SEASONAL CHANGES OF THE HISTOLOGY AND HISTOCHEMISTERY OF THE HAIR FOLLICLES OF BALADI GOATS IN EGYPTIAN DESERT AREAS.

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ABSTRACT

Skin biopsy samples were collected from twenty adult does of Baladi goats, randomly chosen from the main flock raised in Maryout Research Station of the Desert Research Center, at seasonally intervals representing spring, summer, autumn and winter in order to study the histological and histochemical changes in the hair follicle as well as the effect of different climatic conditions on their activities.

In general, there was a significant effect of different seasons on the follicle dimensions. The external diameter of both follicle types was most affected by seasonal variations followed by internal diameter and the least affected was follicle wall thickness.

There was an increase in the external and internal diameters of both primary and secondary follicles found in autumn and spring, while follicle wall thickness showed a noticeable decrease in summer irrespective of follicle types.

The histochemical analysis of the hair follicles showed a high follicle activity in summer in the primary follicle and in autumn in the secondary ones and this could be mainly attributed to the coat type which the animals need to adapt themselves to the changing seasons by controlling the character of the hair coat and development of a suitable coat for different seasons.

Keywords: Goats, hair follicles, seasonal effect, skin histology, histochemistry

INTRODUCTION

Goats have a great ability to tolerate bad weather and poor nutrition where they can survive and reproduce.

The coat cover of goats plays an important role in thermoregulation under both hot and cold conditions and protects animals from solar, sky and bare ground radiation. The insulation properties of the coat are brought about by the fibres beside the still air entrapped between them (Govindiah and Nagarcenkar, 1983) Heat tolerance of animals is closely associated with coat type. The physical characteristics of the coat are important, and so the histological and histochemical investigations of the skin are needed to explain coat structure and to detect the influencing factors. Such information on the effect of season on coat structure in general and on follicle activity in particular are lacking in Egyptian goats.

The present histological and histochemical study of skin of Baladi goats describes the changes in the hair follicle activity. Follicle activity was compared with hair production to count for changes in coat structure under different seasonal conditions.

MATERIALS AND METHODS

The study was carried out on twenty adult does of Baladi goats at the age of 2.0-2.5 years and raised in Maryout Research Station of the Desert Research Center. The station is located at the North-Western desert of Egypt, 35 kilometers southwest of Alexandria.

Skin biopsy samples about one centimeter square were taken at different seasons of the year, spring, summer, autumn and winter from the mid-side position and fixed in calcium formol for about 24 hours (Barker, 1958), washed and left for 24 hours in distilled water then transferred

to 70% ethanol.

Specimens were then dehydrated in an ascending series of ethanol (30 minutes in each of 70%, 80% and 90%ethanol and finally two changes each for 15 minutes in absolute ethanol). The specimens were cleared in benzene for about 30 minutes, infiltrated in paraffin wax at 60°C (4 changes, 20 minutes each) and then embedded in the same paraffin to prepare the blocks. Sections of 6-8 microns in thickness were prepared for both

histological and histochemical studies.

Skin sections were stained by Haematoxylin and Eosin Drury and Wallington, (1980) for histological observations. Histochemically Periodic acid schiff's reaction Mc-Manus and Cason (1950) and Alcian blue at pH 2.5 method Luna (1968) were used for detecting carbohydrates and acid mucopolysaccharides as red and blue colours, respectively. Mercury bromophenol blue Pearse (1968) was used for the demonstration of general proteins as represented by the blue colour. For the nucleic acids demonstration, specimens were stained by methyl green-pyronin Kurnick (1955) giving a blue colour for the DNA, and Gomori (1951) method for alkaline phosphatase enzyme detection at 37C for about 3 hours showing the site activity by the brown colour.

The histological measurements including the hair follicle diameter were undertaken by using Image analyzer (LEICAQ 500 MC) with lens 40/0.65. The follicle wall thickness and secondary/ primary follicles ratios (s/p) were

also calculated.

