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Article

Age-Related Variations in the Population of Active Secondary Hair Follicles, Oxidative Stress and Antioxidant Parameters in Cashmere Goats

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Simple Summary: Previous research has demonstrated that the cashmere production of cashmere goats varies with age. The differences in yield, length, and fineness among different age groups are not proportional, indicating variations in cashmere density, specifically pertaining to the population of active secondary hair follicles. This study investigated age-related variations in cashmere production and the population of active secondary hair follicles in cashmere goats across different age groups, while also exploring the correlation between secondary hair follicle activity and oxidative stress. Results revealed significant age-related variations in cashmere production (yield, staple length, and diameter) as well as the population of active secondary hair follicles among cashmere goats aged 2 to 7 years old. The present study also revealed age-related variations in skin antioxidant capacity and oxidative damage in this age group. Specifically, the younger subgroup (aged 2-4 years old) exhibited significantly higher levels of cashmere production, population of active secondary hair follicles, and antioxidant capacity compared to those aged 5-7 years old. Furthermore, there were significant positive correlations between skin antioxidant capacity and the population of active secondary hair follicles. This study introduces a novel approach for enhancing the activity of secondary hair follicles and improving cashmere production through the regulation of oxidative stress.

Abstract: The objective of this study was to investigate age-related changes in cashmere production and the population of active secondary hair follicles in cashmere goats across different age groups, as well as to explore the association between secondary hair follicle activity and oxidative stress. A total of 104 adult Inner Mongolian ewe goats, aged between 2 and 7 years old, were randomly selected as experimental subjects. Skin samples were collected in August 2020 and cashmere samples were collected in April 2021. The cashmere fiber yield, staple length, and diameter showed age-related variations in cashmere goats aged 2 to 7 years ($P < 0.05$). Cashmere production was higher in goats aged 2-4 years compared to those aged 5-7 years ($P < 0.05$). There were no significant differences in the population of primary and secondary hair follicles among goats aged 2 to 7 years. However, the population of active secondary hair follicles varied significantly with age, with the younger group (aged 2-4 years) having a higher population than those aged 5-7 years ($P < 0.05$). A moderate negative correlation was observed between cashmere fiber diameter and the population of active secondary hair follicles ($P < 0.05$). Age-related variations in skin antioxidant capacity and oxidative damage were observed among cashmere goats aged 2 to 7 years old ($P < 0.05$). Goats aged 2 to 4 years exhibited higher antioxidant capacity and lower oxidative damage ($P < 0.05$). Interestingly, the skin antioxidant capacity and oxidative damage exhibited significant positive and negative correlations, respectively, with the population of active secondary hair follicles ($P < 0.05$). This study presents a novel approach to enhance the activity of secondary hair follicles and improve cashmere production performance through the regulation of oxidative stress.

Keywords: cashmere goat; cashmere production; secondary hair follicle; age; oxidative stress

1. Introduction

The production of cashmere fiber, derived from the secondary hair follicles in the skin of cashmere goats, is crucial in the textile industry and plays a pivotal role in manufacturing high-quality textiles. The quality and economic value of cashmere fiber primarily depend on its length and fineness, with particular emphasis on the latter. Various factors, including genetic factors [1] and non-genetic factors [2] such as nutrition, environment, sex, type of birth, age etc., influence both the yield and quality of cashmere fiber. Therefore, it is of great significance to investigate these influencing factors to enhance both aspects.

The age-related variations in cashmere yield and quality have been extensively studied to assess individual lifetime productivity and optimize flock age structure for enhanced overall productivity. Significant variations were observed in the cashmere yield, length, and fineness of Inner Mongolian cashmere goats aged 1 to 8 years across different age groups [3, 4]. A noticeable decline was observed from the age of 4 or 5 years old onwards, resulting in a substantial decrease in both quantity and quality [3, 4]. Similarly, Shanbei white cashmere goats exhibited significant age-related variations in the yield and quality of their cashmere fiber, with a noticeable decline starting at the age of 4 years old [5, 6]. The differences in cashmere yield, staple length, and fineness between 2-year-old and 5-year-old Shanbei white cashmere goats were found to be 17.47%, 20.00%, and 12.76% respectively [5]. Similarly, the variations in cashmere yield, staple length, and fineness between 2-year-old and 6-year-old Inner Mongolian cashmere goats were observed to be 13.13%, 9.18%, and 3.12% respectively [7]. The cashmere yield is determined by the length, fineness, and density of fiber. However, it should be noted that the variations in cashmere length, fineness, and yield among different age groups of cashmere goats are not directly proportional. This suggests differences in cashmere density, indicating varying populations of active secondary hair follicles. Additionally, previous studies have found a positive correlation between the population of secondary hair follicles in cashmere goats and cashmere yield, as well as a negative correlation with cashmere fineness [8-10]. However, the relationship between the population of active secondary hair follicles in cashmere goats and cashmere fineness remains unknown. The investigation of the age-related changes in active secondary hair follicles and their correlation with cashmere fineness can provide a novel approach to enhance cashmere production performance.

