonal structural variations observed in the poll glands indicates their increased activity during

December-March. These seasonal structural changes were considered as testosterone-dependent (Aguilera-Merlo et al., 2005).

The present results revealed seasonal histological and morphometric variations in the poll gland. During the breeding season the glandular alveoli showed narrow lumina and simple cuboidal or columnar epithelium surrounded by a thin inter-alveolar connective tissue. During non-breeding season the alveoli and intra-lobular ducts were smaller and their epithelium changed to simple squamous or low cuboidal, which resulted in relatively increased luminal diameter and thicker inter-alveolar connective tissue in comparison to those in breeding season. This is in line with the findings of Tingari et al. (1984b).

The histological findings in this study were confirmed by morphometric measurements which showed significantly increased alveolar diameter and epithelial height during breeding season than those in non-breeding season. Also in the breeding season, the inter-alveolar tissue thickness

was significantly decreased. On the other hand, the luminal diameter did not show any significant change in relation to seasonal reproductive activity. Similar findings have also been recorded by Tingari et al. (1984b). According to Singh and Bharadwaj (1978), increased poll gland activity could be indicated by higher alveolar epithelial lining and greatly reduced inter-alveolar connective tissue during December - March in winter as compared to the period between April and August in summer. Moreover, it has been noted that during peak reproductive activity in November and December, the poll gland lobules are separated by thin strands of connective tissue and the alveoli and intra-lobular ducts are taller and lumina are narrower than those during non-reproductive period (Tingari et al., 1984b). However, Taha and Abdalla (1980) found no considerably decreased connective tissue in active poll glands; they however, mentioned that the main structural difference between the active and inactive poll gland resides in the epithelial heights of their secretory units. According to Tingari et al. (1984b) the poll gland secretory phase is characterized by narrow lumina and tall epithelial cells of alveoli and intra-lobular ducts.

The present study showed positive immunoreactivity of S-100 in the epithelial lining and connective tissue of the poll gland. The intensity of the reaction increased during breeding season, especially in supra-nuclear region and apical protruding blebs of the alveolar epithelium. More obvious S-100 binding sites in the supra-nuclear region and apical blebs have also been reported during the rutting season in camel poll gland (Ebada et al., 2012). S-100 reaction in this study was also increased in the myoepithelial cells, glandular stroma and blood vessels during breeding season than in non-breeding season. According to Ebada et al. (2012), variable S-100 immune-reaction was observed in the glandular myoepithelial cells and peri-alveolar connective tissue, whereas weak to moderate reaction appeared in the inter-alveolar connective tissue in camel poll glands. Similarly, Kahn et al. (1985) recorded positive S-100 immunoreaction in the myoepithelial cells of normal salivary glands using immunoperoxidase and immunofluorescence methods. Moreover, a positive reactivity of S-100 in myoepithelial cells has also been expressed in canine sweat glands. According to Heizmann et al. (2002) S-100, (a sub-family of Calcium-binding proteins), is a multifunctional protein with many intracellular and extracellular functions including motility, enzymatic activity, energy metabolism, chemotaxis, neurite extension and secretion. It has also been mentioned that S-100 protein might play a role in the absorptive and secretory activities in the testis (Amselgruber et al., 1994; Cruzana et al., 2003). Further, Ibrahim et al. (2017) stated that S-100 protein improves the secretory and absorptive functions in the epithelial cells and improves smooth muscle contractility in the epididymis during breeding season in camels. Similarly, increased S-

100 immunoreaction in the poll gland epithelial cells in the breeding season observed in this study might indicate increased secretory and absorptive functions. Furthermore, increased intensity of S-100 protein immunoreactions in the glandular myoepithelial cells and tunica media of blood vessels during breeding season recorded in this study could also indicate their increased muscular contractility. Additionally, it has been stated that positive immunoreactivity to S-100 protein in the epididymal vascular endothelium of Egyptian water buffalo indicate its contribution to the processes of transcytosis (Alkafafy et al., 2011b). Therefore, it could also be suggested that the transcytosis process is increased in the poll glands during breeding season as there was increased S-100 immunoreactivity in their vascular tissue.

In the present study there was positive α -SMA immunoreaction in the myoepithelial cells and tunica media of inter-alveolar and interlobular blood vessels of poll glands. The reaction was stronger in the breeding season than in non-breeding season, especially in the myoepithelial cells. The study, however, showed negative α -SMA immunoreactivity in the epithelial cells and weak to negative reaction in the inter-alveolar connective tissue throughout the entire annual reproductive cycle. Positive α -SMA reaction in the myoepithelial cells and inter-alveolar blood vessels of camel poll glands has also been stated by Ebada et al. (2012). Most myoepithelial cells of camel poll glands have been reported to exhibit positive α -SMA reaction (Atoji et al., 1998). Localization of α -SMA has also been reported in myoepithelial cells of mammary gland in bovine (Haaksma et al., 2011) and human (Zancanaro et al., 1999) as well as in rat sweat gland (Gugliotta et al., 1988) and apocrine tubules of Japanese serow (Atoji et al., 1995).

According to Sato et al. (1989), myoepithelial cells are proved to be involved in cellular contraction which results in rapid sweat excretion on stimulation of sweat glands; they added that increased luminal hydrostatic pressure in the walls of sweat glands secretory units can be relieved by the mechanical support of myoepithelial cells. Additionally, the above mentioned authors report that myoepithelial cells are capable of producing both nitric oxide (probably induces relaxation) and acetylcholine which induces contraction. Therefore, increased intensity of immunoreactivity of myoepithelial cells to both S-100 and α -SMA during breeding season could be an indication of increased cellular activity and contractility. They might also play a role in conduction of cellular secretion from the secretory units of poll glands to the lumen and then to the outer surface via the hair follicles.

CONCLUSION

Based on the morphological and immunohistochemical variations, it could be concluded that the camel poll glands are active throughout the year, though their activity increases during cold months of winter and decreases in hot months of summer; this indicates a correlation with the glandular secretion and the male camel sexual activity. Further, the S-100 and α -SMA proteins are suggested to be involved in the control of secretory activities of the poll gland.

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