

# Effects of melatonin administration to cashmere goats on cashmere production and hair follicle characteristics in two consecutive cashmere growth cycles

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## Research

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# Abstract

**Background:** Short-term melatonin treatment of cashmere goats has been shown to increase the quantity and quality of cashmere fibre. However, the long-term effects of melatonin treatment on cashmere production and hair follicle characteristics of these goats has not been reported. Therefore, we investigated changes in cashmere production and in hair follicle populations and their activity in melatonin-treated cashmere goats for two consecutive cashmere growth cycles.

**Methods:** Twenty-four female Inner Mongolian Cashmere goats were randomly allocated to two groups ( $n = 12$ ), one of which received melatonin implants, the other being an untreated control group. Melatonin implants were subcutaneously inserted behind the ear at a dose of 2 mg/kg live weight on two occasions – 30 April and 30 June 2016. Cashmere samples were collected at combing in April of 2017 and 2018. Blood and skin samples were collected monthly between April and September 2016, and during April and September in 2017.

**Results:** Our results showed that melatonin treatment of cashmere goats in one cashmere growth cycle increased ( $P < 0.05$ ) weight, length, density and decreased ( $P < 0.01$ ) diameter of cashmere fibre, however it did not affect cashmere production in the following annual cycle. Melatonin treatment of cashmere goats had no effect on the population of secondary hair follicles for the two consecutive cycles. However, in the first growth cycle following treatment it advanced ( $P < 0.05$ ) the onset of activity of secondary hair follicles by 2 months and it increased ( $P < 0.05$ ) the population of these follicles that were active. Serum melatonin concentrations of the treated goats were elevated, relative to those of the control group ( $P < 0.05$ ), but only during the first growth cycle.

**Conclusions:** In summary, melatonin treatment of cashmere goats in one cashmere growth cycle improved cashmere production for that cycle only, with no residual effects on the following cycle. This makes the technique acceptable to the cashmere goat industry. The improvement in cashmere production following treatment of goats with melatonin appears to involve an acceleration of the annual regeneration of secondary hair follicles and an increased population of active secondary hair follicles in the skin of cashmere goats.

## Background

Cashmere fibre from cashmere goats is produced by skin secondary hair follicles in a sequence of events comprising anagen (active), catagen (quiescent) and telogen (inactive) phases to form a complete secondary hair follicle growth cycle. Cashmere fibre growth is seasonal: growth commences in summer, ceases in winter and the fibres are shed naturally during spring, following which there is a period of growth cessation between spring and summer [1, 2]. Because the value of a cashmere fleece is determined by its weight and by the length and diameter of its fibres, there have been efforts to induce cashmere growth in advance of the normal growth season and during the non-growth period, in order to increase cashmere production and to improve its fibre quality. It has been shown that treatment of adult

cashmere goats with exogenous melatonin prior to or during a cashmere growth cycle can advance the onset of cashmere fibre growth and lead to increased staple length and fibre weight [3–5]. Furthermore, Welch et al. found that melatonin treatment applied in each of two consecutive cashmere growth cycles increased cashmere fibre weight and improved its quality each time [6]. However, it is possible that the stimulatory effects of melatonin treatment on a single cashmere growth cycle may be followed by a compensatory reduction in growth during the subsequent growth cycle. This would mean that there could be no net gain from a single use of melatonin and this creates an uncertainty for farmers that needs to be resolved before the practical application of melatonin treatment can be recommended to the cashmere goat industry. Also, there have been some reports of inconsistent results of melatonin treatment on cashmere fibre weight and staple length, presumably resulting from its effects on secondary hair follicles [3, 5, 7]. Although some of these studies have indicated that melatonin treatments may stimulate secondary hair follicles by accelerating their regeneration from telogen to the anagen phase, it is clear that these and related effects on the population and activity of these follicles require further investigation. Therefore, we hypothesized that melatonin treatment of cashmere goats during one cashmere growth cycle would increase activity of secondary follicles in that growth cycle, thus increasing cashmere production, but would not affect cashmere production in the following growth cycle. In the current study, cashmere production and secondary hair follicle characteristics were evaluated for two consecutive cashmere growth cycles following a melatonin treatment applied in the first cycle. This would enable firm recommendations to be provided to the cashmere goat industry and the detailed histological observations would give further insight into the cellular mechanisms involved in cashmere fibre growth.

## Methods

All experimental procedures in this study were performed in accordance with Guidelines for the Care and Use of Agricultural Animals in Research and Teaching and approved by the Animal Care and Use Committee of China Agricultural University (Beijing, China, permit no. CAU 20150825-2). The experimental goats were reared on a commercial farm (YiWei White Cashmere Goat Farm) located in Inner Mongolia Autonomous Region, China, 39°06'N, 107°59'E, and the study lasted from April 2016 to April 2018.

