Characterization of Embryonic Feather Follicle Development in the Chinese Indigenous Jilin White Goose

W. Wu[1,2], R. F. Xu[2], X. Guo[2], C. H. Li[2] and C. X. Wu[1,] *

[1] Department of Animal Genetics, Breeding and Reproduction, College of Animal Science and Technology
China Agricultural University, Beijing100094, China

ABSTRACT : To investigate goose feather follicle development and difference among the dorsal, ventral, and thoracal tracts during embryonic stage, the present study was conducted on 180 embryos at different ages obtained from the Jilin White goose, a Chinese indigenous breed. The study indicated that the epidermis and dermis of goose embryo formed between embryonic day 10 (E10) and 12 (E12). The thickness of the epidermis remained unchanged until hatching; while the thickness of the dermis increased throughout embryonic development. The primary feather follicles formed around E13-E14 and there were no new primary feather follicles forming after E18. The secondary feather follicles formed coincidently at E18. The density of primary and secondary feather follicles on the ventral and thoracal tracts were significantly higher than those on the dorsal tract (p<0.05). For primary and secondary follicles, the diameter of the feather bulbs and the depth of the feather follicles on the dorsal tract were much greater than those on the thoracal and ventral tracts (p<0.01), respectively; while the difference between the ventral and thoracal tracts was not significant (p>0.05). It is concluded that the Jilin White goose is of a single-follicle group structure, differing from mammals which are of multiple-follicle group structure. (**Key Words** : Goose, Feather Follicle, Embryonic Stage)

INTRODUCTION

The goose is one of the most important, widely spread domestic species within China. The production of high quality down feathers is one of the most interesting goals for goose breeding along with meat and eggs (Tu et al., 2006), so the investigations of goose down characteristics of quality relevant to morphology, physics, and biology have been extensively carried out and reported (Bonser, 1995; Dawson et al., 2000; Taylor et al., 2004; Wilde et al., 2006). The down feathers are made in feather follicles; they are complex epidermal appendages with hierarchical branching and represent a multi-layered topological transformation of keratinocyte sheets. The basics of feather morphogenesis have been described (Lucas et al., 1972), as has a comprehensive description of the developmental biology of feather follicles in chicken was also reported (Yu et al., 2004). In studies of the feather follicle development

so far reported, most attention has intensively been focused on the formation (Dhouailly, 1970; Prum, 1999; Sawyer et al., 2003; Jiang et al., 2004), morphogenesis, differentiation, and maturation of the feather follicles (Dhouailly, 1970; Haake et al., 1984; Yu et al., 2002; Alibardi, 2005; Alibardi, 2006), feather cycling (Paus et al., 1999; Stenn and Paus, 2001; Mou et al., 2006), and molecular expression, and regulative mechanism in chicken (Nohno et al., 1995; Harris et al., 2002; Harris et al., 2005; Yue et al., 2006). However, the development of the primary and secondary feather follicles in the embryonic stage of goose has not been studied widely. Neither the embryonic nor the post-natal development of feather follicle in the indigenous Chinese goose has been reported so far.

This paper, therefore, presents some values for feather follicle development of the Jilin White goose in China.

MATERIALS AND METHODS

Sampling and histological processing

Jilin White goose eggs were obtained from Jilin Agricultural University in Jilin Province, Northeast of China, and incubated in one incubator according to the routine procedure. Totally, 180 embryos of goose at

different embryonic ages from E10, E12, and gradually to E30 were used.

Skin samples were obtained from goose embryos during E10-E30 every 2 d. The first sampling started at E10. Before sampling, the embryos were extracted from the egg and immersed into 10% neutral buffered formalin for 20-24 h to fix the skin tissue. The skin samples of approximately 1.5 cm^2 were excised from the right midst of the dorsal, ventral, and thoracal tracts and stored in 10% buffered formalin for further histological processing. After the initial sampling (at E10) further skin specimen were taken as same as possible to the original sampling site.

Skin tissues were prepared for histological sectioning as described by Parry et al. (1992). Dehydration, clearing in xylol, and embedding with wax was carried out in an automated tissue processor (ThermoShandon Pathcentre). The tissues were embedded in paraffin wax at 70°C using the KD-BM. The paraffin blocks were stored under refrigeration before sectioning; then serial longitudinal and cross sections of skin were cut at the desired thickness of 5 μm using a microtome, and floating the sections on a 45°C water bath containing distilled water. Transfer the sections onto the pure slides, and then allow the slides to dry overnight and store slides at room temperature until ready for use. After the sections were mounted on slides and a modification of the stain combination of haematoxylin and eosin was employed to stain the sections.