The maximum activity of secondary hair follicles in the skin of one-year-old Inner Mongolian cashmere goats, as well as Liaoning cashmere goats, was reported to be 81% [11], 69% [12], and 70% [13] respectively. The peak activity of secondary hair follicles in Inner Mongolian cashmere goats aged 2-4 years was found to be 80% in our previous study [14]. Numerous studies have shown that excessive reactive oxygen species can cause oxidative stress-induced damage, leading to inhibited hair follicle development and reduced follicle activity in both rodents and humans [15, 16]. Previous studies have shown that exogenous melatonin can reactivate dormant secondary hair follicles, restoring their activity and increasing the population of active ones [14, 17], indicating a potential relationship between oxidative stress and the activity of secondary hair follicles. However, the relationship between the activity of secondary hair follicles in cashmere goats and skin oxidative stress remains elusive. Therefore, we hypothesized that the activity of secondary hair follicles in cashmere goats undergoes age-related alterations and is correlated with oxidative stress. In this study, we conducted an analysis of age-related variations in cashmere production performance, including yield, length, and fineness, across different age groups of goats. Additionally, the investigation examined the population of active secondary hair follicles in goat skin along with the activity of skin antioxidant enzymes and oxidative stress damage. Furthermore, this study explored the relationship between the population of active secondary hair follicles and antioxidant enzyme activity as well as oxidative stress.

2. Materials and Methods

The experimental procedures were approved by the Institutional Animal Care and Use Committee of Shanxi Agricultural University (Taigu, China) under permission number SXAU-EAW-2021G. VQ.0080050143. The experiment was conducted at YiWei White Cashmere Goat Farm located in Inner Mongolia Autonomous Region, China (39° 06' N, 107° 59' E).

2.1. Animals, Experimental design, and Management

A total of 104 adult ewe goats were randomly selected from a flock of 220 Inner Mongolian cashmere goats, including 16 two-year-olds, 16 three-year-olds, 18 four-year-olds, 20 five-year-olds, and 17 each of six-year-olds and seven-year-olds. The animals grazed in the pasture from 0800 to 1700 hours and were fed in an open barn from 1800 to 0700 hours year-round. The animals were bred in October, gave birth in March, and separated from their offspring in July. The animals were given additional supplementary concentrates from January to June to meet their nutrient requirements during gestation and lactation. The amount of supplementary feeding gradually increased from 0.275 kg/day per goat in January to 0.40 kg/day in April, and further increased to 0.55 kg/day in May and June. The supplementary feed was obtained from Baotou Jiuzhoudadi Biotech Company (Baotou, China) and consisted of a 70% corn and 30% concentrate mixture.

2.2. Sample Collection

In August 2020, two skin biopsies were obtained from the right mid-side flank region using a 1 cm trephine, with the sampling positions of the two skins in close proximity to each other. The skin sample was placed onto tissue processing cassettes for tissue fixation, while another sample was frozen in liquid nitrogen and stored in a -80°C freezer for tissue homogenization. After fixation in a 4% neutral formalin solution for 24 hours, the skin samples were dehydrated using varying concentrations of ethanol and then embedded in paraffin for transverse sectioning. Fleece samples were collected from the left mid-side flank region of each goat within a 10 cm × 10 cm area to assess fiber staple length and diameter after shearing in April 2021. The cashmere weight of each goat was recorded following combing in April 2021.

2.3. Measurements of Cashmere Fiber Performance

The cashmere fibers were manually separated from fleece samples containing a mixture of coarse hairs and cashmere fibers before washing. They were then washed with carbon tetrachloride and distilled water, followed by air drying in a draught cupboard. The staple length of cashmere fibers was determined using the ruler method, following the established protocol outlined by Yang et al [18]. One hundred individual fibers were measured to determine their staple length in each sample. The diameter of cashmere fibers was determined using an optical microscopic projection technique outlined by Peterson and Gherardi [19]. In each sample, the diameter of 200 individual fibers was quantified using an Optic Fiber Diameter Analyzer (CU-6, Beijing United Vision Technical Company, Beijing, China).

