

Histomorphometrical Variations of Hair Follicles of *Bakhtiari* Sheep at Different Skin Areas

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Abstract

The purpose of this study was to determine the histomorphometrical variations of hair follicles at different skin areas in *Bakhtiari* sheep. Twelve healthy adult sheep (6 males and 6 females) of the *Bakhtiari* breed, aged from 1 to 2 years, were selected from the abattoir of Shahrekord. Skin samples were taken from different areas, fixed and stained with haematoxylin and eosin. By using lattice line graticule (5x5) and ocular micrometer, the following histomorphometric indices were tested: number of primary and secondary follicles per one mm²; depth and width of primary and secondary follicles; ratios of number, depth and width of secondary to primary follicles; and width of primary and secondary hairs. In both sexes, the means of all indices varied among areas of skin. This breed may be characterized by the secondary to primary follicles ratio of 0.17-1.89, number of primary follicles per mm² of 2-12, number of secondary follicles per mm² of 0.67-9.67, depth of primary follicles of 760-1766.67 µm, depth of secondary follicles of 227.33-670 µm, width of primary follicles of 126.67-210, and width of secondary follicles of 51.67-128.33 µm. The results showed that except the width of primary follicles, width of secondary follicles and ratios of depth of secondary follicles to primary follicles, the means of all histomorphometric indices were affected by sex ($p < 0.05$). It can be concluded that *Bakhtiari* sheep possess coarse wool in this age.

Keywords: Bakhtiari sheep, hair follicles, histomorphometry, skin, variations

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บทคัดย่อ

ความแตกต่างทางจุลสัณฐานวิทยาของรูขุมขนแกะ *Bakhtiari* จากผิวหนังบริเวณต่างๆ

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วัตถุประสงค์ของการศึกษาครั้งนี้ เพื่อศึกษาความแตกต่างทางจุลสัณฐานวิทยาของรูขุมขนจากผิวหนังบริเวณต่างๆ ของแกะ *Bakhtiari* ทำการศึกษาโดยคัดเลือกแกะโตเต็มวัย พันธุ์ *Bakhtiari* ที่มีสุขภาพแข็งแรง จำนวน 12 ตัว (เพศผู้และเพศเมีย เพศละ 6 ตัว) ช่วงอายุ 1-2 ปีจากโรงฆ่าสัตว์ของเมือง Shahrekord ทำการเก็บตัวอย่างผิวหนังจากบริเวณต่างๆ และย้อมด้วยสีฮีมาทอกซิลินและอีโอซิน ในการวัดดัชนีทางจุลสัณฐานวิทยาใช้สเกล graticule (ขนาด 5 x 5) ร่วมกับ ocular micrometer จากนั้นวัดดัชนีจุลสัณฐานวิทยา ได้แก่ จำนวนของรูขุมปฐมภูมิและทุติยภูมิ อัตราส่วน ความลึกและความกว้างของรูขุมขนแบบทุติยภูมิต่อปฐมภูมิ และความกว้างของรูขุมปฐมภูมิและทุติยภูมิ จากการศึกษาในแกะทั้งสองเพศ ค่าเฉลี่ยของดัชนีทั้งหมดแตกต่างกันขึ้นอยู่กับตำแหน่งที่เก็บผิวหนัง สำหรับแกะพันธุ์นี้มีลักษณะเฉพาะคือ อัตราส่วนของรูขุมขนทุติยภูมิต่อปฐมภูมิ มีค่า 0.17-1.89 จำนวนของรูขุมปฐมภูมิจำนวน 2-12 ต่อตารางมิลลิเมตร จำนวนของรูขุมทุติยภูมิ 0.67-9.67 ต่อตารางมิลลิเมตร ความลึกของรูขุมขนปฐมภูมิ 760-1766.67 ไมโครเมตร ความลึกของรูขุมขนทุติยภูมิ 227.33-670 ไมโครเมตร ความกว้างของรูขุมขนปฐมภูมิและทุติยภูมิ 126.67-210 และ 51.67-128.33 ไมโครเมตร ผลการศึกษาชี้ให้เห็นว่า นอกจากความกว้างของรูขุมขนปฐมภูมิและทุติยภูมิ และอัตราส่วนของความลึกของรูขุมขนทุติยภูมิต่อปฐมภูมิแล้ว ค่าเฉลี่ยของดัชนีทั้งหมดมีความแตกต่างระหว่างเพศ ($p < 0.05$) อาจสรุปได้ว่าแกะ *Bakhtiari* ในช่วงอายุนี้นี้มีขนหยาบ