Observation and measurement

Using a microscope (JNOEC), the distribution of primary and secondary feather follicles were first observed from the skin sections at a magnification×40. The number of primary and secondary feather follicles was counted from 10 fields of each cross section at a magnification ×100, and an actual field area of 5.25 mm^2. At a magnification ×400, and an actual field area of 0.325 mm^2, the thickness of the epidermis and dermis, diameter of primary and secondary feather bulbs, and depth of primary and secondary feather follicles were measured from 20 fields from serial longitudinal sections of the same skin, and the fine structures of secretive glands beside the primary and secondary feather follicles could be clearly observed from the cross section.

The following measurements were calculated for each cross section from each embryo at each age: (a) density of primary feather follicles as folls/cm^2 (Pf); and (b) density of secondary feather follicles as folls/cm^2 (Sf). And from these, the Sf/Pf ratio was obtained. Shrinkage of skin samples during processing was corrected in the following way. The mean diameter (of 2 measurements) of processed samples was measured, and the correction factor calculated on the basis of shrinkage from the 1.0 cm diameter biopsy for each individual embryo at each age. Skin sections for
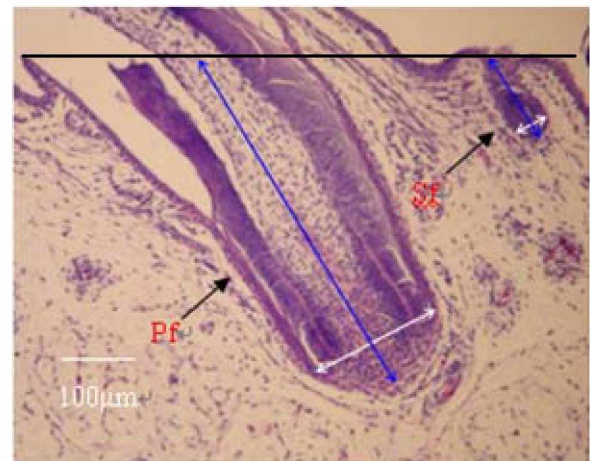

Figure 1. The longitudinal section of feather follicles on thoracal tracts at E22. The diameter of primary and secondary feather bulbs is indicated by the white arrows. The depth of primary and secondary feather bulbs is indicated by the blue arrows. The bigger one is primary feather follicle (Pf). The other is secondary feather follicle (Sf). The surface of skin is indicated by the black line.

measurement were selected at or above (primary) sebaceous gland level, because those below the sebaceous gland were sometimes found to pass through the follicle bulb, and not the fibre, particularly in the younger embryos. The position of measurement on sections is indicated as Figure 1.

Statistical analyses

The following statistical model was used to analyze the relationship between the factors of age (Snedecor and Cochran, 1967), and sampling sites of the embryos and observed values using GLM procedure of SPSS13.0.

$$Y_{ijk} = \mu + \alpha_i + \beta_j + e_{ijk}$$

Where, Y_{ijk} is phenotypic value of the target trait, such as follicle density, μ is population mean, α_i is the fixed effect of the ith factor of ages of embryonic goose (i = 12, 14, 16,..., 30); β_j, the fixed effect of the jth factor of sampling sites of the embryos sampled (j = 1, 2, 3) and e_{ijk} is random error effect of each observation. The developmental curves of epidermis, dermis, and primary and secondary feather follicles were drawn from the data, respectively.