2.4. Calculations of Hair Follicle Population

The skin cross-sectional samples were prepared following the protocol described by Yang et al [14]. In brief, a Leica RM 2235 rotary microtome (Leica, Germany) was used to obtain 5 µm thick serial sections parallel to the skin surface while the structure of hair follicles was continuously observed and documented. Transverse section collection continued until 5-10 primary follicles accompanied by sebaceous glands became visible. The skin sections were stained using a modified Saccic method, following Nixon's protocol [20]. Hair follicle sections were captured using a microscope camera (Leica ICC 50W, Leica, Germany), and the quantification of follicles was done by capturing ten microscopic fields per sample. The hair follicles in each image were counted within the designated area, excluding those that were cut on the bottom and right edges. The primary and secondary hair follicles can be distinguished by their size, spatial arrangement, and presence of

accessory structures. In summary, the primary hair follicles are larger than the secondary ones. Within a group of follicles, the primary ones are linearly arranged on one side with the secondary ones surrounding them on the opposite side. Additionally, sebaceous glands and arrector pili muscles accompany the primary hair follicles. Hair follicles, both active and inactive, were quantified separately using the methodology described by Nixon [20]. Active follicles were defined as those with a fiber or a fiber canal, while inactive follicles lacked a fiber canal or were filled with root sheath cells. In addition to the S:P ratio, indicators such as the density index (DI) and number (FN) were calculated to assess follicle population. Consequently, primary hair follicle-related indicators included the primary follicle density index (PFDI) and number (PFN), while secondary hair follicle-related indicators included secondary follicle density index (SFDI), number (SFN), and S:P ratio. The present study does not calculate indicators of active primary hair follicle, as the focus is not on primary hair follicles and coarse hairs, which have asynchronous activity. Indicators related to the population of active secondary hair follicles include ASFDI (active secondary follicle density index), ASFN (active secondary follicle number), *Sf:P* (ratio of active secondary follicles to primary follicles), and PASF (percentage of active secondary follicles). The typical structure of hair follicles in the skin of cashmere goats is depicted in Figure 1.



Figure 1. The typical structure of hair follicles in the skin of cashmere goats (10×). → This symbol indicates the primary hair follicle; ---▶ this symbol indicates the inactive secondary hair follicle; ----▶ this symbol indicates the active secondary hair follicle.

2.5. Statistical Analysis

The statistical analyses were conducted using SAS 9.2 software from SAS Institute Inc (Cary, NC, USA). The cashmere production performance of cashmere goats aged 2 to 7 years old, including yield, staple length, and diameter, was analyzed using one-way ANOVA. Similarly, various indicators related to primary hair follicle population (PFDI, PFN), secondary hair follicle population (SFDI, SFN, S:P), active secondary hair follicle population (ASFDI, ASFN, *Sf:P*, PASF), as well as serum antioxidant capacity and oxidative damage were evaluated using one-way ANOVA. The relationship between cashmere fiber diameter and indicators associated with primary hair follicle population (PFDI, PFN), secondary hair follicle population (SFDI, SFN, S:P), and active secondary hair follicle population (ASFDI, ASFN, *Sf:P*, PASF) was examined using the CORR procedure of Pearson correlation analysis. Additionally, Pearson correlation analysis was performed to investigate the relationship between indicators of active secondary hair follicle population and antioxidant capacity as well as oxidative damage. Correlation coefficients were interpreted based on these criteria: $|r| \geq 0.5$ indicated a strong correlation; $0.3 \leq |r| < 0.5$ indicated a moderate correlation; $0.1 \leq |r| < 0.3$ indicated a weak correlation; and $|r| < 0.1$ indicated no correlation [21]. The data are presented as mean \pm standard deviation (mean \pm SD). Statistical significance was set at $P < 0.05$ for significance and $P < 0.01$ for extreme significance.

3. Results

3.1. Cashmere Fiber Yield, Staple Length, and Diameter in Cashmere Goats Aged 2 to 7 Years Old

The cashmere fiber yield, staple length, and diameter in cashmere goats aged 2 to 7 years old exhibited significant age-related variations ($P < 0.05$; Figure 2). There was no statistically significant difference observed in the cashmere production performance of 2-4-year-old goats ($P > 0.05$; Figure 2). Similarly, the cashmere production performance of 5-7-year-old goats did not show any statistically significant difference ($P > 0.05$; Figure 2). However, there was a significant difference between the cashmere production performance of goats aged 2-4 years and those aged 5-7 years ($P < 0.05$). The cashmere yield of 2-4-year-old goats was found to be higher by 23.23% (760.7 ± 108.6 g vs 617.3 ± 88.1 g), while their staple length was observed to be longer by 15.10% (8.92 ± 1.00 cm vs 7.75 ± 0.51 cm) compared to that of the older ones aged 5-7-year-old respectively. The cashmere diameter of younger goats (aged 2-4-year-old) decreased by about 4.61% compared with that of older ones (aged 5-7-year-old) (14.91 ± 0.72 μ m vs 15.63 ± 0.80 μ m).