Data were statistically analyezd according to SAS (1995) using General Linear Models (GLM) classification followed by Duncan's multiple range test to examine the significance between means.

RESULTS AND DISCUSSION

Hair follicle structure

The hair follicle of Baladi goat skin was observed as a tubular epidermal structure divided vertically into three main regions from the base towards the skin surface. The first region was a rounded bulb containing the papilla (Plate 1), the second was the part in which the hair fibre was enclosed by the inner sheath, and the third was the region into which the follicle glands opened (Plate 2). Transversely the follicle wall was divided into two distinct layers, the inner and outer root sheaths (Plate 3). The inner root sheath itself was divided into three concentric layers that grew up with the fibre from the

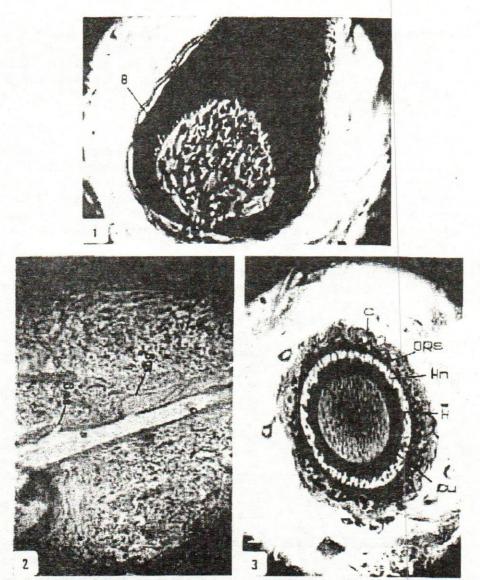


Plate 1: L. S. of the hair follicle in the skin of Baladi goat showing the bulb, B enclosing a dermal papilla, P.(Hx. E., X400).

Plate 2: V. S. of the skin of Baladi goat showing longitudinally cut hair follicle with sweat gland duct opening, Sw G and sebaceous gland with its opening, Sb G.(Alcian blue, X100).

Plate 3: V. S. of a hair follicle in the skin of Baladi goat showing the connective tissue sheath, C; outer root sheath, ORS; Henle's layer, Hn; Huxley's layer, H and cuticle, Cu. (Hx. E., X400).

follicle bulb. The inner layer is a thin cuticle which interlocks with the fibre cuticle. In the middle is the Huxley layer and on the outside the Henle layer. The follicle was surrounded by three indistinct dermal layers, the glassy membrane, an inner circular layer and an outer longitudinal layer of connective tissue (Plate 3). The above results are in agreement with those reported by El-Sayed et. al., (1998).

Hair follicle dimensions:

Table (1) showed the average values of the external and internal diameters and wall thickness of both primary and secondary follicles in the Baladi goat skin. The data were represented graphically in figure (1) and statistically analyzed in (Table 2) which revealed a highly significant (P< 0.01) effect of different seasons. Generally, external diameter of both follicle types was most affected by fluctuating temperatures followed by internal diameter. and the least affected was the follicle wall thickness.

Table(1): Mean values ±SE of external, internal and wall thickness of both primary and secondary follicles(µ) during different caseane