RESULTS

Observation of the feather follicle development

At E10 the surface of the goose embryo was smooth and transparent, covered by a layer of mucous membrane. It could be deduced that the mucous membrane was the early stage of skin. At this age, the skin of the goose embryo had not yet formed completely, so it was impossible to obtain
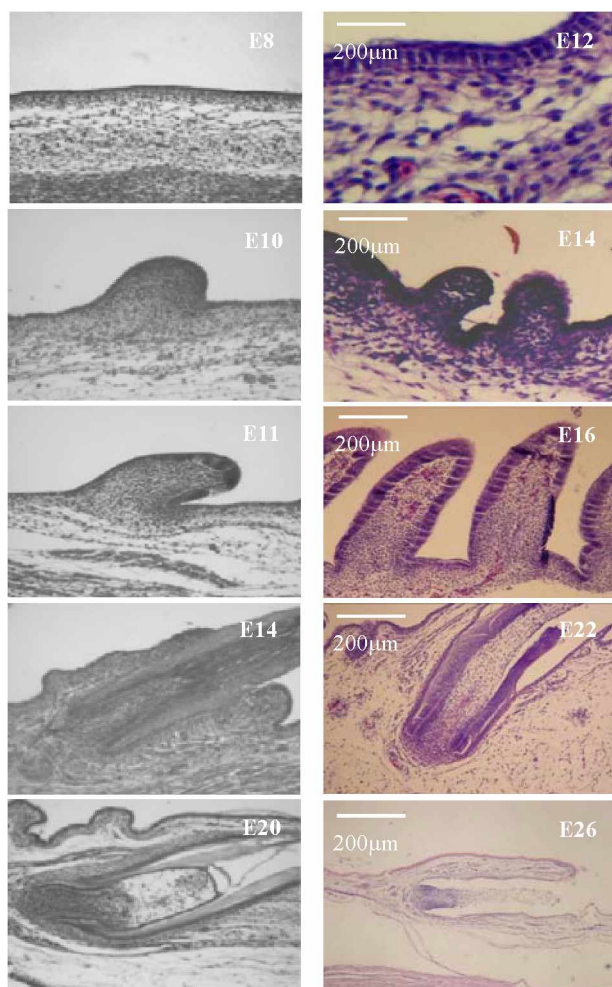
Figure 2. The process of feather formation. A-E, for chicken: At E8, dense dermis starts to form. At E10, feather buds form. At E11, buds have elongated. At E14, buds invaginate to form feather follicles. At E20, a new feather is forming (Widelitz et al., 2003). F-J, for goose: At E12, it is flat. At E14 short buds are forming. At E16, long buds are forming. At E22, feather follicles form. At E26, a new feather is forming.

skin samples to make slices. At E12, the feather placodes (short bud) were clearly visible on the skin surface, the feather placodes of the dorsal tracts were of different sizes; while the feather placodes on the thoracal and ventral tracts were smaller and distributed regularly. By E14, the feather placodes on the dorsal tracts had started to protrude, whereas those of the other tracts had not. No feather placodes were observed in the midline of the ventral tracts at this age, while towards to the legs, the diameter of feather placode became smaller. At E16, the dorsal and sides of the thoracal and ventral tracts were covered by feathers, and at E18, the feather placode displayed an uneven pattern on the skin surface.
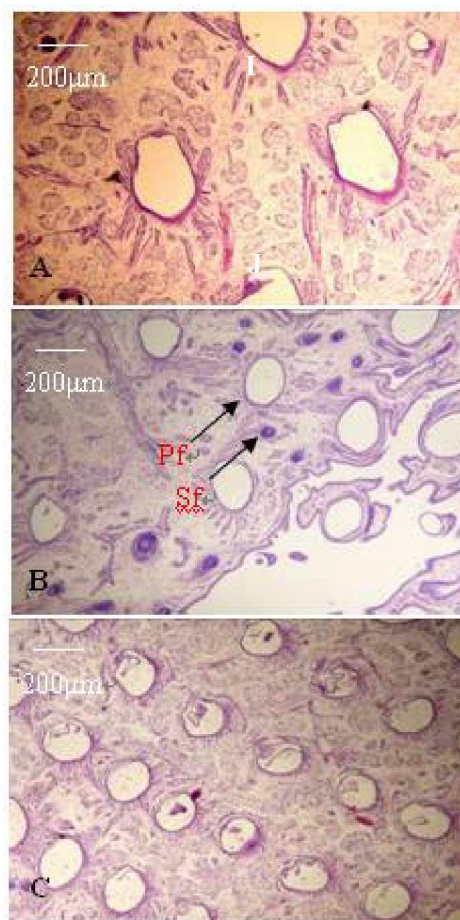
Histological observations of the embryonic feather

Figure 3. Patterning of feather follicles from dorsal (A), ventral (B), and thoracal (C) tracts at E22, ×40. The patterning is regular and linear, not clustered.

follicle patterning

Figure 2 showed morphological changes of feather follicle (longitudinal sections), and we compared them with those of chicken (Figure 2A-E). We found that the process of goose feather follicle formation was similar to that of chicken.