## Animals, Experimental Design And Management

Twenty-four female Inner Mongolian Cashmere goats were randomly allocated to two groups (n = 12): melatonin-treated (Melatonin) and control (Control) that were balanced for age, live weight and cashmere yield. Capsules containing melatonin were subcutaneously inserted behind an ear in cashmere goats on April 30 and June 30, 2016 at a dosage of 2 mg/kg live weight, which is based on our previous studies [3, 8]. The melatonin implants were purchased from Kangtai Biotechnology Co., Ltd (Beijing, China) and can release melatonin sustainably for two months. During the experimental period, all goats were on pasture from 0800 to 1700 h daily and were housed in an open barn from 1800 to 0700 h under natural photoperiodic conditions. The grassland in the area is part of semi-desertification grassland and included primary vegetation such as *Caragana stenophylla poiark*, *Caragana rorsninskii kom*, *Agriopyron cristutum*

*gaertn*, *Agriopyron cristutum* *schut*, *Alium polyrhizum* *turcz*, *Artemisia frigidi* *willd*, *Artemisia ordosica* *praschen*, *Stipa breviflora* *griseb*, *Haloxylon ammodendron* *bunge*. The additional supplementary concentrates were provided to the goats from January to June, which was 0.275 kg/d per goat in January, gradually increased to 0.4 kg/d in April and subsequently increased to 0.55 kg/d in May and June to meet the needs of the animals during gestation and subsequent lactation. The supplementary concentrate consisted of 70% corn and 30% concentrate feed and was purchased from a local feed company (Baotou Jiuzhoudadi Biotech Company, Baotou, China). The does were mated in October, kidded in March and weaned in July. The experimental cashmere goats were combed on April 30, 2017 and April 30, 2018. The time when melatonin implants were inserted, and the estimated release period of melatonin are shown in Fig. 1.

## Sample Collection

Blood samples were collected one day before melatonin treatment and then were collected monthly on May, June, July, August, September 2016. In addition, blood samples were collected on April 30 and September 30, 2017. Blood samples were taken from a jugular vein of each goat via plain vacutainer tubes under low red-light conditions (< 3 lux) at night between 2300 and 2400 h. The blood samples were allowed to coagulate at room temperature before centrifugation at 3000 × g for 20 min. Serum was collected and stored at – 20 °C until analysis of melatonin concentrations. Total cashmere fleece weight of each goat was recorded at combing in April 2017 and April 2018. Before combing, cashmere samples within an area of 10 cm × 10 cm were shorn from the left midside of each goat close to the skin for the measurement of fibre staple length and diameter. Skin biopsies of each goat were obtained from the right midside flank region one day before melatonin treatment and then were collected monthly on May, June, July, August, September 2016. In addition, skin biopsies were collected on April and September 2017. Skin biopsies were excised as described by Duan [3] for analysis of hair follicle parameters.

## Hormone Analysis

Serum melatonin concentration was determined in duplicate by radioimmunoassay using a commercial RIA kit (BAR-3300, LDN, Nordhorn, Germany) which had a sensitivity of 2.3 pg/ml and intra- and inter-assay CVs of 9.7%~13.4% and 8.0%~13.3%, respectively.

## Cashmere Fibre Measurements

Cashmere fibre staple length was measured with a ruler as described by Yang [8] and cashmere fibre diameter was measured by an optical microscopic projection method as described by Duan [3]. Cashmere fibre density was determined by measuring the active secondary hair follicle density in the skin obtained on September when the active secondary hair follicle numbers reach their seasonal maximum and agree with the cashmere fibre numbers.

# Hair Follicle Parameters

Skin samples were embedded in paraffin after dehydration via a series gradient of ethanol solutions, and sections were sliced into three 5  $\mu\text{m}$  sections and then stained by a modified Saccpic method [9]. The sub-epidermal level immediately below the sebaceous glands was selected for carrying out microscopical observations. In this study, primary hair follicles and secondary hair follicles were evaluated separately. They are easily distinguished from each other based on their specific characteristics as described by Yang [8]. Hair follicles were classed as active or inactive according to their size, shape, staining and the presence or absence of an inner root sheath. The basic criterion for distinguishing an active from an inactive follicle is the presence of a fibre and inner root sheath cells. In an active follicle stained with the Saccpic technique a yellow stained fibre is surrounded by a distinct bright red-stained inner root sheath, whereas non-active follicles were identified by the absence of these features and the presence of a quiescent hair germ or a fibre with a brush end.

## Statistical analysis

All statistical analysis was performed with SAS statistical software (version 9.2; SAS Institute Inc., Cary, NC, USA). The significance of differences in cashmere production (cashmere weight, fibre staple length, fibre diameter and fibre density) between melatonin-treated and control cashmere goats was analyzed by the independent sample *t*-tests procedure. Statistical analysis of melatonin concentrations and hair follicle parameters (follicle density, ratio of secondary to primary follicles, percentage of active hair follicles) were performed with the repeated measures ANOVA procedure, and data are presented as least squares means  $\pm$  SEM. Melatonin treatment was between-subject effect and time was within-subject effect. The difference was considered significant at  $P < 0.05$  and a tendency was declared at  $0.05 < P < 0.10$ .

