

# Ultrastructural Characteristics of Neonate Scalp Hair

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## Abstract

**Background/Objectives:** There are many differences between the hair from neonate and those from children and adults.

**Methods/ Statistical Analysis:** The microstructural characteristics and external shape of neonate hairs were investigated using scanning electron microscopy and transmission electron microscopy. **Findings:** The thickness of the terminal end of neonate hairs was  $\sim 2.5 \mu\text{m}$ , with a slightly round shape, and one cuticle cell surrounded the cortex. Different from normal adult hair, a cuticle layer and cortex are present in neonate hair, but there is no medulla. Cuticle cells in neonate hair showed a typical structure. However, the exocuticle, endocuticle, A layer, and cell membrane complex exhibited markedly different electron densities. The cortex is composed of polygonal cortical cells and was clearly observed by the boundary of these cells. The cytoplasm of keratinized cortical cells is filled with macrofibrils and melanin granules around the cuticle layer and cortex regions. **Applications/Improvements:** Neonate hair follicles were  $\sim 0.5 \text{ mm}$  in size, fivefold smaller than adult hair follicles. Neonate hair follicles had a structure identical to that of anagen phase adult hair follicles but differed in size.

**Keywords:** Hair Cortex, Hair Cuticle, Hair Follicle, Neonate Hair, SEM, TEM

## 1. Introduction

Neonate hair is the hair that develops after 7-8 months of pregnancy within the uterus. The first hair, known as lanugo, begins to grow on the entire body of the embryo in uterus beginning at 3-4 months of pregnancy and falls out at 7 or 8 months of pregnancy. Lanugo is the first hair to develop in the hair follicle, does not have a medulla or melanin granules. A new hair follicle is formed where the lanugo falls out and terminal hair starts to grow, and vellus grows on the skin. Vellus is thin and short and is distributed on the skin, except the scalp, on which it exists only in humans among mammals. Neonate hair is terminal hair that grows in the uterus prior to 2-3 months of birth<sup>1,2</sup>.

Newborn babies are infants within 4 weeks after birth, the neonate hair of which has minimal damage from external environmental factors.

Hair is damaged by sunlight, friction, and environmental pollution as it grows, and is also damaged

by physiochemical procedures such as application of permanent wave, hair coloring, and bleaching at hair salons<sup>3-8</sup>. Hair keratin decomposition and deterioration of elasticity and strength of cortex occur as weathering takes place, in which the hair surface becomes rough and melanin granules decompose, resulting in loss of color<sup>9-11</sup>.

The terminal hair shaft of growing children or adults is composed of three layers: the cuticle layer, cortex, and medulla<sup>12,13</sup>.

The cuticle layer of hair surrounds the cortex and plays a protective role. Undamaged cuticle layers of normal hair have a smooth surface, refract light, and reduce the friction between hairs. The cuticle layer differs depending on hair thickness, but it is composed of ca. 4-10 cuticle cells and is spread along the major axis of hair growth. Cuticle cells overlap in the cuticle layer with a plate structure, and each cuticle cell is composed of an epicuticle, A layer, exocuticle, and endocuticle. The epicuticle is the cell membrane of cuticle cells, which gives shine to hair. The A layer and exocuticle are rich in cystine, and have consid-

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erable strength due to cross-linking. The endocuticle has a low cystine content, and so is mechanically weak<sup>14,15</sup>.

The cortex provides elasticity and strength to hair; long cortical cells are located in close proximity to each other. Cortical cells are closely attached in the direction of the major axis of hair. Macrofibrils with diameters of  $\sim 0.5 \mu\text{m}$  fill these cells and 2 nm microfibrils are aligned in the same structure as fingerprints in macrofibrils<sup>13</sup>. Microfibrils within macrofibrils play a significant role in the elasticity and flexibility of hair and the sulfur-containing proteins between these microfibrils support microfilaments. Melanin granules are spread around or between the macrofibrils of cortical cells. Melanin granules are of an elongated rice shape with a diameter of  $\sim 0.3\text{-}0.5 \mu\text{m}$ .

Medullas are located in the center of hair fibers and are aligned with the major axis of fibers. Medullas are keratinized cells involved hair formation and are  $\sim 5\text{-}10 \mu\text{m}$  holes containing air<sup>16-18</sup>. Medullas do not have a continuous hole following the major axis of hair, and a single hair may contain portions with and without medulla. Also, hairs with and without medulla are mixed in the scalp.