The serial longitudinal and cross sections were observed in goose embryos from E12 to E26 (Figures 2F-J and 3). The patterning of feather follicles from different tracts was shown in Figure 3. The patterning was regularly linear. And we can see from Figure 3 that, the size of feather follicles on dorsal tracts was much bigger than that on the ventral and thoracal tracts, but the density of feather follicles on dorsal tracts was less than that on the other two tracts.

Figure 4 showed that the patterning of goose feather follicles was simple different from that of goat (hair follicle group), maybe it was the difference between fowl and mammal.

Thickness of epidermis and dermis

Figure 5 showed results of changes in the thickness of epidermis and dermis of the sampled skins at each age of
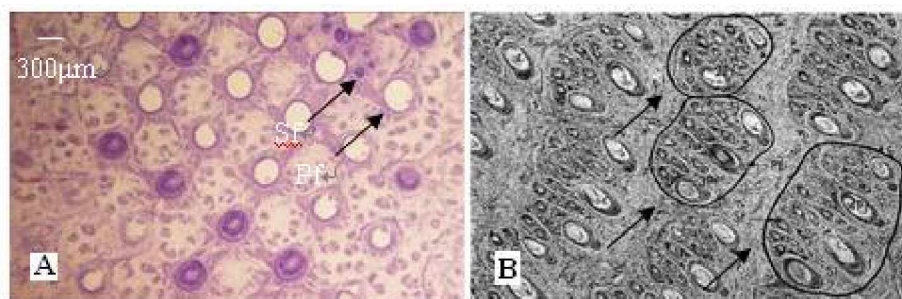
Figure 4. Cross sections of feather and hair follicles. (A) Cross section of goose feather follicles at E20, on the dorsal tracts. The patterning of feather follicles is regularly linear, and there is no follicle group structure. Primary and secondary follicles are indicated by the arrows. (B) Cross section of Australian Cashmere goat hair follicles (×125) (Parry et al., 1992). Hair follicle groups are indicated by the arrows.
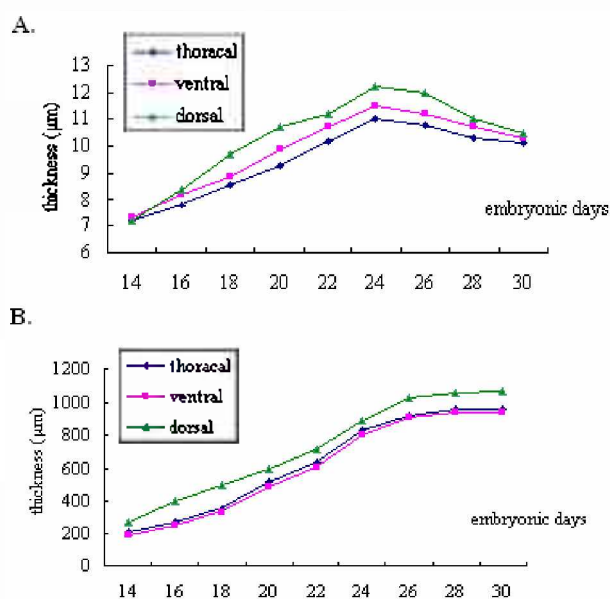

Figure 5. Changes in the thickness of epidermis (A) and dermis (B). The thickness of epidermis is maximal at E24, but the thickness of dermis keeps increasing.

embryonic stage. The thickness of epidermis on the thoracal tracts increased significantly (p<0.05) from 7.2±1.3 μm at E14 to 11.0±1.6 μm at E24, and then gradually declined to 10.8±2.3, 10.3±1.3, and 10.1±1.3 μm at E26, E28, and E30. The thickness of dermis on the thoracal tracts increased significantly (p<0.05) from 204.4±14.3 μm at E14 to 924.8±131.6 μm at E28, and then declined to 890.6±82.3 μm at E30. Similar changes in the thickness of epidermis and dermis were also observed on the ventral and dorsal tracts of the embryonic skin, respectively.