## Results

### Cashmere weight and cashmere fibre characteristics in two successive cashmere growth cycles

Cashmere production of melatonin-treated goats obtained at the April 2017 combing was higher than that of the control group ( $P < 0.05$  or  $P < 0.01$ ; Table 1). Melatonin-treated goats had a mean fleece weight that was 121 g higher, which is an 18% increase ( $P = 0.04$ ) over that of the control group. The mean cashmere fibre staple length of melatonin-treated goats at the April 2017 combing was 1.57 cm longer ( $P = 0.01$ ) than that of the control goats. On this date, mean cashmere fibre diameter of the Melatonin goats was 0.81  $\mu\text{m}$  finer ( $P < 0.01$ ) and their cashmere fibre density was 15% greater ( $P < 0.05$ ) than those of the Control group. However, at the April 2018 combing there was no difference in cashmere production performance between the two groups ( $P > 0.1$ , Table 1).

Table 1

Effect of melatonin administration to cashmere goats in one cashmere growth cycle on cashmere production for two consecutive cashmere growth cycles

Cashmere production	Treatment		<i>P</i> -value
	Control	Melatonin	
Cashmere yield, g			
April 2017	671 ± 138	792 ± 125	0.04
April 2018	588 ± 62	605 ± 91	0.65
Cashmere staple length, cm			
April 2017	8.79 ± 1.43	10.36 ± 0.84	0.01
April 2018	8.84 ± 0.94	8.45 ± 0.80	0.40
Cashmere diameter, μm			
April 2017	14.82 ± 0.47	14.01 ± 0.60	< 0.01
April 2018	14.73 ± 0.57	14.75 ± 0.55	0.95
Cashmere density, n/mm <sup>2</sup>			
April 2017	27.08 ± 2.38	31.21 ± 4.09	0.03
April 2018	30.72 ± 1.55	30.37 ± 1.00	0.85

## Populations of primary and secondary hair follicles in two successive cashmere growth cycles

In this study, the hair follicle density (number of follicles per square millimeter of skin) and S:P (ratio of secondary hair follicle to primary hair follicle) were used as an indicator of the population of hair follicles in the skin of cashmere goats. There was no time-related change ( $P > 0.05$ ) in primary or secondary hair follicle density throughout the two successive cashmere growth cycles, averaging  $(2.56 \pm 0.03)/\text{mm}^2$  and  $(30.14 \pm 0.25)/\text{mm}^2$ , respectively (Table 2). Similarly, there was no effect of time ( $P > 0.05$ ) on S:P throughout the two successive cashmere growth cycles, averaging  $11.99 \pm 0.16$  (Table 2). In addition, there was no treatment-related difference ( $P > 0.05$ ) in primary hair follicle density, secondary hair follicle density and S:P throughout the two successive cashmere growth cycles (Table 2; Fig. 2).

Table 2

Effect of melatonin administration to cashmere goats in one cashmere growth cycle on primary and secondary hair follicle numbers for two consecutive cashmere growth cycles

Parameter <sup>1</sup>	Treatment		SEM <sup>2</sup>	<i>P</i> -value		
	Control	Melatonin		Treatment	Time	Treatment × Time
PFD	2.62	2.49	0.05	0.39	0.37	0.87
SFD	30.16	30.11	0.50	0.47	1.00	0.44
S:P	11.80	12.19	0.23	0.58	0.12	0.61

<sup>1</sup> Abbreviations: PFD, Primary follicle density; SFD, Secondary follicle density; S:P, ratio of secondary follicle to primary follicle.

<sup>2</sup> Standard error of the least square mean.

## Population of active secondary hair follicles in two successive cashmere growth cycles

In general, the population of active secondary hair follicles showed a similar time-related change in melatonin-treated and control cashmere goats between April and September, which increased with time and had the highest value in September, as indicated by three parameters: active secondary hair follicle density ( $P < 0.01$ ), ratio of active secondary follicles to primary follicles (Sf:P.  $P < 0.01$ ) and percentage of active secondary follicles (Fig. 3). The point of time when the population of active secondary hair follicles was higher than the April value occurred one month earlier for the Melatonin group than for the Control group (Fig. 2; Fig. 3). At the onset of the study, there were no between-group differences in active secondary hair follicle density (Fig. 3A). However, there was a time×treatment interaction ( $P = 0.03$ ) for the active secondary hair follicle density (Fig. 3A). The mean active secondary hair follicle density in the skin of Melatonin goats was higher than that of the Control group on May 2016 (Fig. 2B; Fig. 2b;  $P < 0.01$ ), June 2016 (Fig. 2C; Fig. 2c;  $P < 0.01$ ), July 2016 (Fig. 2D; Fig. 2d;  $P < 0.01$ ), August 2016 (Fig. 2E; Fig. 2e;  $P < 0.05$ ) and September 2016 (Fig. 2F; Fig. 2f;  $P < 0.01$ ) (Fig. 3A). There was no difference in the ratio of active secondary to primary follicles between the Melatonin and Control groups (Fig. 3B) at the beginning of the study. However, there was a time×treatment interaction ( $P = 0.04$ ) for this parameter following melatonin treatment (Fig. 3B), the Sf:P ratio for the Melatonin group being higher than that of the Control group on May 2016 (Fig. 2B; Fig. 2b;  $P < 0.05$ ), June 2016 (Fig. 2C; Fig. 2c;  $P < 0.01$ ), July 2016 (Fig. 2D; Fig. 2d;  $P < 0.05$ ) and September 2016 (Fig. 2F; Fig. 2f;  $P < 0.05$ ) (Fig. 3B). The percentage of active secondary hair follicles in the skin of cashmere goats was similar for Melatonin and Control groups prior to melatonin administration, averaging  $(43.33 \pm 5.36)$  % (mean  $\pm$  SD) (Fig. 3C). However, following melatonin treatment there was also a time×treatment interaction ( $P < 0.01$ ) for this parameter (Fig. 3C), it