คำสำคัญ: แกะ *Bakhtiari* รูขุมขน จุลสัณฐานวิทยา ผิวหนัง ความแตกต่าง

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Introduction

Iran possesses 20 breeds of sheep. More than 96% of Iranian sheep are fat-tailed (Kiyanzad et al., 2003). One of the main Iranian fat-tailed and native breeds is *Bakhtiari*, which is found in Charmahal va Bakhtiari province, where sheep production contributes significantly to the agricultural economy (Mobini, 2012). Skin has considerable economic value with regards to leather, fur and wool industries (Konig and Liebich, 2004). Hairs or wool fibers are produced from hair follicles and are divided into two groups as primary and secondary hairs (Kurtdele, 2002; Özfiliz et al., 2002). Quality and quantity characteristics of wool vary according to age, which is related to the growth physiology of sheep (Mobini, 2012). The value of hair and wool produced by sheep is mainly determined by histomorphometry of hairs and follicles (McDonald et al., 1987). In available literature, there is a lack of data characterizing the histomorphometric properties of the hair follicles in *Bakhtiari* sheep. The objective of this study was to describe the histomorphometric properties in this breed and evaluate their variations among different areas of skin in both sexes.

Materials and Methods

Twelve clinically healthy adult sheep of the *Bakhtiari* breed (6 males and 6 females), aged from 1 to 2 years, were selected according to their phenotypic features from the abattoir of Shahrekord. Immediately after slaughter, samples of skins, each of 3 cm², were taken from eight regions; belly, neck, leg, rump, flank, forearm, shoulder and hip. Skin samples were fixed in 10% neutral buffered formalin solution for 24-48 hours and then submitted to the dehydration process with alcohol and embedded in Paraplast. Transverse sections (5 µm thick) were cut parallel to the surface of the skin at midsebaceous gland level and stained with H&E (Kiernan, 1999). By using lattice line graticule (5x5) and ocular micrometer, the following histomorphometric indices were measured: number of primary (NP) and secondary follicles (NS) per one mm²; depth of primary (HP) and secondary follicles (HS); width of primary (WPH) and secondary hairs (WSH) and their follicles (WP and WS); and ratios of number (NS/P), depth (HS/P) and width of secondary follicles to primary follicles (WS/P).

Data were analyzed by one-way ANOVA, using the SPSS/PC statistic software (version 18) for windows. All values were expressed as mean±SD. Differences were considered significant when $p < 0.05$ (Duncan's multiple-range test).

Results and Discussion

Results showed that except the width of primary (WP) and secondary follicles (WS) (Table 1), and depth of secondary follicles to primary follicles (HS/P) (Table 2), the means of all histomorphometric indices were affected by sex. With the exception of hip, the mean numbers of primary follicles (NP) in male sheep were higher than females.

In the present study, the only sex-related significant difference in the NS value (number of secondary follicles per one mm²) was found in hip skin (Fig 1), which was higher in females than compared to males ($p < 0.05$).

With the exception of neck, HP values (depth

of primary follicles) in other areas of skin in rams were higher than ewes. The results also showed that the males had a significantly higher depth of secondary follicles (HS) and WPH value (width of primary hairs) in flank skin than females (Fig 2).

In the hip skin, the ratio of number of secondary follicles to primary follicles (NS/P value) was higher in the female sheep, whereas the males had a higher value of WS/P (the ratio of width of secondary follicles to primary follicles). The results also revealed that the values of width of secondary hairs (WSH) in neck (Fig 3) and rump (Fig 4) areas were affected by sex, and the males had higher values than females ($p < 0.05$). Significant sex-based differences were also

Table 1 Mean values of NP and NS (per one mm²), HP, HS, WP and WS (µm) in different skin areas of male (M) and female (F) Bakhtiari sheep (mean±SD).