Feather follicle density and Sf/Pf ratio

Figure 6 showed results of changes in the primary and secondary feather follicle densities of different sampling sites at each age of embryonic stage. Density of primary feather follicle on the thoracal tracts increased significantly
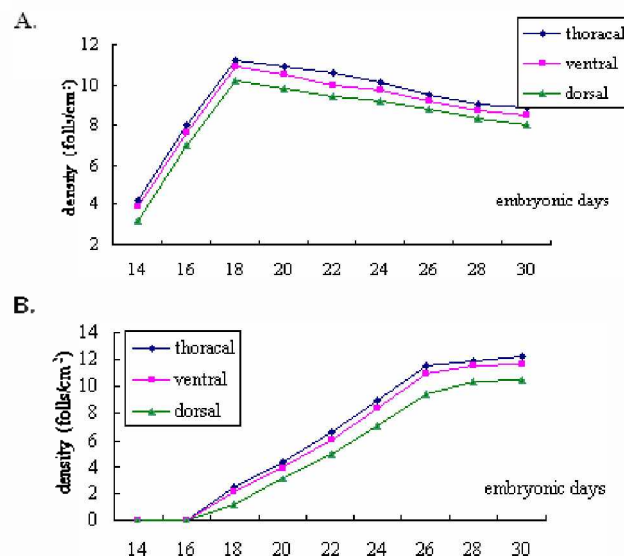

Figure 6. Changes in the density of primary (A) and secondary (B) feather follicles. follicles/cm² (folls/cm²).

(p<0.01) from 4.2±0.5 folls/cm² at **E14** to 11.2±0.6 folls/cm² at E18, and then declined from 10.9±1.1 folls/cm² at E20 to 8.9±0.9 folls/cm² at E30 (p<0.05), and density of secondary feather follicle increased to 2.4±0.2 folls/cm² at E18, then up to 12.2±2.3 folls/cm² at E30 significantly (p<0.01). The changes of feather follicle density observed on ventral and dorsal tracts tend to be similar to that on the thoracal tracts, respectively. The results indicated the density of primary and secondary feather follicles on the thoracal and ventral tracts were significantly higher than that on the dorsal (p<0.05). Generally, Sf/Pf ratio increased from 0 at E16 to 0.176 at E18, and then up to 1.366 at E30.

Diameter of feather bulbs

Figure 7 presented results of changes in the diameter of primary and secondary feather bulbs at each age of embryonic stage. The bulb diameter of primary feather follicles kept increasing during embryonic stage. On the thoracal tracts, it increased significantly (p<0.01) from
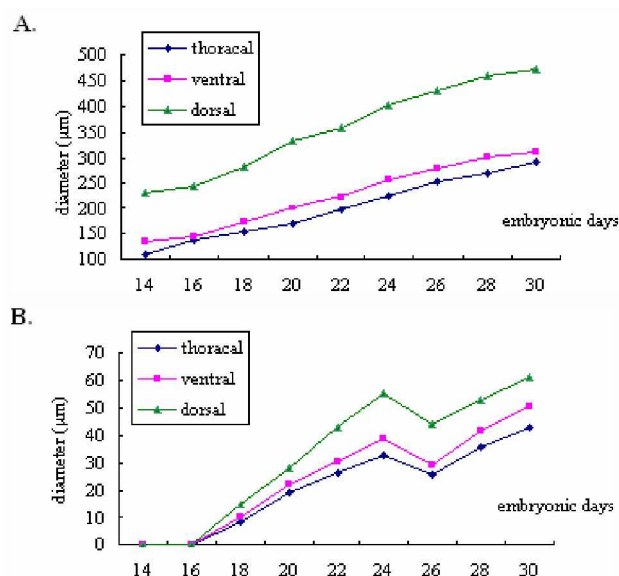
Figure 7. Changes in the diameter of primary (A) and secondary (B) feather bulbs.

Figure 8. Changes in the depth of primary (A) and secondary (B) feather follicles.

133.1±19.4 μm at E14 to 310.8±48.0 μm at E30. The bulb diameter of secondary feather follicles on the same site increased significantly (p<0.01) from 10.0±1.1 μm at E18 to 38.8±5.2 μm at E24; while declined to 29.6±10.7 μm at E26 (p<0.01), and then increased to 41.5±16.4, 50.7±17.2 μm at E28, E30 (p<0.01). Similar changes for diameter of primary and secondary feather bulbs were also observed on ventral and dorsal tracts of the goose embryos, respectively. However, the diameter of primary and secondary feather bulbs on the dorsal tracts were significantly higher than those on the thoracal and ventral tracts, respectively (p<0.01); while the difference between the thoracal and ventral tracts was not significant at this embryonic stage (p>0.05).