The structure, physiochemical change, and characteristics of adult hair have been reported<sup>19-22</sup>. In Korea, research on the characteristics of children's hair<sup>23</sup>, mummy hair<sup>24-26</sup>, morphological changes in the hair of deceased persons<sup>9</sup>, and the weathering of hair<sup>7</sup> has been conducted, but no morphological and ultrastructural research has focused on neonate hair.

In this study, SEM and TEM were used to compare neonate and adult hair in terms of their fine structural characteristics.

## 2. Material and Methods

### 2.1 Materials

Hair was extracted from the frontal region of five newborns within 1-4 weeks after birth. For extraction of samples, tweezers were used to pull hair from the scalp and samples were excised from hair.

### 2.2 Scanning Electron Microscopy (SEM)

After extracting hair from newborns, the samples were immediately prefixed for 1 h with 2.5% paraformaldehyde-glutaraldehyde (4°C, phosphate buffer, pH 7.4), cleansed twice for 15 min with phosphate buffer (4°C, 0.4 M phosphate buffer, pH 7.4), and postfixed for 1 h with

1% OsO<sub>4</sub> (4°C, phosphate buffer). After fixation, hair was cleansed twice for 15 min in phosphate buffer (4°C, 0.4 M phosphate buffer, pH 7.4), dehydrated in a graded ethanol series, and substituted to isoamyl acetate. The treated material was dried in a critical-point dryer (Hitachi SCP-II) and arranged on a stub with carbon tape. An IB-5 ion coater (Eiko, Japan) was then used to coat samples with platinum to 20 nm thickness, followed by visualization by SEM (S-4700, Hitachi, Japan) at 15 kV.

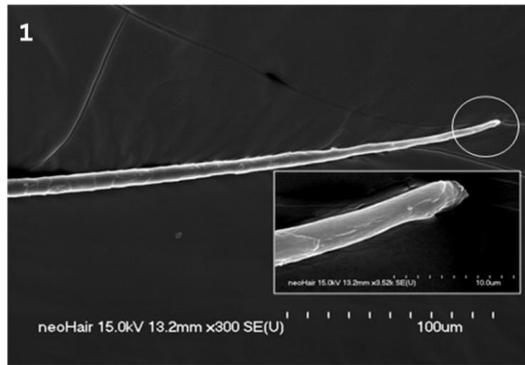
### 2.3 Transmission Electron Microscopy (TEM)

Extracted neonate hair samples were prefixed for 1 h with 2.5% paraformaldehyde-glutaraldehyde (4°C, phosphate buffer, pH 7.4), cleansed twice for 15 min with phosphate buffer (4°C, 0.4 M phosphate buffer, pH 7.4), and postfixed for 1 h with 1% OsO<sub>4</sub> (4°C, 0.4 M phosphate buffer, pH 7.4). After fixation, the material was cleansed twice with phosphate buffer and dehydrated in a graded ethanol series. Dehydrated tissues were substituted with propylene oxide, embedded in Epon-Araldite solution, and polymerized for 36 h in a 60°C vacuum-drying oven (Yamato, DPF-31, Japan). Embedded tissues were manufactured into semi-thin sections using an ultramicrotome (LKB-2088) and dyed for 2 min on a hot plate (60°C) with 1% toluidine blue (1% borax). Dyed sections were cleansed with distilled water and visualized using an optical microscope (Olympus CH30). To observe the microstructure of hair shafts, ultra-thin sections were generated, attached to a copper grid, dyed with uranyl acetate and lead citrate, and observed by TEM (H-7500, Hitachi, Japan) at 100 kV.

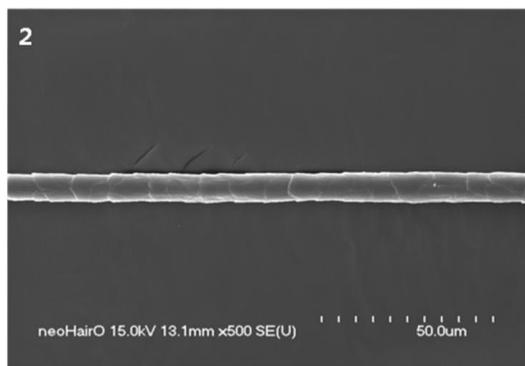
## 3. Results

The terminal end of neonate hair showed a slightly round shape by low-magnification SEM; the hair diameter of this part was  $\sim 2.5 \mu\text{m}$ . At the terminal end, one long cuticle cell surrounded the cortex (Figure. 1). Hair formed in the early stage is very thin and a wide area of scale exposed in the cuticle layer covered the cortex of the hair surface. Neonate hair became thicker as it became closer to the fundus of the scalp, starting from the terminal end; in contrast, the area of the scale on the hair surface decreased (Figure. 2).