Seasons							
Follicle dimensions	Season	Primary follic Mean ± SE	le	Secondary follicle Mean ± SE			
	Spring	164.06 ± 3.34	а	35.40 ± 0.63	b		
-	Summer	148.32 ± 3.34	b	31.21 ± 0.54	C		
External diameter µ	Autumn	163.16 ± 3.33	а	39.35 ± 0.58	а		
	Winter	150.85 ± 3.34	SE Mean ± SE 34 a 35.40 ± 0.63 34 b 31.21 ± 0.54 33 a 39.35 ± 0.58 34 b 36.86 ± 0.65 39 ab 17.03 ± 0.30 5 bc 15.56 ± 0.26 38 a 19.24 ± 0.28 9 c 17.88 ± 0.31 0 a 9.18 ± 0.2 7.82 ± 0.20 ab 10.05 ± 0.22	b			
	Spring	101.01 ± 2.39	ab	17.03 ± 0.30	C		
	Summer	95.03 ± 2.45	bc	15.56 ± 0.26	d		
Internal diameter µ	Autumn	104.73 ± 2.38	а	19.24 ± 0.28	а		
	Winter	92.64 ± 2.39	C	17.88 ± 0.31	b		
	Spring	31.52 ± 0.90	а	9.18 ± 0.2	b		
Wall thickness μ	Summer	26.64 ± 0.93	b	7.82 ± 0.20	C		
	Autumn	29.21 ± 0.90	ab	10.05 ± 0.22	а		
	Winter	29.10 ± 0.90	b	7.82 ± 0.20	ab		

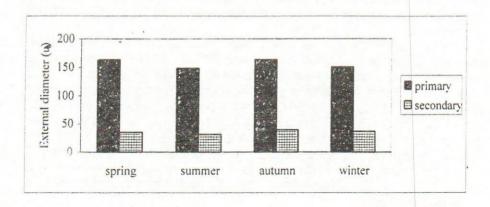
In each parameter, means within the same column followed by different letters differed significantly (P<0.05).

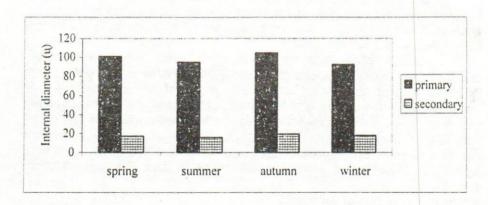
Table (2): Analysis of variance of the effect of season on external diameter, internal diameter and wall thickness of both primary and secondary follicles.

s.o.v	External diameter			Internal diameter				Wall Thickness					
	Primary F. Sec		Sec	ondary F. P		Primary F. Se		Secondary F.		Primary F.		Secondary F.	
	FD	MS	FD	MS	FD	MS	FD	MS	FD	MS	FD	MS	
Season	3	12531.33**	3	4888.33**	3	5792.69**	3	988.40**	3	737.27**	3	378.67**	
Error	751	2138.75	1481	130.74	751	1093.63	1481	30.56	751	155.91	1481	18.66	

S.O.V: Source of variance.

**= Significant at (p<0.01). MS: Mean squares.





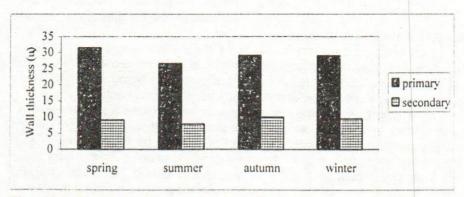


Fig. (1): The external and internal diameters and wall thickness of primary and secondary follicles during different seasons.

The previous results are similar to those reported by Guirgis et. al. (1981) who added that the pattern of increase or decrease in the follicle dimension corresponded with a similar pattern of change in ambient temperature.

The size of the follicle varied throughout different seasons and that variation is connected with its productivity. Rudall (1955) also found that the

papilla size is less relative to the bulb size in winter than in summer.

It was found that follicle wall thickness varied directly with seasonal changes (Table1). Priestley (1967) recorded significant variations in the inner root sheath thickness ,represented the winter thinning in herdwick sheep ,brought about by the changes in the huxley's layer that occurred with season. These variations appear very likely to be linked with the type of keratin produced . The winter thinning in the fibre was accompanied by a thickening of the Huxley's layer in the inner root sheath in primary follicles which remained active.

Seasons affected the direction of dividing cells, where in winter Huxley's layer became thick, due to the proliferation of the cells in fibre matrix towards fibre length rather than fibre thickness. Similar results were reported

by Henderson and Sabine (1992).