Region		Indices					
		NP	NS	HP	HS	WP	WS
Belly	M	4.67±1.15 ^a	8.0±4.0	1010.0±87.18	313.33±83.86	153.33±20.82	88.33±31.75
	F	2.0±1.0 ^a	1.67±1.53	1000.0±286.88	256.67±45.09	166.67±23.09	90.0±20.90
Neck	M	10.0±1.0 ^b	5.0±2.0	916.67±105.99 ^f	306.67±75.72	183.33±35.12	98.33±18.93
	F	7.0±1.0 ^b	2.0±1.0	1458.33±228.82 ^f	413.33±334.86	170.0±34.64	83.33±5.77
Leg	M	4.67±1.53	1.33±1.53	1733.30±101.16 ^g	670.0±202.98	210.0±10.0	125.0±37.75
	F	4.67±2.52	3.67±2.52	1183.30±64.29 ^g	390.0±191.57	196.67±28.87	78.33±45.37
Rump	M	10.0±1.0 ^c	5.33±1.53	1471.70±150.69	503.33±165.02	156.67±20.82	100.0±45.82
	F	4.33±1.15 ^c	1.67±2.08	1343.30±196.55	446.67±100.17	203.33±45.09	128.33±36.17
Flank	M	8.33±0.58	8.0±3.60	1330.0±215.17	430.0±10.0 ⁱ	133.33±30.55	66.67±11.55
	F	8.0±3.60	9.67±2.52	950.0±478.43	258.33±67.88 ^j	153.33±51.32	51.67±12.58
Forearm	M	7.33±4.04	2.33±1.15	1466.67±392.47 ^h	333.33±83.27	160.0±30.0	96.67±41.63
	F	5.33±3.21	3.0±2.64	760.0±70.0 ^h	227.33±107.15	140.0±26.46	100.0±20.0
Shoulder	M	5.0±2.64	1.33±0.58	1553.33±690.39	376.67±45.09	176.67±25.17	101.67±7.64
	F	2.0±0.0	1.67±0.58	1766.67±438.22	360.0±87.18	163.33±51.32	110.0±10.0
Hip	M	4.33±2.52 ^d	0.67±1.15 ^e	1173.33±145.72 ⁱ	256.67±60.28	126.67±25.17	83.33±11.55
	F	12.0±4.0 ^d	9.67±1.53 ^e	906.67±51.32 ⁱ	290.0±52.91	161.67±2.89	71.67±16.07

Same small letters within a column differ significantly ($p < 0.05$).

Table 2 Mean values of NS/P, HS/P, WS/P, WPH and WSH in different skin areas of male (M) and female (F) Bakhtiari sheep (mean±SD).

Region		Indices				
		NS/P	HS/P	WS/P	WPH	WSH
Belly	M	1.89±1.17	0.31±0.07	0.58±0.19	70.0±10.0	40.0±10.0
	F	1.22±1.57	0.27±0.08	0.54±0.06	70.0±10.0	36.67±11.55
Neck	M	0.50±0.19	0.34±0.10	0.54±0.06	86.67±15.27	71.67±7.64 ^d
	F	0.30±0.15	0.31±0.30	0.50±0.06	108.33±7.64	35.0±8.66 ^d
Leg	M	0.25±0.31	0.39±0.12	0.60±0.19	170.0±17.32	88.33±20.21
	F	1.26±1.52	0.33±0.17	0.38±0.16	123.33±37.86	50.0±26.46
Rump	M	0.53±0.10	0.35±0.15	0.66±0.36	66.67±11.55	256.67±70.94 ^e
	F	0.51±0.72	0.34±0.11	0.67±0.34	95.0±31.22	98.0±26.51 ^e
Flank	M	0.98±0.48	0.33±0.05	0.51±0.09	83.33±5.77 ^c	20.0±0.0
	F	1.58±1.25	0.29±0.09	0.38±0.20	53.33±11.55 ^c	33.33±11.55
Forearm	M	0.35±0.22	0.25±0.13	0.63±0.35	80.0±36.05	60.0±30.0
	F	0.76±0.70	0.30±0.14	0.73±0.23	61.67±24.66	53.33±13.32
Shoulder	M	0.32±0.18	0.28±0.12	0.58±0.07	75.0±13.23	63.33±15.27
	F	0.83±0.29	0.21±0.05	0.72±0.25	85.0±15.0	67.0±3.46
Hip	M	0.17±0.29 ^a	0.22±0.05	0.66±0.05 ^b	96.67±15.27	42.33±18.61
	F	0.85±0.20 ^a	0.32±0.08	0.44±0.09 ^b	111.67±10.41	40.67±1.15

Same small letters within a column differ significantly ($p < 0.05$).