In the middle region of the extracted hair, the end part of the exposed scale of the cuticle cells that form the



**Figure 1.** SEM image of the terminal end of a neonate hair. Inset: magnification of circle.

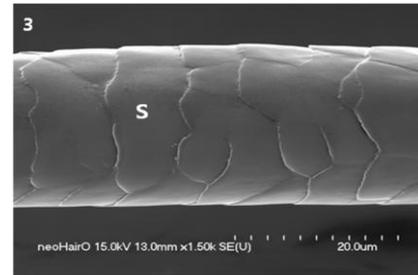


**Figure 2.** SEM image of a neonate hair.

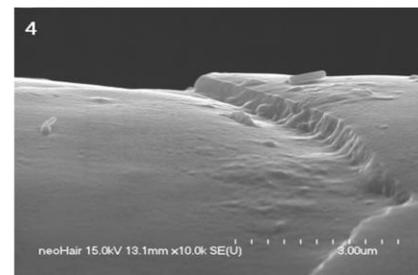
cuticle layer showed a round shape, and the surface was clean and smooth without damaged regions (Figure. 3). In high-magnification SEM, the exposed surface of the cuticle cells that surround the outermost surface of hair was relatively smooth, but the edge was slightly damaged and exhibited breakage (Figure. 4).

Hair growing close to the neonate scalp showed a diameter of  $\sim 34 \mu\text{m}$  and the surface was slightly rough. Also, the diameter between the scale exposed to the surface and scale was  $10\text{-}12 \mu\text{m}$ , which was smaller than the scale exposed to the terminal end or middle of the hair (Figure. 5).

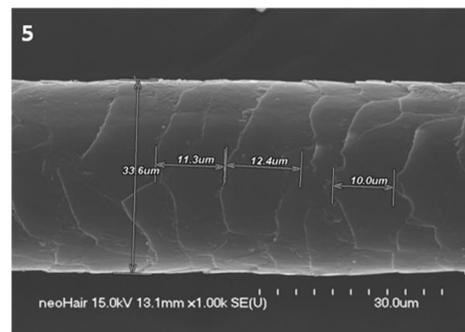
As result of SEM setting the hair of newborn baby mothers as the control group, the hair had a diameter of  $\sim 81 \mu\text{m}$  and the diameter between scale exposed to the surface and scale was  $\sim 7\text{-}8 \mu\text{m}$  (Figure. 6). Regarding the hair close to the scalp, adult hair was  $\sim 2.5$ -fold thicker than neonate hair and the length between exposed scale of hair surface was about 1.5-fold greater in neonate hair (Figures. 5, 6).



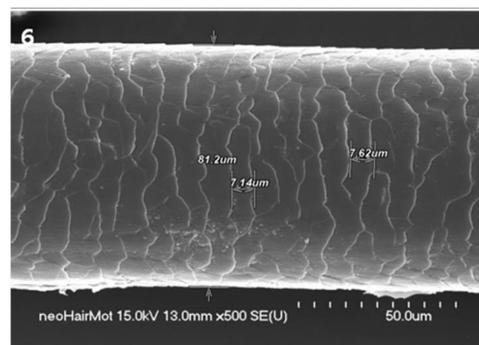
**Figure 3.** Middle region of a neonate hair with a smooth surface. S: scale.



**Figure 4.** High-magnification SEM image of figure 3. Cuticle cells are partially broken.



**Figure 5.** Neonate hair close to the scalp. Thickness of hair is approximately  $34 \mu\text{m}$ .



**Figure 6.** Normal hair of the mother. Thickness of hair is approximately  $81 \mu\text{m}$ .

By SEM, the cross section of neonate hair was circular, the cuticle layer and cortex were clearly separated, and no medulla was evident (Figure. 7).