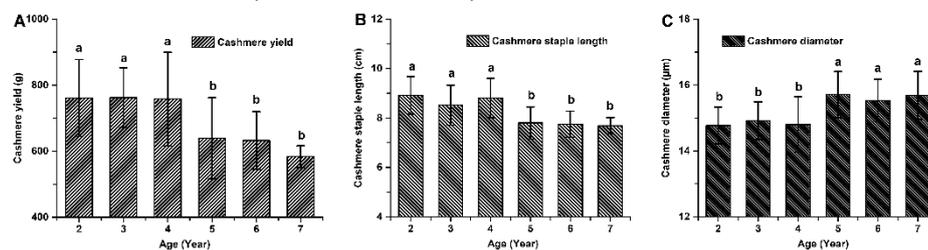


Figure 2. Cashmere production performance in cashmere goats aged 2 to 7 years old. (A) cashmere yield. (B) cashmere staple length. (C) cashmere diameter.

3.2. Population of Active Secondary Hair Follicles in Cashmere Goats Aged 2 to 7 Years Old

The indicators associated with the primary hair follicle population (PFDI and PFN), showed no significant differences among cashmere goats aged 2 to 7 years old ($P > 0.05$; Figure 3). The average values of PFDI and PFN in cashmere goats aged 2 to 7 years old were recorded as 35.87 ± 4.36 and 3.39 ± 0.43 respectively. Similarly, there were no significant differences observed in the indicators associated with the secondary hair follicle population (SFDI, SFN, and S:P) ($P > 0.05$; Figure 4). Additionally, the mean values of SFDI, SFN, and S:P in cashmere goats aged between 2 to 7 years old were determined as follows: SFDI, 449.35 ± 56.50 ; SFN, (42.31 ± 3.67) million; S:P, 12.55 ± 1.80 . The indicators associated with the active secondary hair follicle population, including ASFDI, ASFN, PASF and Sf:P, showed significant variations ($P < 0.05$; Figure 5). The ASFDI of cashmere goats aged 2-4 years was found to be 18.23% higher (319.35 ± 34.46 vs 270.10 ± 41.99) compared to that of cashmere goats aged 5-7 years ($P < 0.05$). Additionally, the younger age group (aged 2-4 years old) exhibited a significantly higher ASFN and PASF by 22.38% (31.06 ± 3.73 vs 25.38 ± 4.01) and by 20.15% (82.04 ± 5.00 vs 68.28 ± 5.81), respectively ($P < 0.05$). Furthermore, the Sf:P ratio in cashmere goats aged 2-4 years was 32.47% higher (9.22 ± 1.21 vs 6.96 ± 0.77) compared to those aged 5-7 years ($P < 0.05$).

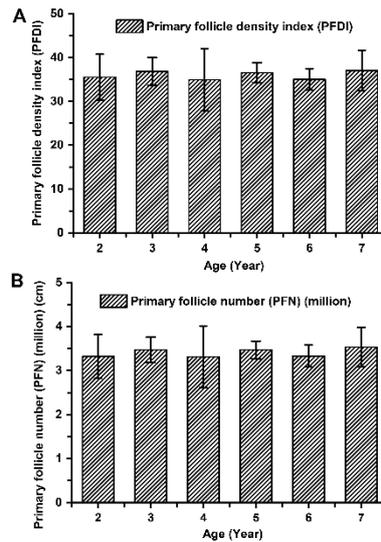


Figure 3. The population of primary hair follicles among cashmere goats aged 2 to 7 years. (A) Primary follicle density index among cashmere goats aged 2 to 7 years. (B) Primary follicle number among cashmere goats aged 2 to 7 years.

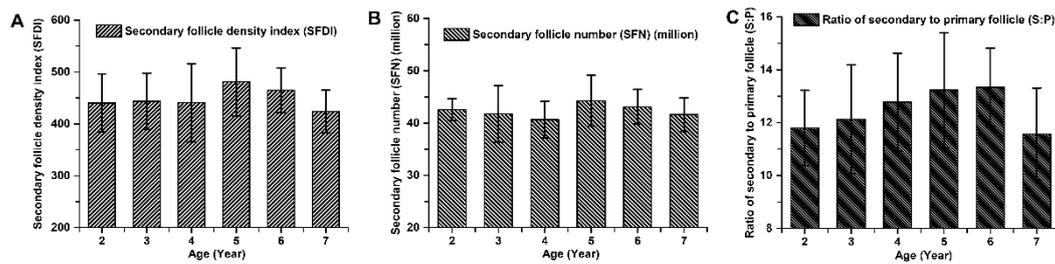


Figure 4. The population of secondary hair follicles among cashmere goats aged 2 to 7 years. (A) Secondary follicle density index among cashmere goats aged 2 to 7 years. (B) Secondary follicle number among cashmere goats aged 2 to 7 years. (C) Ratio of secondary to primary follicle among cashmere goats aged 2 to 7 years.