Follicle activity was essentially associated with fibre diameter. However, secondary follicles showed more activity in summer months which were not evident in the coat until the beginning of the winter months. This might be attributed to the fact that many of the newly-produced cells were producing inner and outer root sheaths and a lengthening of the follicle rather than fibre (Henderson and Sabine, 1992). There would be a lag period between renewal of the follicle activity and manifestation of this in the fibre above skin level, and this period would depend upon the rate of follicle activity.

Histochemistry of hair follicles:

DNA content

Studies of the DNA in the follicle bulb cells of both primary and secondary follicles (Table 3) were verified the previous results. The DNA content of bulb cells would support the cell division in the follicle bulb and represents the follicle activity.

In winter, the primary follicle bulbs showed low amount of DNA (Plate 4), while the intense reaction was recorded at summer season (Plate 5). On the other hand, the secondary follicle bulbs had few amounts of DNA in the spring (Plate 6) while the highest content was observed in autumn (Plate 7).

The hair growth cycle in the goat appeared to have a simple activity in summer and inactivity in winter. Both primary and secondary fibres would follow a similar cycle. In winter follicle activity was found to decline as most of fibres completed their growth to give a dense coat composed of long guard hair. The winter coat was shed during spring. Guard hair was shed and regrew from primary follicles giving a continuous cover and no moult was obvious because new guard hairs grew before all the old ones had shed. The only manifestation of shedding was a shortening of the coat. In secondary follicles, under coat was mostly shed in summer months but regrowth was not detected in fleece samples until mid winter. Nixon et al. (1991) suggested

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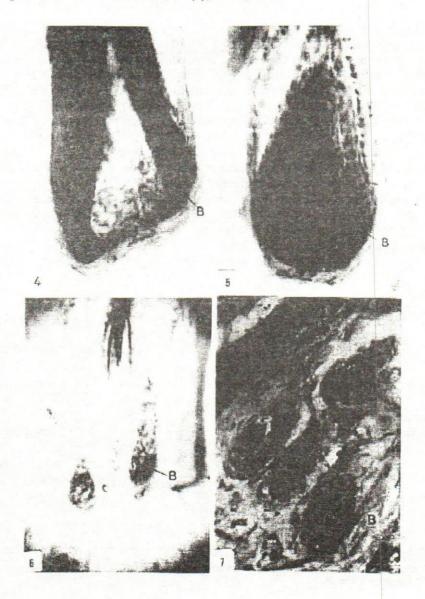


Plate 4: L. S. of a primary follicle bulb (B) of the Baladi goat stained with Methyl green-pyronin, showing the concentration of DNA at winter season. X400.

- Plate 5: L. S. of a primary follicle bulb(B) of the Baladi goat stained with Methyl green-pyronin, showing the concentration of DNA at summer season. X400.
- Plate 6: T. S. of secondary follicle bulb (B) of the Baladi goat skin stained with Methyl green-pyronin, showing the concentration of DNA at spring season. X400.
- Plate 7: T. S. of secondary follicle bulb (B) of the Baladi goat skin stained with Methyl green-pyronin, showing the concentration of DNA at autumn season. X400.

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that some secondary follicles remained in telogen for several months after cashmere was shed. However, other secondary follicles underwent a spring fibre growth cycle which produced only minute fibres which barely projected above the skin surface, and were not detectable in the fleece samples. They were shed in summer, when virtually all secondary follicles became active to grow cashmere over summer-autumn, culminating in the dense winter coat and they referred as vellus fibres (Rook, 1970) and Montagna and Parackal (1974).

Table (3): Histochemical reactions in hair follicles, sebaceous and sweat

glands in goat at different seasons.