Depth of feather follicles

It revealed in Figure 8 that the depth of primary and secondary feather follicles kept increasing during embryonic stage. The primary feather follicles on the thoracal tracts increased from 11.9±3.8 μm at E14 to 677.6±79.5 μm at E30 (p<0.01). The depth of secondary feather follicles on the same site increased from 45.7±6.4 μm at E18 to 202.5±19.8 μm at E30 (p<0.01). Similar changes for depth of primary and secondary feather follicles occurred on ventral and dorsal tracts of the goose embryos, respectively. However, the depth of primary and secondary feather follicles on the dorsal tract were significantly higher than those on the thoracal and ventral tracts, respectively (p<0.01); while the difference between the thoracal and ventral tracts was not significant at the embryonic stage (p>0.05).
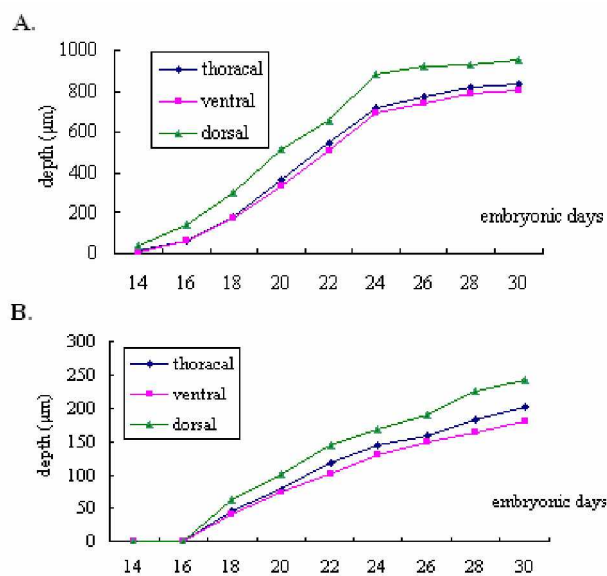
DISCUSSION

Development and patterning of feather follicles in goose

Although the general features and detailed development and growth of feather follicles in chicken, duck and other avian have been well described (Prum, 1999; Yu et al., 2002; 2004; Sawyer et al., 2003), only less information is available for goose species (Lilja, 1981). This study has provided a comprehensive description of the development of feather follicles of the Jilin White goose, a Chinese indigenous breed. Comparing with the feather formation in chicken (Figure 2), we found that the features of the epithelium and embryonic feather development were similar to the chicken reported (Widelitz et al., 2003). At the same time, difference in feather follicle development between goose and chicken were clearly observed also. In goose embryo, the dense dermis started to form At E12; the presence of feather placodes was obvious along the length of the skin at E14; buds elongated at E16 and invaginated to form feather follicles at E22; while in chicken, the corresponding events of feather follicle development were observed at E8, E10, E11 and E14, respectively. Thus the development of feather follicles in goose embryos was slower than that of feather follicles in chicken, presumably because the incubation period of the Jilin White goose is longer (31 d) than that of chicken.

The present study showed that for each embryo, the feather buds on the dorsal tract were little larger and longer than those on the thoracal and ventral tracts observed at E12; it suggested that the feather follicles completely formed earlier on the dorsal tract than those on the thoracal and ventral tracts. For any tracts, we could clearly determine the anterior-posterior axis due to the direction the

feather buds were leaning; this finding was similar to the observations in chicken.

Different from the wool follicle development (Figure 4), in sheep and goat, the fleece was based on the primary trio or five follicle grouping, and all primary wool follicles but few secondary wool follicles were mature (fibre-bearing) at birth and the number of secondary wool follicles increased 10 fold in the first 57 d after birth (Parry et al., 1992). In goose, all primary (generating contour feather) and few secondary feather follicles (generating down feather) formed completely during embryonic stage, but the former was formed 5-6 d earlier than the later. Moreover, the geese were of single-follicle group structure, distinct to animals of multiple-follicle group structure, such as in goat: the primary and secondary feather follicles of goose developed and located independently (Figure 1), while the secondary follicles derived from the primary follicles in goat.