being higher for the Melatonin group than the Control group on May 2016 (Fig. 2B; Fig. 2b;  $P < 0.05$ ), June 2016 (Fig. 2C; Fig. 2c;  $P < 0.01$ ), July 2016 (Fig. 2D; Fig. 2d;  $P < 0.01$ ), August 2016 (Fig. 2E; Fig. 2e;  $P < 0.01$ ) (Fig. 3C).

## Serum Melatonin Concentration In Two Successive Cashmere Growth Cycles

In general, serum melatonin concentration showed a similar pattern of seasonal change in both Melatonin and Control groups, with a general increase between April and September (Fig. 4). Prior to melatonin implantation, mean serum melatonin concentrations were not different ( $P > 0.05$ ) between the two groups (Fig. 4). Following melatonin administration, there was a time\*treatment interaction ( $P < 0.01$ ) (Fig. 4) with the Melatonin group having a higher mean serum melatonin concentration than the Control group in May 2016 ( $P = 0.03$ ), June 2016 ( $P < 0.01$ ), July 2016 ( $P < 0.01$ ), August 2016 ( $P < 0.01$ ) and September 2016 ( $P < 0.01$ ) (Fig. 4). However, there was no difference ( $P > 0.1$ ) in mean serum melatonin concentration between the two groups in April and September 2017 (Fig. 4).

## Discussion

It has been shown that treatment of cashmere goats with melatonin during the period when the fleece is not growing can advance the onset of seasonal fleece growth and improve cashmere production in terms of fleece weight and staple length [3, 7, 10]. However, whether such treatment with melatonin in one season has any impact on the growth of the cashmere fleece or effects on population and activity of secondary hair follicles in the subsequent season has not been investigated. Therefore, in this study we have evaluated cashmere production performance and the population and activity of secondary hair follicles for two consecutive cashmere growth cycles following a treatment of cashmere goats with melatonin that was limited to the first growth cycle. The results showed that although melatonin treatment improved cashmere production performance in the immediate growth cycle, there was no residual impact on cashmere production in the subsequent growth cycle. This rules out any potential negative impact of melatonin treatment on cashmere growth cycles in the years subsequent to that when the treatment was applied, thus paving the way for its practical application in the cashmere goat industry.

Other findings of the study provide more insight on the specific effects of melatonin treatment on the biology of secondary hair follicles. Although treatment of cashmere goats with melatonin during the cashmere non-growth period had no effect on the total population of secondary hair follicles, it did accelerate the regeneration process of these follicles from telogen stage through to anagen stage. Importantly, it enhanced their activity and increased the number of the population that were active. These effects resulted in an increase in cashmere fibre weight and staple length, and a decrease in cashmere fibre diameter. Thus, we provide some histological explanation for the beneficial effects of melatonin treatment on cashmere production in these goats.

The magnitude of the stimulatory effect on cashmere fleece weight recorded here (18%) following treatment of cashmere goats with melatonin is modest when compared with responses variously reported as between 20% and 54% in other published studies [3, 5, 11, 12]. However, the increase in cashmere fibre staple length (1.57 cm) that resulted from the melatonin treatment is well within the range of 1.25 ~ 2.02 cm that has been reported from similar studies [3, 11, 12]. Effects of melatonin treatment on cashmere fibre diameter appear to be more inconsistent, ranging from no effect [11–13] to significant reductions, i.e. 0.6  $\mu\text{m}$  [3], 0.70  $\mu\text{m}$  [5] and 0.81  $\mu\text{m}$  in the present study. It is likely that these inconsistencies, especially for cashmere fibre diameter, resulting from studies involving use of melatonin in cashmere goats are related to factors such as nutrition level, age and genetic background of the goats [14, 15]. The effects of melatonin treatment on cashmere fleece weight, or even from elevation of endogenous melatonin by use of artificial lighting [16], result from an increased population of active fibre-producing follicles. This accords with the present finding of increased numbers and activity of active of cashmere-bearing secondary hair follicles.