Histochemical	Conne	Pr. Fo	ollicle	Sec. Fo	ollicle	Seb	Sw. Gland	
parameter	Season	O.r.sh	l.r.sh	O.r.sh	I.r.sh	gland.		
Carbohydrate	Spring	++	+	++	±	±	++	
substances	Summer	+++	++	+++	±	±	+++	
	Autumn	++	++	+++	±	±	+++	
	Winter	+	+	+	\pm	\pm	±	
Acid-	Spring	+++	++	+++	+	±	+	
mucopolysacch-rides	Summer	+++	++	+++	+	±	++	
	Autumn	++	+	++	+	±	++	
	Winter	++	-	±	\pm	±	++	
Proteins	Spring	+++	++	+++	++	+	++	
	Summer	+++	+++	++	++	+	+++	
	Autumn	+++	+++	+++	+++	++	++	
	Winter	++	+	+++	++	++	++	
Alkaline	Spring	+	+	±	±	+	+	
phosphatase	Summer	++	+++	++	++	±	++	
	Autumn	+	++	+++	+++	+	+	
	Winter	±	+	++	++	++	±	
		Pr.Follicle bulb		Sec.Follicie bulb			0	
DNA	Spring	++		+				
	Summer	+++		++				
	Autumn	+++		+++				
	Winter	-	-		++			

Negative; ± Traces; + Few; ++ Moderate; +++ Intense.

Pr.Primary; Sec.Secondary; Seb.Sebaceous; Sw.Sweat; O.r.sh.Outer root sheath; I.r.sh.Inner root sheath.

General carbohydrates content (PAS):

The general carbohydrate content in different skin structures are shown in Table (3) at different seasons. Generally, the carbohydrates were more concentrated in the outer root sheath than in the inner root sheath (Plate 8) and also in the lower third of the hair follicle (Plate 9). In both primary and secondary follicles the outer root sheath showed higher

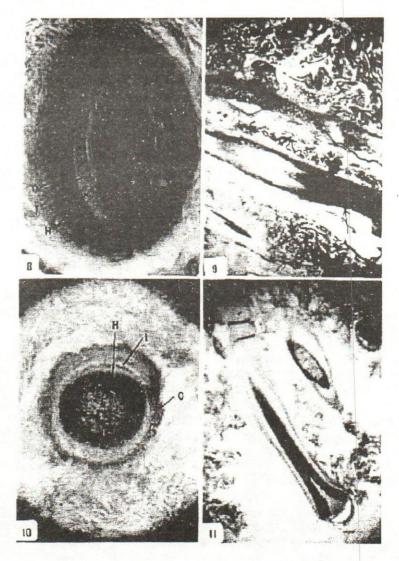


Plate 8: T. S. of a hair follicle stained with PAS method, showing the distribution of general carbohydrates in the different follicle sheaths. H, hair; I, inner root sheath; O, outer sheath. X400.

- Plate 9: V. S. of skin of Baladi goat stained with PAS method, showing the presence of the general carbohydrates in the lower third of a longitudinally cut hair follicle. X100.
- Plate 10: T. S. of a hair follicle stained with Alcian blue, showing the distribution of the acid mucopolysacchrides in the different follicle sheaths. H. hair; I, inner root sheath; O, outer sheath. X400.
- Plate 11: V. S. of skin of Baladi goat stained with Alcian blue, showing the presence of the acid mucopolysacchrides in the lower third of a longitudinally cut hair follicle. X100.

carbohydrate contents in summer while the lowest carbohydrate content was recorded in winter (Table3).

Table (3) indicates that both summer and autumn seasons showed moderate amounts of carbohydrate in the inner root sheath of primary follicles whereas few amounts were found in both spring and winter. In secondary follicles, there were only traces of carbohydrates in the inner root sheath at different seasons.

The strong reaction of PAS in the outer root sheath of both primary and secondary follicles in Baladi goats was similar to that observed in the skin of kashmir sheep by Tej Sharma (1982) and as mentioned by Parmar et al. (1988). The carbohydrates, as a sign of follicle activity, was in accordance with the assumption of Montagna (1956) who assumed that the carbohydrates in the outer root sheath was the source of energy for protein synthesis during hair growth.

In sweat glands, the carbohydrate content showed the highest level in summer and autumn, the minimum content was noticed in winter, while a moderate amount was found in spring (Table 3).