Quantitative description for the development and variations of feather follicles

Goose feathers have an important commercial value, and down feathers are extensively used as high quality insulation materials in both clothing and bedding as for a given level of warmth, because they are lighter and more compressible. The production and quality of down feathers are determined by the development of secondary feather follicles. Therefore, researches on the development of feather follicles of goose have attracted more attention. In goose, each of primary and secondary feather follicles generated from the separated, independent feather primordia that first lead to the formation of feather buds (Jiang et al., 2004), but the formation of secondary feather follicles was confirmed to be 5-6 d later than that of the primary follicles in the present study. In goat, the wool follicles were arranged in a specific group of primary and secondary follicles, and the follicle groups generally consisted of three primary follicles with a varying number of secondary follicles in wedge-shaped groups between the central and lateral primaries (Parry et al., 1992).

Up to the present time, quantitative description for the developing feather follicles of goose is reported very limitedly. To quantificationally describe the development of feather follicles of embryonic goose, some parameters were primarily introduced in this paper, including primary and secondary feather follicle density (Sf, Pf), Sf/Pf ratio, diameter of feather bulbs and depth of feather follicles that were generally used to characterize the wool follicle development in sheep and goat (Koul et al., 1987). In this article, the studies of feather follicle density and Sf/Pf ratio in embryonic goose revealed that the decrease in Pf density from E18 to E30 is likely to be due to two reasons: (a) an increase in body size; (b) no or less new primary feather follicles formation happens; meanwhile, the Sf density was

increasing significantly, although due to the continuing skin expansion, and two developmental peaks of secondary feather follicles were observed. The first developmental peak emerged at E26, and the other peak appeared at 48 d after its birth (Wu et al., unpublished data). The increasing of Sf density suggested that the feather buds forming the secondary feather follicles were undergoing an active cell proliferation, migration and differentiation stage up to 48 d after birth (Wu et al., unpublished data). It was also testified by the constant increase in Sf/Pf ratio. This result provided very valuable information for us to interfere in the development of down feathers in goose.

Morphogenesis of feather follicle initiated from the basal epidermal placode appearing above a condensation of dermal cell (Prum, 1999), the increasing of the dermal condensation and the thickening of the epidermal played an essential role in the induction of feather follicles (Wolpert, 1998). In this study, the thickness of the epidermis and dermis of the skin of the embryonic goose were highest at E24 and E26, respectively. Correspondingly, as mentioned above, the first density peak of secondary feather follicles occurred at E26, and the diameter of secondary feather bulbs reached the first highest point at E24, and then sharply descended to the lowest point at E26 during embryonic stage. Moreover, the depth of primary feather follicle came to the highest point at E26. Did they happen only coincidentally? From the result, we could preliminarily conclude that the higher thickness of the epidermis and dermis during the period between E24 and E26 might be required by the morphogenesis and formation of a larger number of secondary feather follicles and the maturation of primary feather follicles. The possible explanation may be due to the following reasons: (a) the feather buds were undergoing active morphogenesis to become the secondary feather follicles as the aforementioned. The feather follicle was an invaginated epidermis surrounding the feather filament cylinder with pulp inside (Yu et al., 2004). (b) The epidermal layer had been thickening and beginning to differentiate to generate different cell types. The various cells were essential for the formation of secondary feather follicles. (c) Although the formation of primary feather follicle was finished about at E18, but the feather follicles were under continuing growth and maturation. The proper thickness of the epidermis and dermis provided the spatial organization for the development of primary feather follicle.

Clearly, the diameter of feather bulbs was associated with the diameter of rachis; the depth of feather follicle was associated with the length of calamus, and the depth was also related to the nutrition requirement of the developmental feather follicles. As for the smallest diameter of secondary feather follicle at E26, it was mainly caused by the presence of a large number of the novel secondary follicles which generally had smaller diameters than those

of mature ones. It also strongly supported the conclusion that the development of secondary feather follicle reached the apex at E26.

We can conclude that: (a) The morphogenesis of primary feather follicles finished during embryonic stage, but that of secondary feather follicles was still happening. (b) The depth and diameter of the primary and secondary feather follicles kept increasing before birth. (c) There was no follicle groups observed in goose, but in goat and sheep, they have trio or five secondary wool follicles rounding the primary wool follicle. The study on the modulating mechanism of formation of the secondary follicle in goose is under going.

ACKNOWLEDGMENTS

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