In the current study, the population of primary hair follicles in the skin was the same for both melatonin-treated and control cashmere goats and did not change over two consecutive cashmere growth cycles. This helps to confirm the view that the population of primary hair follicles in cashmere goats is established at birth and is not affected subsequently by treatment of adult cashmere goats with exogenous melatonin [8, 17–19]. In the case of secondary hair follicles, previous studies have also shown no effect of melatonin treatment on their total population, and thus no effect on S:P ratio [20, 21]. This finding was strengthened by the present study which confirmed this to be the case for two consecutive growth cycles. However, in the case of postnatal cashmere goats we have shown previously that treatment of these animals with melatonin does increase the number of secondary hair follicles and raises the S:P ratio [8]. This difference between postnatal animals and adults relates to the development of secondary hair follicle population in the skin. Morphogenesis of secondary hair follicles is initiated during embryonic development and their final population is not established until they mature and commence formation of cashmere fibre at about 3 to 6 months after birth [8, 22, 23]. It is not surprising then, that secondary hair follicle populations of postnatal animals can respond to stimulatory treatments whereas those of their adult counterparts are not able to.

Although melatonin treatment of adult cashmere goats does not affect the total population of secondary hair follicles, it does increase the population that is active [20, 21, 24], as does exposure of goats to artificial short-day photoperiods [11, 18, 25]. This indicates that melatonin treatment speeds up the transition of secondary hair follicles from telogen to the anagen phase, thus promoting onset of their activity. In vitro studies have also shown that melatonin exposure promoted the proliferation of goat secondary hair follicle stem cells and fibre elongation and growth [10, 26]. These findings are all supported by the current study where the density of active secondary hair follicles and the S:P ratio of active hair follicles were higher in the melatonin-treated cashmere goats. As well as the effect of greater numbers of active secondary hair follicles causing an increase in cashmere fleece weight, Merchantt et al [27] have proposed that extra growth of cashmere fibre also arises from an extended duration of the growing period. In the current study, the population of active secondary hair follicles in melatonin-treated

cashmere goats was higher than that of the controls during the growing period (August and September 2016) when follicles are fully active. However, the populations were similar in both groups of goats at the end of the growing season, suggesting that the extra cashmere growth resulted from the enhanced activity of follicles during the growth period, rather than from an extension of the growth period duration. It is possible that during the growing period, melatonin treatment of the goats has not only accelerated the maturation (telogen to anagen progression) of the follicles but has also activated some secondary follicles that may have remained inactive in normal circumstances.

Treatment of cashmere goats with melatonin elevated serum concentrations of this hormone at the sampling points in the initial cashmere growth cycle. However, the finding that there was no difference in serum melatonin concentrations between Melatonin and Control goats in the following year, during the subsequent growth cycle, indicates that the provision of exogenous melatonin to these goats has not affected endogenous secretion of this hormone in the following year. This observation reinforces those of Foldes et al [28] and Matsumoto et al [29] who also found that administration of exogenous melatonin did not affect secretion of the endogenous hormone.

## **Conclusions**

Treatment of cashmere goats with exogenous melatonin during the period of seasonal cessation of cashmere growth improves cashmere production in terms of increased fleece weight and staple length, and improves its quality by reducing fibre diameter. This effect of melatonin on cashmere production is confined to the growth cycle immediately following the treatment and does not lead to enhanced or reduced effects on the cashmere growth cycle of the following year, which makes such treatment acceptable from the cashmere goat industry point of view. These beneficial effects of melatonin treatment on production of cashmere fibre result from its acceleratory effect on regeneration of secondary hair follicles (telogen through anagen phase) plus its elevation of the population of active secondary follicles, some of which may be due to reactivation of follicles that normally remain inactive during the natural growth period.

## **Declarations**

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### **Authors' contributions**

CHY, CXZ and WZ designed the research; CHY, CHD, ZYW, YL, YYL and XJF performed the research; CHY conducted data analysis and prepared the initial draft; CHD provided technical and material support and aided in editing of the manuscript; CXZ and WZ conducted critical analysis. All authors critically revised the manuscript and gave final approval for submission.

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## Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Ethics approval

All experimental procedures in this study were performed in accordance with Guidelines for the Care and Use of Agricultural Animals in Research and Teaching and approved by the Animal Care and Use Committee of China Agricultural University (Beijing, China, permit no. CAU 20150825-2).