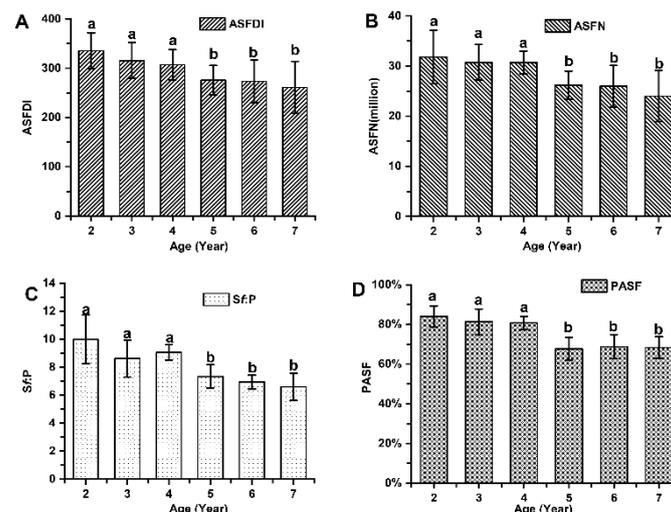


Figure 5. The population of active secondary hair follicles among cashmere goats aged 2 to 7 years. (A) Active secondary follicle density index among cashmere goats aged 2 to 7 years. (B) Active secondary follicle number among cashmere goats aged 2 to 7 years. (C) Ratio of active secondary to primary follicle among cashmere goats aged 2 to 7 years. (D) Ratio of active secondary to primary follicle among cashmere goats aged 2 to 7 years.

primary follicle among cashmere goats aged 2 to 7 years. (D) Percentage of active secondary follicle among cashmere goats aged 2 to 7 years.

3.3. Relationship between Population of Primary and Secondary Hair Follicles and Cashmere Fiber Diameter in Cashmere Goats Aged 2 to 7 Years

In the present study, there were no significant correlations between cashmere fiber diameter and indicators of primary hair follicle population (PFDI and PFN) in cashmere goats aged 2 to 7 years old ($P > 0.05$; Table 1). However, a significant negative moderate correlation was found between cashmere fiber diameter and indicators of secondary hair follicle population (SFDI, SFN, and S:P) in the same age group of cashmere goats ($P < 0.05$; Table 1). Additionally, a significant negative moderate correlation was observed between cashmere fiber diameter and indicators of active secondary hair follicle population (ASFDI, ASFN, and Sf:P) in the same age group of cashmere goats ($P < 0.05$; Table 1).

Table 1. The correlation coefficient among the population of primary follicles, secondary follicles, active secondary follicles, and cashmere diameter.

Item	PFDI	PFN	SFDI	SFN	S:P	ASFDI	ASFN	Sf:P	PASf
Cashmere fiber diameter	0.164	0.155	-0.374	-0.348	-0.330	-0.376	-0.328	-0.370	-0.397
<i>P</i> value	0.283	0.310	0.023	0.032	0.033	0.018	0.036	0.024	0.010

3.4. Serum Antioxidant Capacity and Oxidative Damage in Cashmere Goats Aged 2-7 Years Old

Significant variations in skin antioxidant capacity were observed among cashmere goats aged 2 to 7 years old ($P < 0.05$; Figure 6). Cashmere goats aged 2 to 4 years exhibited significantly higher levels of superoxide dismutase (SOD) by 22.87% (108.53 ± 4.87 vs 88.33 ± 4.76), glutathione peroxidase (GSH-Px) by 10.29% (961.23 ± 31.77 vs 871.52 ± 28.37), and total antioxidant capacity (TAOC) by 14.34% (12.20 ± 0.54 vs 10.67 ± 0.92) compared to those aged 5 to 7 years ($P < 0.05$). Furthermore, significant differences in oxidative damage were found among cashmere goats aged 2 to 7 years old, with the younger age group (2 to 4 years old) showing a remarkable decrease of MDA levels by approximately 16.73% (4.38 ± 0.41 vs 5.26 ± 0.65) compared to the older age group (5 to 7 years old) ($P < 0.05$).