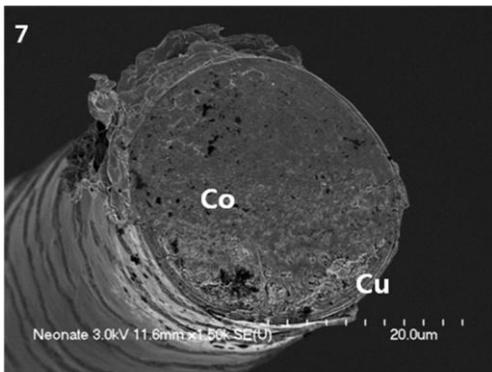
In high magnification SEM the cuticle layer was composed of four cuticle cells and melanin granules were concentrated in the cortex region close to the cuticle layer (Figure. 8). Melanin granules were of an elongated rice shape with several pits on the surface. The major axis of granules was 0.7  $\mu\text{m}$  in length, and that of the minor axis was 0.4  $\mu\text{m}$ .

The cuticle layer and cortex of neonate hair were closely aligned (Figure. 9). In the cuticle layer, cuticle cells had a lamellar structure and exhibited different electron densities and structures. The endocuticle showed a high electron density in which nucleus residue and traces of destroyed cell organelles were present. The exocuticle had a relatively low electron density and no damaged regions were observed. Also, a layer existed

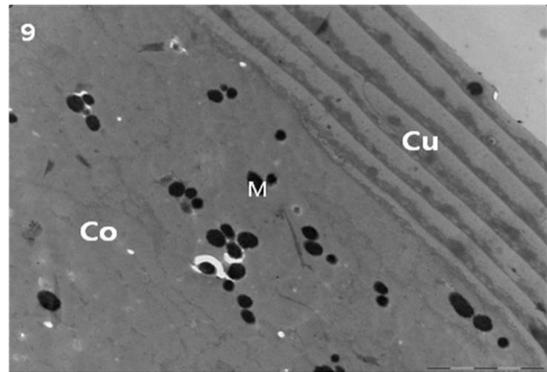
above the boundary of exocuticles; this layer had a higher electron density than the exocuticle and is traversing by constant thickness along the membrane of cuticle cells (Figure. 10).

Cuticle cells were firmly attached with cells with cell membrane complex in between, the thickness of which was 20 nm. Cell membrane complexes were separated into three layers: two black-belt-shaped  $\delta$  layers with high electron density separated by a  $\beta$  layer with low electron density (Figure. 10).

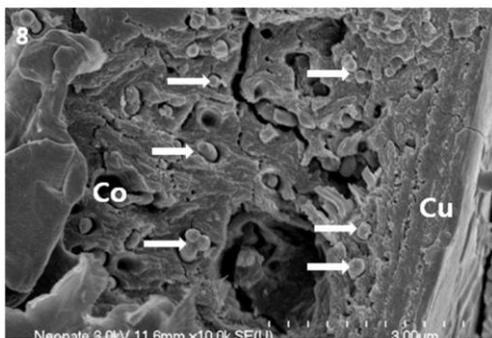
In low-magnification TEM images, the cortex was filled with cortical cells and the boundaries between these cells were clearly observed by cell membrane. Cortical cells exhibited an irregular polygon shape and keratinized cytopla are filled with macrofibrils and melanin granules. Also, destroyed nucleus and residue of cellular organelles were observed; these were amorphous and had a high electron density (Figure. 11).



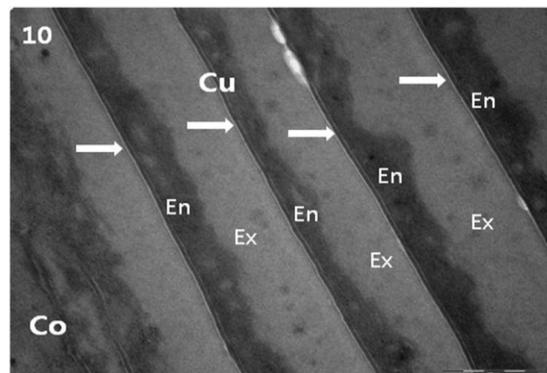
**Figure 7.** Transverse section of a neonate hair. The hair cross-section is circular and the cuticle layer(Cu) and cortex(Co) is clearly visible.