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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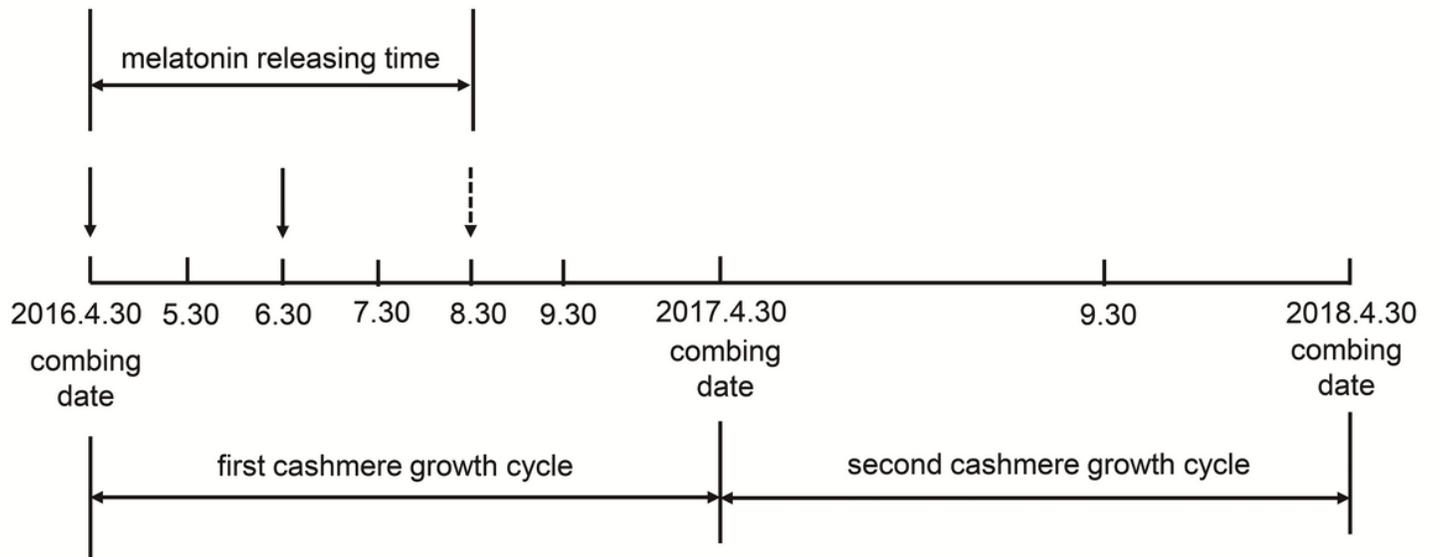
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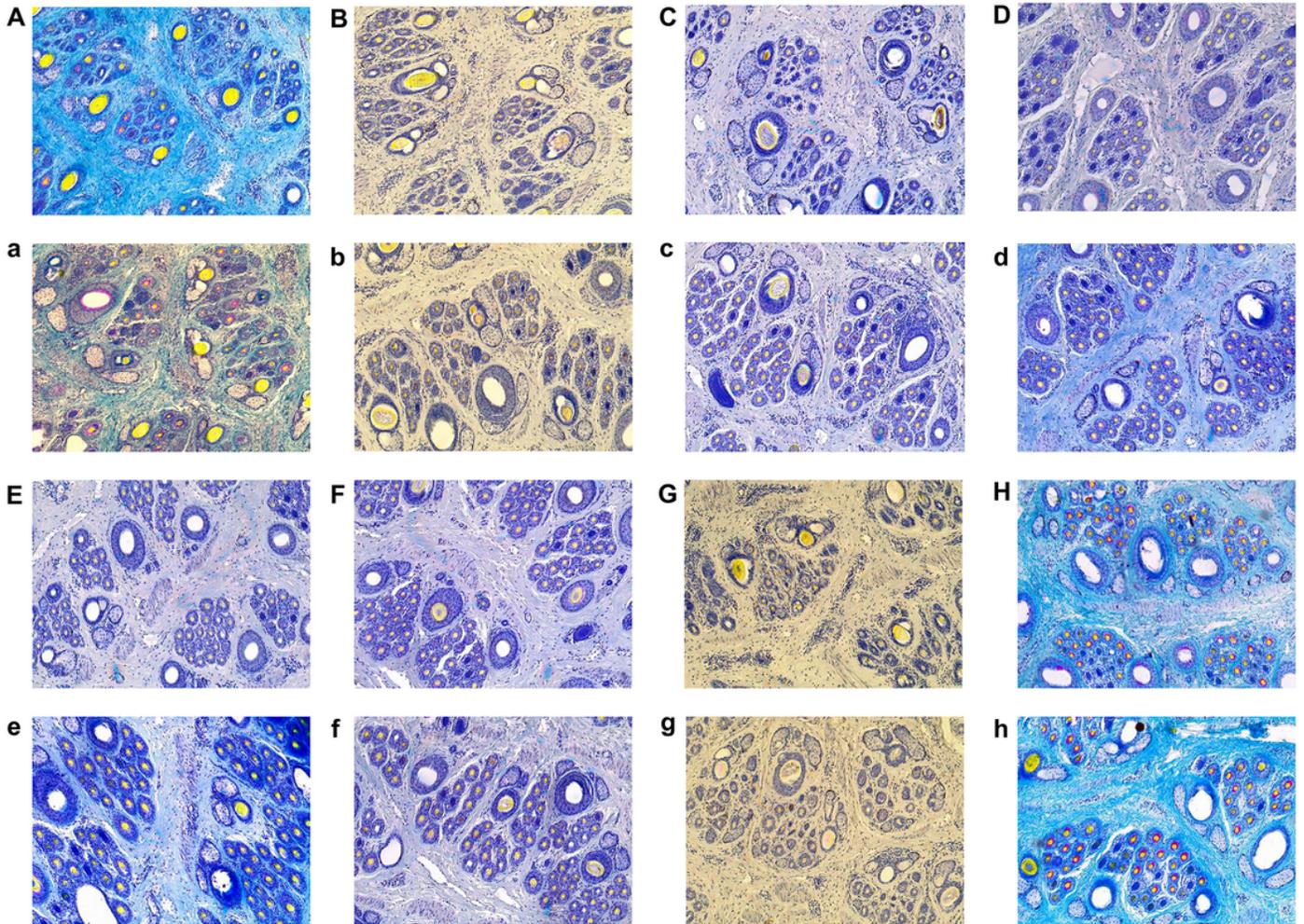
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## Figures