These results could be explained that sweat glands were more active during summer and their activity decreased in winter as an adaptive mechanism.

The sebaceous glands were found to show a faint reaction of carbohydrates at all seasons (Table 3) since most of its content was the sebum, mainly of a lipoidal material. Traces of carbohydrates as represented by PAS positive cell membranes and the connective tissue layers surrounded the sebaceous glands. However, the sweat glands showed higher PAS activity than that of sebaceous glands. In this respect, the present results were in harmony with those of Kamel et al. (1986) and Parmar et al. (1988) in their studies on the skin of Camels and goats.

-Acid mucopolyscchride

The presence of acid mucopolysaccharide was dominant in the outer root sheath (Plate 10) specially in the lower third of the hair follicle (Plate 11). It is clear from (Table 3) that the outer root sheath of primary follicles showed the highest acid mucopolysaccharide content in both spring and summer which decreased to moderate amounts in autumn and winter. In case of inner root sheath the acid mucopolysaccharide content decreased from moderate amounts in spring and summer to low amount in autumn and was absent in winter. In the secondary follicles the outer root sheath showed the highest content of acid mucopolysaccharide in spring and summer and moderate amounts in autumn (Table 3), and a very slight amount in winter. Whereas, the inner root sheath had few amounts in both spring and summer and very scanty amounts in autumn and spring.

Intense reaction of PAS positive substances and acid mucopolysaccharides was observed in the outer root sheath than in the inner root sheath which might be due to enhanced energy requirements for active cellular proliferation (Parmar et al., 1988).

The sweat glands showed moderate amounts of mucopolysaccharides in summer, autumn and winter (Table 3). Parmar et al. (1988) found that in sweat gland of goats, the secretory cells showed mild staining while the luminal secretion revealed strong staining for acid mucopolysaccharides. Sorenson and Prasad (1973) also reported that acid mucopolysaccharides were also found in the sweat gland of the horse. Whereas Singh et al. (1976) reported negative reaction in the sweat gland of buffalo calf.

The sebaceous gland showed trace amounts of acid mucopolysaccharides at all different seasons (Table 3). Parmar et al., (1988) found a positive reaction in goats and this was in corroboration with those reported by Prasad and Sinha (1979) in the buffaloes. Generally, in addition to the use of carbohydrates to provide energy and the synthesis of amino acids, a third important use is the synthesis of ribose to form part of the RNA and DNA, molecules involved in protein synthesis.

-General protein

The distribution of the general proteins as demonstrated by the bromophenol blue stain in the different skin structures of the Baladi goat showed that both outer and inner root sheaths of the hair follicles possessed high amounts of proteins (Table 3).

Plates (12,13,14 and 15) illustrated that the outer root sheath of primary follicles showed the highest protein content in spring, and moderate amount in winter. Meanwhile, the inner root sheath showed the highest intense reaction for proteins in summer, and the lowest amount in winter.

While in the secondary follicles, the outer root sheath showed the highest protein content in spring, autumn and winter. A moderate amount of proteins was found in summer. On the other hand the inner root sheath had moderate amounts in spring, summer and winter. However, intense protein reaction was recorded in autumn.

Parmar et al. (1988) stated that the protein content was larger in the outer root sheath than that of the inner root sheath of the skin follicles of goats and this was probably due to the increased protein synthesis in the cellular proliferation.

In Baladi goats, the sebaceous glands had trace of protein, as those detected by Parmar *et al.* (1988), in goats. In the case of sweat glands, they had few amount of proteins in spring and summer, which slightly increased in autumn and winter (Table 3). Parmar *et al.* (1988) recorded moderate amounts of proteins in sweat glands in goats.

-Alkaline phosphatase

The vitality of hair follicles as represented by the site activity of alkaline phosphates content was higher in summer in the case of primary follicles and in autumn in the case of secondary follicles (Table 3). The alkaline phosphatase activity was intense in the inner root sheaths than those of the outer root sheaths of both primary and secondary follicles.