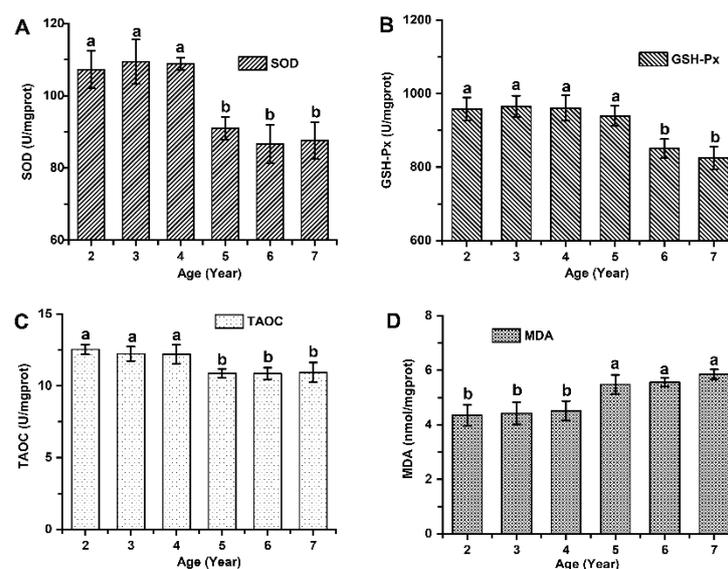


Figure 6. Skin antioxidant capacity and oxidative damage among cashmere goats aged 2 to 7 years old. (A) The activity of SOD enzyme among cashmere goats aged 2 to 7 years old. (B) The activity of

GSH-Px enzyme among cashmere goats aged 2 to 7 years old. (C) The level of TAOC among cashmere goats aged 2 to 7 years old. (D) The content of MDA among cashmere goats aged 2 to 7 years old.

3.5. Association between Antioxidant Capacity and Active Secondary Hair Follicle Population in Cashmere Goats Aged 2 to 7 Years Old

The SOD activity showed significant positive correlations with indicators of active secondary hair follicle population, including ASFDI, ASFN, and Sf:P ratio ($P < 0.05$; Table 2). There was a trend indicating a moderate positive correlation between SOD activity and PASF ($P = 0.051$; Table 2). Additionally, the GSH-Px activity showed significant positive correlations with indicators of active secondary hair follicle population such as ASFDI, ASFN, PASF, and Sf:P ratio ($P < 0.05$; Table 2). Moreover, the TAOC levels demonstrated significant positive correlations with indicators of active secondary hair follicle population like ASFDI, ASFN, and Sf:P ratio ($P < 0.05$; Table 2). Similarly, there were significant negative correlations between MDA content and indicators of active secondary hair follicle population, including ASFDI, ASFN, PASF, and Sf:P ratio ($P < 0.05$; Table 2).

Table 2. The correlation coefficient between the population of active secondary follicles and skin antioxidant capacity and oxidative damage among cashmere goats aged 2 to 7 years old.

Item	ASFDI	ASFN	PASF	Sf:P
SOD	0.462(0.030)	0.432(0.045)	0.421(0.051)	0.594(0.020)
GSH-Px	0.462(0.030)	0.434(0.044)	0.456(0.033)	0.555(0.039)
TAOC	0.412(0.045)	0.489(0.040)	0.378(0.069)	0.556(0.039)
MDA	-0.510(0.013)	-0.490(0.018)	-0.445(0.033)	-0.575(0.010)

Note: P -values are represented in parentheses.

4. Discussion

Previous studies have demonstrated significant variations in cashmere yield, staple length, and fineness among cashmere goats of different ages, indicating differences in cashmere density due to the population of active secondary hair follicles in the skin [3, 7]. Additionally, researches on hair follicle development and growth in mice and humans have demonstrated that the excessive generation of reactive oxygen species (ROS), leading to oxidative stress, plays a crucial role in the progression of hair follicle atrophy, diminished activity, and eventual degeneration [16, 22, 23]. However, the correlation between secondary hair follicle activity in cashmere goats of different ages and antioxidant capacity as well as oxidative stress damage remains unknown. The present study reveals variations in cashmere production performance among different age groups of cashmere goats, with significantly higher performance observed in 2-4-year-old goats compared to those aged 5-7 years. Similar alterations were found across different age groups regarding the population of active secondary hair follicles in their skin. Age-dependent changes were observed for antioxidant enzyme activity with significantly higher levels detected in 2-4-year-old goats compared to those aged 5-7 years. Conversely, age-related changes were observed for MDA levels - a biomarker reflecting oxidative stress-induced damage - exhibiting an inverse pattern. Importantly, correlation analysis results revealed a significant negative correlation between secondary hair follicle activity and skin oxidative stress damage.