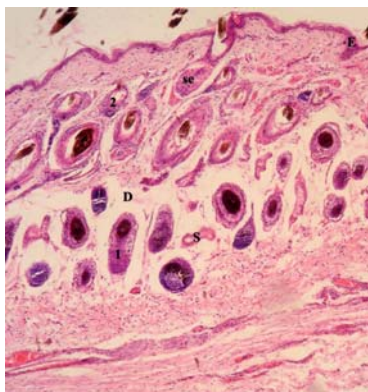


Figure 1 The hip skin of *Bakhtiari* ewes aged 1-2 years. Epidermis (E), dermis (D), sebaceous glands (se), sweat glands (S), primary (1) and secondary hair follicles (2). H&E, x40.

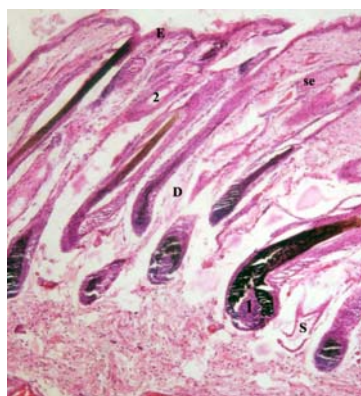


Figure 2 The flank skin of *Bakhtiari* rams aged 1-2 years. Epidermis (E), dermis (D), sebaceous glands (se), sweat glands (S), primary (1) and secondary hair follicles (2). H&E, x40.

reported in Lori (Abbasi et al., 2008), Merino (Andrews et al., 1998) and Madras red (Mir Shabir et al., 2011). Yeruham et al. (1997) concluded that the gender-related changes of skin were attributed to endogenous androgen stimulation at puberty.



Figure 3 The neck skin of *Bakhtiari* rams aged 1-2 years. Epidermis (E), dermis (D), sebaceous glands (se), sweat glands (S), primary (1) and secondary hair follicles (2). H&E, x100.

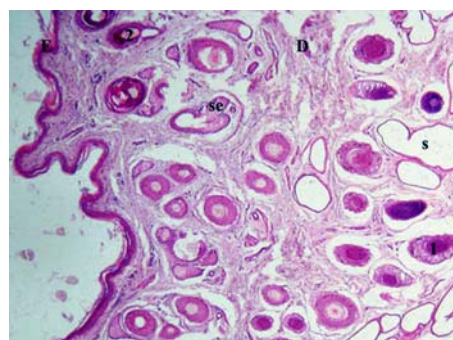


Figure 4 The rump skin of *Bakhtiari* rams aged 1-2 years. Epidermis (E), dermis (D), sebaceous glands (se), sweat glands (S), primary (1) and secondary hair follicles (2). H&E x 40.

The means of NP and NS per one mm² were varied among areas of skin in *Bakhtiari* sheep (2-12 and 0.67-9.67, respectively). These findings were similar to those of Abbasi et al. (2008) in Lori (3.22 and 7.63, respectively), Kocamis and Aslan (2004) in Tuj breed (7.4-8.64 and 37.1-40.32, respectively), and Kurtdele and Asti (1999) in German Black Head, Hampshire Down, Lincoln Longwool, White Karaman, Awassi and Konya Merino (26-38, 24-25, 20-38, 15-36, 13-16 and 44-72, respectively).