**Figure 9.** TEM image of a transverse section of a neonate hair. Cu: cuticle layer, Co: cortex, M: melanin granule.



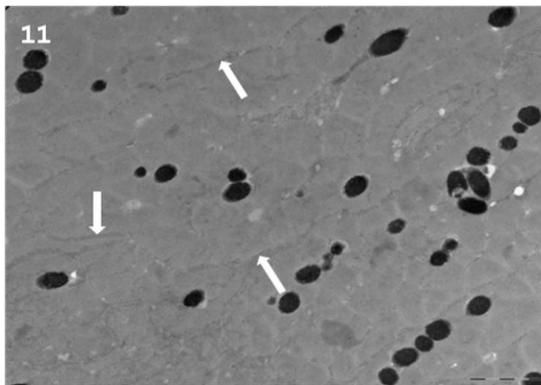
**Figure 8.** High-magnification SEM image of figure 7. Melanin granules(Arrows) are distributed around the cortex(Co) near the cuticle layer(Cu).



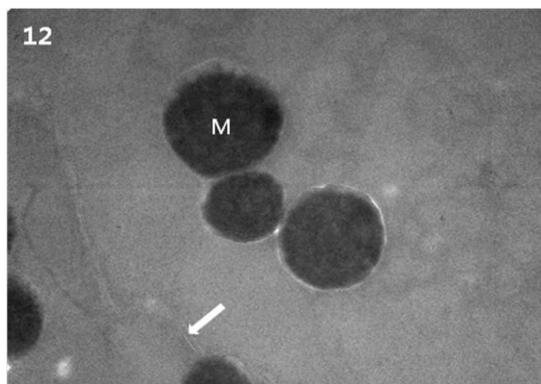
**Figure 10.** High-magnification TEM image of the cuticle layer. Cu: cuticle layer, Co: cortex, En: endocuticle, Ex: exocuticle.

By low-magnification TEM of a transverse section, macrofibrils were polygonal and microfibrils were aligned in the same structure as fingerprints in macrofibrils. Also, melanin granules between macrofibrils were aligned in the same direction as the major axis of hair. Rice-shaped melanin granules showed different diameters depending on the cut region (Figure. 12).

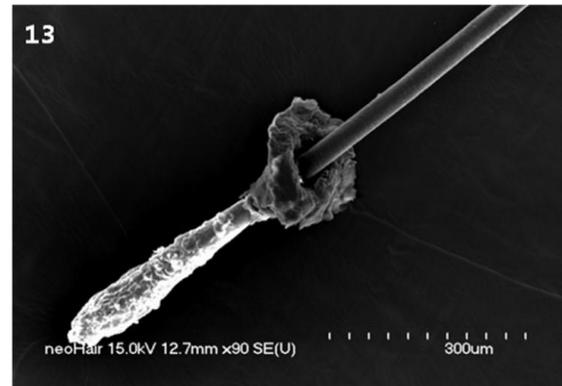
Hair follicles artificially pulled out from the temporal region of the heads of newborns were of ~0.5 mm length, ivory white in color, and the fundus was slightly expanded, forming a hair bulb. The surface of the hair follicle separated from the scalp was relatively smooth, but no glassy basement membrane was evident, and outer root sheaths surrounded the hair follicle surface (Figure.13). Also, hair follicles were spread over the scalp and epithelial cells of the epidermis were attached to the region of exposed hair shafts (Figure. 14).



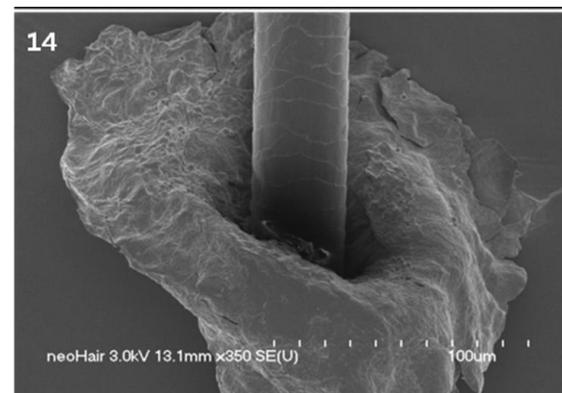
**Figure 11.** TEM image of the cortex. Arrows: membrane of a cortical cells.



**Figure 12.** High-magnification TEM image of a melanin granules(M) and macrofibril from the cortex. Arrow: cortical cell membrane.



**Figure 13.** Low-magnification SEM image of a neonate hair follicle.



**Figure 14.** Magnified SEM image of figure 13.

## 4. Discussion

The thickness of normal adult hair is generally 80-110  $\mu\text{m}$ ; however, this varies among individuals. The thickness of neonate hair was approximately 25  $\mu\text{m}$ , which is threefold thinner than normal adult hair. This is because normal hair goes through an anagen phase for 3 to 5 years, but neonate hair follicles undergo hair follicle embryogenesis and differentiation in the uterus for ~4 months after development of a mature hair follicle.