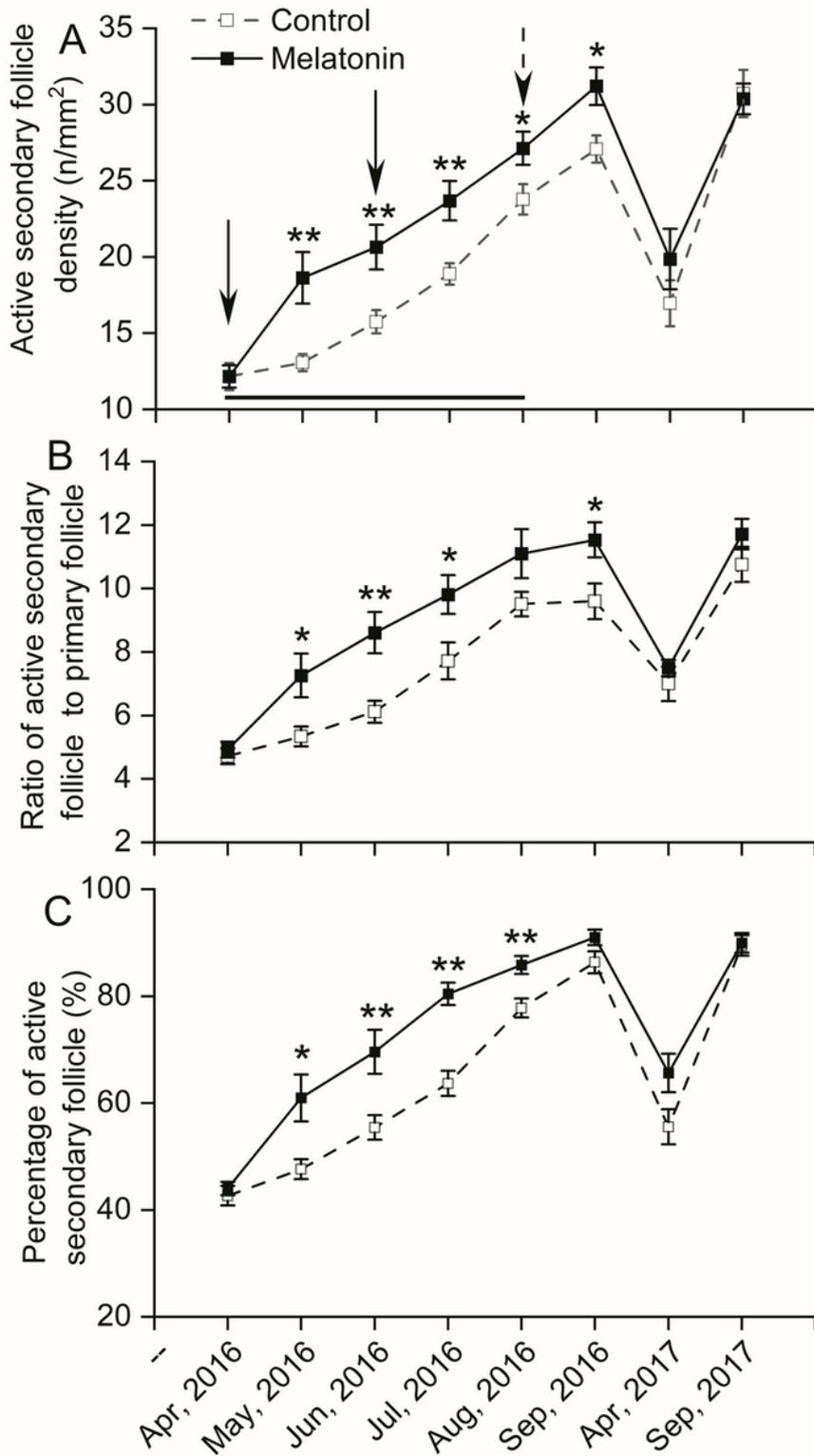
**Figure 1**

Sketch map of the experimental design of the effect of melatonin administration to cashmere goats in one cashmere growth cycle on hair follicle characteristics and cashmere production for two consecutive cashmere growth cycles. The solid arrow above the horizontal timeline indicates the time when exogenous melatonin implants were inserted subcutaneously. The dotted arrow above the horizontal timeline indicates the time when exogenous melatonin implants release the melatonin completely.