In the primary follicles, the outer root sheath showed traces of alkaline phosphatase activity in winter and moderately increased in summer. In case of inner root sheath the alkaline phosphatase activity was low in both

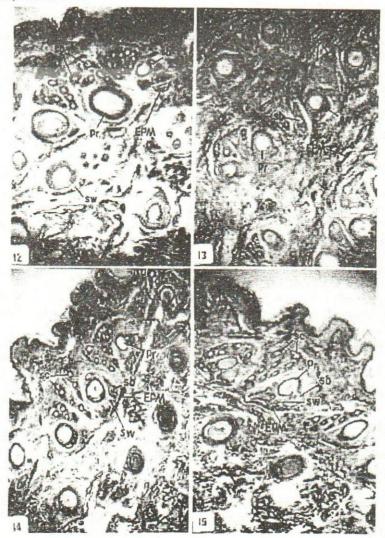


Plate 12: V. S. of skin of Baladi goat stained with bromphenol blue, showing the distribution of the general proteins in the different skin structures at spring season. Pr, primary follicle; Sc, secondary follicle; Sw, sweat gland; Sb, sebaceous gland; EPM, erector pili muscle. X400.

Plate 13: V. S. of skin of Baladi goat stained with bromphenol blue, showing the distribution of the general proteins in the different skin structures at Summer season. Pr, primary follicle; Sc, secondary follicle; Sw, sweat gland; Sb, sebaceous gland; EPM, erector pili muscle. X400.

Plate 14: V. S. of skin of Baladi goat stained with bromphenol blue, showing the distribution of the general proteins in the different skin structures at winter season. Pr, primary follicle; Sc, secondary follicle; Sw, sweat gland; Sb, sebaceous gland; EPM, erector pili muscle, X400.

Plate 15 V. S. of skin of Baladi goat stained with bromphenol blue, showing the distribution of the general proteins in the different skin structures at autumn season. Pr, primary follicle; Sc, secondary follicle; Sw, sweat gland; Sb, sebaceous gland; EPM, erector pili muscle. X400.

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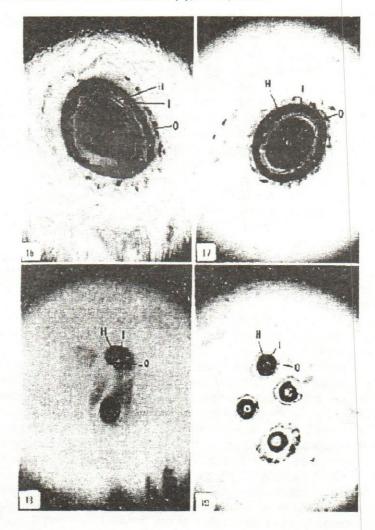


Plate 16: T. S. of a primary hair follicle stained with Gomori method, showing the distribution of the activity of alkaline phosphatase enzyme in the different follicle sheaths at winter season. H, hair; I, inner root sheath; O, outer sheath. X400.

- Plate 17: T. S. of a primary hair follicle stained with Gomori method, showing the distribution of the activity of alkaline phosphatase enzyme in the different follicle sheaths at summerseason. H, hair; I, inner root sheath; O, outer sheath. X400.
- Plate 18: T. S. of secondary hair follicles stained with Gomori method, showing the distribution of the sight activity of alkaline phosphatase enzyme in the different follicle sheaths at spring season. H, hair; I, inner root sheath; O, outer sheath. X400.
- Plate 19: T. S. of secondary hair follicles stained with Gomori method, showing the distribution of the sight activity of alkaline phosphatase enzyme in the different follicle sheaths at autumn season. H, hair; I, inner root sheath; O, outer sheath. X400.

spring and winter, while the intense reaction was found in summer (Plates 16,17).

The outer and inner root sheath of secondary follicles showed traces of alkaline phosphatase activity in the spring and an intense reaction was recorded in autumn (Plates 18 and 19).