The cortex occupies about 80% of normal hair and the surrounding cuticle layer occupies about 15%. Cells are keratinized along the major axis of hair in the core where medulla exists forming an empty hole<sup>15</sup>. In this study, the cuticle layer and cortex were evident in neonate hair, but no medulla was observed. The lack of a medulla does in neonate hair is likely because hair growth occurs in the presence of amniotic fluid in the uterus.

Cuticle cells are classified into the endocuticle, A layer, exocuticle, and epicuticle according to their microstructural characteristics and cystine content. The epicuticle is the cell membrane of cuticle cells, which gives shine to hair. The A layer, exocuticle, and epicuticle, which comprise most of the cuticle cells, have cystine contents of 30%, 15%, and 3%, respectively<sup>11,27</sup>.

Cuticle cells in neonate hair showed a typical structure; i.e., the exocuticle, epicuticle, A layer, and cell membrane complex were present and exhibited differing electron densities.

Cell membrane complexes seal the gap between cuticle cells and are composed of one  $\delta$  layer and two  $\beta$  layers. The  $\delta$  layer is composed of protein and polysaccharide and the  $\beta$  layer is composed of 18-methyleicosanoic acid, which is covalently bonded to a protein molecule and reduces friction with the hydrophobic hair surface<sup>14,27</sup>.

The cortex of neonate hair is filled with long, polygonal cortical cells. These cells contain macrofibrils and melanin granules. Macrofibrils are composed of isomer crystals and microfibrils separated by an amorphous matrix.

Chang & Lee<sup>28</sup> compared keratinizing cortical cells with a cloth pencil case. The cloth cover material was compared with the cortical cell, the writing pencils with macrofibrils, and the eraser with melanin granules. Thus macrofibrils were covered by a membrane with a diameter of approximately 0.5  $\mu\text{m}$  and contained microfibrils. For example, a lead mechanical pencil is the macrofibril and the lead is the microfibril.

Artificially formed holes between macrofibrils and around melanin granules exist in cuticle cells and the cortex of the cuticle layer of normal<sup>13</sup> explained this as a natural weathering phenomenon that occurs due to keratinization caused by continuous exposure to external environmental factors. However, artificially formed holes were not observed in the cuticle layer or cortex of neonate hair.

Anagen phase hair follicles are composed of active hair bulbs and non-active cells, and are classified as infundibulum and isthmus. The infundibulum is the top part of a hair follicle, where pores are open on the scalp surface and is connected to the secretory vessels of sebaceous glands. The isthmus is the region in which the hair follicle thins from the hair bulb and the arrector pili muscle is connected to the outer root sheath epithelial cells. Hair bulbs contain active cells and can be thought of as a hair-shaft factory<sup>29</sup>. Neonate hair follicles showed a struc-

ture identical to that of anagen-phase adult hair follicles but differed in size<sup>30</sup>. reported that anagen-phase hair follicles in the adult scalp have a length of approximately 2.5 mm with a slightly expanded fundus and form hair bulbs. The neonate hair follicles were approximately 0.5 mm in length, ca. fivefold smaller than adult hair.

## 5. Conclusions

Neonate hair is the first terminal hair that occurs after hair follicles are created. These have a rounded end and a thickness of approximately 2.5  $\mu\text{m}$ . The thickness of hair close to the neonate scalp is about 25  $\mu\text{m}$ , more than threefold thinner than normal adult hair. Neonate hair was approximately 2.5 fold thinner than normal adult hair and the length between scales exposed on the hair surface was 2.5 fold thinner than that of adults. Indeed, cuticle cells that comprise the surface of neonate hair had a greater exposed area than the cuticle cells of normal adult hair. Neonate hair becomes thicker as it becomes closer to the scalp, starting from the terminal end. Hair close to the scalp showed a diameter of approximately 25  $\mu\text{m}$  and the cuticle layer close to the scalp was composed of 4-6 cuticle cells. The cortex was filled with keratinized cortical cells and no medulla was observed in the core region of the cortex. In conclusion, hair growing in the uterus comprised only a cortex and cuticle layer, no medulla was present.

## 6. Acknowledgements

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