The findings of previous studies have demonstrated age-related variations in cashmere yield, staple length, and fineness among cashmere goats of varying ages. The staple length of cashmere fiber in Longdong cashmere goats aged 2-4 years was found to be significantly higher compared to those aged 5-7 years [24]. Similarly, the present study found that the staple length of cashmere fiber in Inner Mongolian cashmere goats aged 2-4 years was 15.10% higher than those aged 5-7 years. Previous studies have demonstrated a significant increase in the cashmere yield of Inner Mongolian cashmere goats aged 2-4 years [3], Shanbei white cashmere goats aged 2-4 years [25], and Longdong cashmere goats aged 2-4 years [24] compared to those aged 5-7 years, while there has been a notable decrease in the diameter of the cashmere fiber. The present study revealed a comparable increase in

cashmere yield, with the cashmere yield of Inner Mongolian cashmere goats aged 2-4 years being 23.23% higher than that of the goats aged 5-7 years. The present study, however, found that the cashmere diameter in Inner Mongolian cashmere goats aged 5-7 years was 4.61% higher than that of goats aged 2-4 years. The diameter of cashmere fibers in Inner Mongolian cashmere goats was reported to undergo a significant increase beyond the age of 5 years [7]. The inconsistent findings regarding the variation in cashmere diameter of cashmere goats at different ages may be attributed to the nutrient level during the period of secondary hair follicle reconstruction. Our previous research showed that cashmere goats with high body weight gain during the period of secondary hair follicle reconstitution exhibited a greater cashmere diameter compared to those with low body weight gain [26]. The cashmere yield depends on the length, diameter, and density of the fiber; however, these factors do not exhibit a proportional relationship across different ages of cashmere goats. This implies that variations in the population of active secondary hair follicles within the skin contribute to disparities in cashmere density among different age groups.

The morphogenesis of secondary hair follicles in the skin of cashmere goats has been confirmed to commence during the fetal stage and reach maturity at 3-6 months postpartum [18, 27, 28]. Upon reaching maturity, the population of secondary hair follicles remains constant, subsequently undergoing a cyclical process comprising catagen, telogen, and anagen [12, 29]. It was reported that the skin S:P ratio remained consistent across different age groups (6 months, 1 year, and 2 years) for both Longdong [30] and Inner Mongolian cashmere goats aged 1-3 years old [27], suggesting a stable population of secondary hair follicles. In addition, the skin S:P ratio of one-year-old Inner Mongolian and Liaoning cashmere goats remains consistent throughout the year without significant variation [13]. In the present study, no statistically significant variations were observed in the population of secondary hair follicles in the skin of cashmere goats across different age groups, as indicated by SFDI, SFN, and S:P. This finding further validates the precision of the slicing technique in this study and establishes a solid foundation for quantitative assessment of active secondary hair follicles. The activity of secondary hair follicles in the skin of cashmere goat has been confirmed to peak during the months of August and September [11, 12, 31]. The activity of secondary hair follicles in the skin of one-year-old Inner Mongolian cashmere goats, as well as Liaoning cashmere goats, was reported to be 81% [11], 69% [12], and 70% [13] respectively. In the present study, the activity of secondary hair follicles in the skin of Inner Mongolian cashmere goats aged 2-7 years ranged from 67.70% to 84.02%. Importantly, the findings of this study revealed significant age-related variations in the population of active secondary hair follicles, as indicated by ASFDI, ASFN, PASF, and S_f:P ratio. The population of active secondary hair follicles in goats aged 2-4 years was significantly higher compared to those aged 5-7 years. The findings of this study demonstrate that not all secondary hair follicles in cashmere goats undergo transition from telogen to anagen, with some secondary hair follicles undergoing atresia, thereby resulting in reduced activity of secondary hair follicles and cashmere yield.