A reaction of alkaline phosphatase activity was obtained in the outer sheath and the papillae of the follicle. The blood vessels too, gave a reaction and this might suggest that the reaction in the outer root sheath and the papillae had arisen by diffusion of the enzyme from the blood vessels Ryder and Stephenson (1968).

The sweat glands also play an important role in the physiological equilibrium of the animal. The alkaline phosphates activity, observed in the sweat glands of the Baladi goats skin (Table 3), was in accordance with those reported by Parmar *et al.* (1988) in goats which was also represented in the report of Takagi and Tagava (1959) in the horse.

The present study showed that the vitality of the sweat glands, represented by alkaline phosphates activity, gradually increased reaching the maximum rate at summer season (Table 3). Such activity was essential for heat regulation.

On the other hand ,the sebaceous glands had a weak stain for alkaline phasphatase enzyme activity in summer , and showed moderate amounts in winter (Table 3).

It was observed, from the previous histochemical studies, that goats could adapt themselves to the changing seasons by controlling the character of the hair coat and development of a suitable coat for different climates. The activity of the hair follicles as represented by shedding and regrowth of fibres was involved in this process. While, it was a relatively slow operation and the animal requires advanced warning for seasonal changes. Monthly changes in the temperatures could give signs of approaching hot or cold season but these might be erratic in comparison with day length. Therefore, photoperiod had been considered to play an important role in controlling the character of the coat by modifying neuro - secretory rhythms via the pineal gland, which would ultimately affect the initiation of hair growth Lynch and Russel, (1990).

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التغيرات الموسمية في التركيب الهستولوجي و الهستوكيميائي لبصيلات الشعر في الماعز البلدي في المناطق الصحراوية

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أجريت الدراسة على عينات جلد من عدد ٢٠ من إناث الماعز البلدي بمتوسط ٢-٣,٥ سنة بمحطة بحوث مريوط على بعد ٣٥ كيلومتر جنوب غرب الإسكندرية. وقد تم تجهيز عينات الجلد للفحص هستولوجيا و هستوكيميائيا لدراسة مكونات الجلد و كيفية تأثرها بالاختلافات في فصــول السنة المختلفة (شتاء ربيع صيف خريف).

أظهرت نتائج الدراسة تأثيرا معنويا واضحا لفصول السنة المختلفة على القياسات الهستولوجية للبصيلات الشعرية. فقد وجد أن هناك زيادة في الأقطار الداخلية و الخارجية لكل من البصيلات الشعرية الأولية و الثانوية في كل من فصلى الخريف و الربيع بينما وجد أن هناك نقص ملحوظ في سمك جدار البصيلات في فصل الصيف عنة في فصل الشتاء حيث يزداد سمك جدار البصيلات و قد وجد أن ذلك يرجع الى ازدياد سمك طبقة هاكسلى المتلازم مع صغر قطر الألياف الشعرية.

و قد بينت الدراسة أيضا أن معدل نشاط بصيلات الشعر الأولية يزداد في فصل الصيف عنه في فصول السنة الأخرى أما بصيلات الشعر الثانوية فهي تظهر نشاطا عاليا في فصل الخريف و قد تأكدت هذه النتائج هستوكيميائيا من خلال دراسة وصفية لمعدلات تركيز أنزيم الفوسفات القاعدي و بعض المكونات الكيميائية الأخرى في طبقات كل من بصيلات الشعر الأولية و الثانوية خلال فصول السنة المختلفة.

أوضحت الدراسة ان معدل نمو الشعر يرتبط بمعدل انقسام الخلايا في الجزء النامي مــن البصيلات الشعرية من خلال دراسة معدلات تركيز الحمض النووي الدي أكسى ريبوزي في هـذا الجزء من البصيلات وقد وجد ان معدلات النمو للشعر تزداد في فصل الصيف بالنسبة للبصيلات الأولية وفي فصل الخريف بالنسبة للبصيلات الثانوية.