**Figure 3**

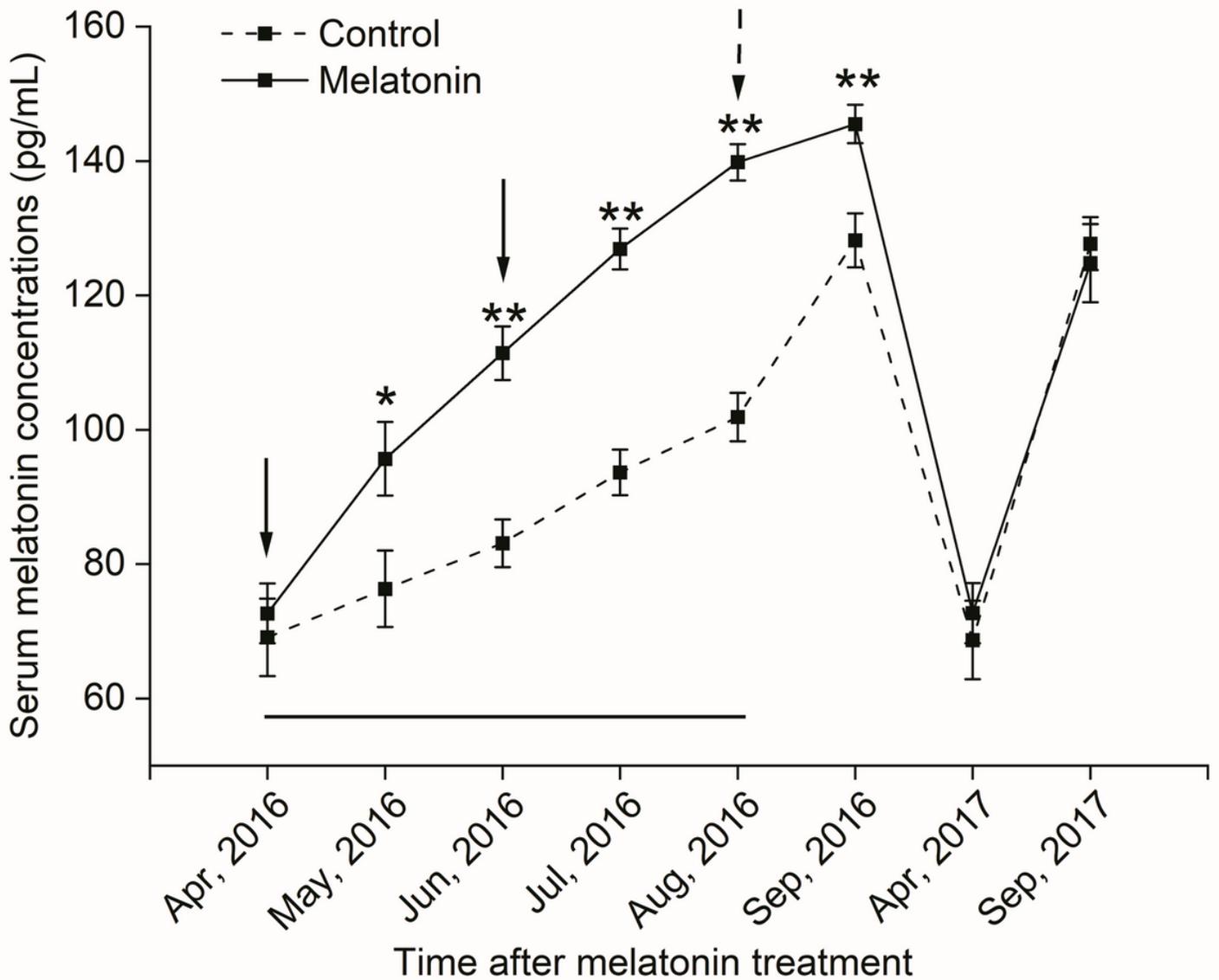
Representative hair follicle groups at horizontal sections stained by Saccpic method both in control and melatonin group (40×). A-H, Representative hair follicle groups at horizontal sections in the skin of melatonin-treated cashmere goats on April 2016 (A), May 2016 (B), June 2016 (C), July 2016 (D), August 2016 (E), September 2016 (F), April 2017 (G), September 2017 (H). a-h, Representative hair follicle groups at horizontal sections in the skin of control cashmere goats on April 2016 (a), May 2016 (b), June 2016 (c), July 2016 (d), August 2016 (e), September 2016 (f), April 2017 (g), September 2017 (h).



**Figure 5**

Effect of melatonin administration to cashmere goats in in one cashmere growth cycle on active status of secondary hair follicles for two consecutive cashmere growth cycles (—■—, melatonin group, n = 12; —□—, control group, n = 12). The solid arrow indicates the time when exogenous melatonin implants were inserted subcutaneously. The dotted arrow indicates the time when exogenous melatonin implants

release the melatonin completely. The solid line above the X-axis indicates the time period that exogenous melatonin functions. Data are presented as least square means  $\pm$  SEM.



**Figure 7**

Effect of melatonin administration to cashmere goats in in one cashmere growth cycle on serum melatonin concentrations for two consecutive cashmere growth cycles (—■—, melatonin group, n = 12; —□—, control group, n = 12). The solid arrow indicates the time when exogenous melatonin implants were inserted subcutaneously. The dotted arrow indicates the time when exogenous melatonin implants release the melatonin completely. The solid line above the X-axis indicates the time period that exogenous melatonin play roles. Data are presented as least square means  $\pm$  SEM.