The NP and NS values in flank skin in *Bakhtiari* sheep were 8-8.33 and 8-9.67 respectively (Table 1), while in other Iranian sheep breeds were, 3.2-3.6 and 12.1-13.8 (Ansari-Renani et al., 2011). In other sheep breeds such as Lori (Abbasi et al., 2008), Duben, Kotel, Stranja, Sakar and Central Rodopi (Raichev and Khristova, 1990), and Omani native (Mahgoub et al., 2010), the NP and NS values were 3.13 and 7.3, 3.3 and 16.6, 2.9 and 17.1, 3.6 and 12.5, 3.2 and 12.9, 2.5 and 10.7, 22.9 and 12.8, respectively. When the NP and NS values in shoulder area of skin in *Bakhtiari* sheep (2-5 and 1.33-1.67, respectively) were compared with those in Lori (3.22 and 7.42, respectively), it was determined that the *Bakhtiari* sheep had lesser numbers of secondary follicles in this skin area than Lori sheep (Abbasi et al., 2008). Genkovski and Gerchev (2007) reported that in Tsigai ewes, the NP of shoulder area ranged from 3.64-4.15, which was much higher than *Bakhtiari* ewes (2.0).

In the present study, the WP and WS values in shoulder area of skin in ewes were 163.33 and 110 μ m respectively, but in Tsigai ewes ranged from 104.2-119.4 μ m and 74.7-81.0 μ m, respectively (Genkovski and Gerchev, 2007). Also, these values in flank skin of ewes were 153.33 and 51.67 μ m respectively, but in Duben, Kotel, Stranja, Sakar and Central Rodopi ewes were 113.4 and 86.4 μ m, 140.9 and 79.4 μ m, 145.6 and 76.9 μ m, 148.6 and 82.7 μ m and 134.6 and 80.6 μ m, respectively (Raichev and Khristova, 1990).

The results showed that the least HP (760 μ m) and HS values (227.33 μ m) were recorded in forearm area of skin. The maximum HP (1766.67 μ m) and HS values (670 μ m) were recorded in shoulder and leg area, respectively. Mir Shabir et al. (2011) stated that in Madras red sheep, the minimum and maximum depths of primary follicles (784.33 and

1935.0 μm , respectively) were in the neck dorsal and ventral region, respectively. The HP (1766.67 μm) and HS values (360 μm) in the shoulder skin in *Bakhtiari* ewes were 1553.33 μm and 450.0 μm , respectively, but in Tsigai ewes ranged from 1695.4 to 1735.8 μm and 1095.0 to 1142.0 μm , respectively (Genkovski and Gerchev, 2007).

The WPH value in this study ranged from 53.33-123.33 μm , whereas the WSH value ranged from 20-256.67 μm (Table 2). This value in Omani native ewes was 45.9 μm (Mahgoub et al., 2010). The WPH between species can vary from 10-250 μm and are influenced by the metabolic and nutritional state of the animal (Jones, 2001).

The present study showed that in *Bakhtiari* sheep, the mean NS/P ratio was 0.17 to 1.89, which in other Iranian sheep breeds were 3.7- 3.5 (Ansari-Renani et al., 2011). In some other sheep breeds such as Omani native (Mahgoub et al., 2010), Barki, Sannen, Togenburg, Lori (Abbasi et al., 2008), and Merino hybrids (Andrews et al., 1998), the mean NS/P ratios were 0.6, 2.4, 3.9, 2.4, 2.26 and 4.8, respectively. The highest NS/P was found in Merino sheep, which mounted to 16.5 (Gifford et al., 1995). In comparison of the NS/P ratio of flank skin in *Bakhtiari* ewes (1.58) with those of Raichev and Khristova (1990) in Duben (5.2), Kotel (4.8), Stranja (4.2), Sakar (3.9) and Central Rodopi (4.2), it was seen that the NS/P ratio was lower in *Bakhtiari* ewes. When the NS/P ratio of the shoulder skin in *Bakhtiari* ewes (0.83) were compared with that in Tsigai ewes (1.36-1.47), it was found that the NS/P ratio in the shoulder skin of *Bakhtiari* sheep was lesser than Tsigai sheep (Genkovski and Gerchev, 2007).

It is well established that the quality of wool depends on NS/P ratio as higher ratio refers to better quality for the wool (Hynd, 1995) and this ratio is hereditary. However, it can also be influenced by diet considerably (Gifford et al., 1995). In conclusion, the lower NS/P ratio showed that the wool quality in *Bakhtiari* sheep, like other Iranian breeds (Ansari-Renani et al., 2011), could be classified as carpet wool breeds.

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