The fineness of cashmere fiber plays a crucial role in determining both the quality and economic value of cashmere. Previous studies have confirmed a negative correlation between the fineness of cashmere fiber and the population of secondary hair follicles in the skin of cashmere goats [9]. Increasing the population of secondary hair follicles is an important approach to reducing the fineness of cashmere fiber [17, 18]. The fineness of cashmere fiber exhibited a significant negative correlation with the skin S:P ratio in one-year-old Shanbei white cashmere goats ($r = -0.434$) [10] and 6-month-old Inner Mongolian cashmere goats ($r = -0.488$) [32]. Similarly, a negative correlation ($r = -0.330$) was observed between the fineness of cashmere fiber and the skin S:P ratio in Inner Mongolian cashmere goats aged 2-7 years in this study. Furthermore, the secondary hair follicle population indicators in Inner Mongolian cashmere goats aged 2-7 years, such as SFDI and SFN, exhibited a negative correlation with the fineness of cashmere fiber ($r = -0.374$ and $r = -0.348$ respectively). Our findings provide further evidence of a negative correlation between the population of secondary hair follicles and the fineness of cashmere fiber, irrespective of the age of cashmere goats. Interestingly, the correlation analysis results of the present study revealed a significant moderate negative correlation between the population of active secondary hair follicles and cashmere fiber diameter.

The findings of the present study suggest that the variation in active secondary hair follicle populations among cashmere goats of different ages contributes partially to variations in cashmere yield, rather than differences in the overall populations of secondary hair follicles. In addition, the findings suggest that, in addition to increasing the population of total secondary hair follicles, enhancing the metabolic activity of secondary hair follicles can increase cashmere yield in individual cashmere goats. Therefore, it is crucial to elucidate the underlying mechanisms of age-related atresia and decline in secondary hair follicle activity in order to implement interventions aimed at enhancing secondary hair follicle activity and cashmere performance.

The hair follicles are frequently exposed to high levels of reactive oxygen species (ROS), causing oxidative stress and subsequent apoptosis in the progenitor cells and dermal papilla cells, ultimately leading to structural impairment [15]. Previous studies demonstrated that the function of reactive oxygen species (ROS) in hair follicles is dualistic: a low level of ROS can stimulate the transition from telogen to anagen, while a high level of ROS can hinder this process and induce regression of hair follicles [22]. The excessive production of reactive oxygen species (ROS) induced by obesity can initiate the activation of inflammatory signals and abnormal accumulation of ROS and lipids in murine hair follicle stem cells, thereby impairing the metabolic activity of hair follicles [33]. During the transition from telogen to early anagen stage, dermal papilla cells release signals that stimulate metabolic activity in hair progenitor cells, promoting proliferation and generating reactive oxygen species (ROS); failure to promptly eliminate these ROS can lead to oxidative stress [16, 34]. In the present study, the antioxidant enzyme activity in the skin of 2-4-year-old cashmere goats was significantly higher than that observed in 5-7-year-old goats, while the MDA content showed a significant decrease compared to the latter group. The findings of this study align with those observed in rodent models, including rats and mice, as well as in avian species such as laying hens. The activity of serum SOD decreased significantly in aged rats compared to young rats, while the MDA content increased significantly [35]. The activity of SOD enzyme in the intervertebral disc homogenate [36] and brain [37] decreased significantly in aged mice compared to the young and adult groups, while the concentration of MDA increased significantly. The activities of SOD enzyme and CAT enzyme in testicular tissue gradually declined from 8 months of age in mice [38]. During the late laying period, there was a notable decrease in laying performance along with decreased levels of GSH content, T-AOC, total superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST) in ovarian tissue of hens [39]. Conversely, MDA content as well as hydrogen peroxide and ROS levels exhibited a significant increase when compared to the initial and peak laying periods [40]. In the present study, the changes in antioxidant enzyme activity and oxidative stress injury in the skin of cashmere goats at different ages were consistent with variations in active secondary hair follicles. The results of correlation analysis revealed significant positive correlations between the indicators of active secondary hair follicle population (ASFDI, ASFN, and S_f:P ratio) and skin antioxidant enzyme activity. Additionally, these indicators showed notable negative associations with skin malondialdehyde (MDA) content. Our findings suggest a preliminary correlation between oxidative stress and diminished activity of secondary hair follicles in cashmere goats. However, the relationship between oxidative stress and the activity of secondary hair follicles in the skin of cashmere goats, as well as the underlying mechanism, requires further investigation.

5. Conclusions

The cashmere fiber yield, staple length, and diameter showed significant age-related variations in cashmere goats aged 2 to 7 years. The population of active secondary hair follicles and skin antioxidant capacity exhibited significant age-related variations among cashmere goats aged 2 to 7 years old, with the younger group (aged 2-4 years) having a significantly higher level than those aged 5 – 7 years. Importantly, antioxidant capacity and oxidative damage exhibited significant positive and negative correlations, respectively, with the population of active secondary hair follicles. This study presents a novel approach to enhance the activity of secondary hair follicles and improve cashmere production performance through the regulation of oxidative stress. Further investigation

is required to explore the relationship between oxidative stress and the activity of secondary hair follicles in the skin of cashmere goats, as well as the underlying mechanism.

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