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Short communication: Temporal changes in the skin morphology of dairy cows during the periparturient period

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ABSTRACT

Management of dairy cow productivity requires monitoring of their nutritional status by visual observation. It has been suggested that changes in hair coat appearance are among the indicators of nutritional state in dairy cows. Temporal changes in the skin morphology in cows, however, have not been reported. In this study, we examined the changes in the skin of dairy cows that occur during the peripartum period. Seven pluriparous cows were used. Skin samples were collected at 28 d before the due date and 28 d and 56 d after calving for morphological examination. Hair follicle width was 108.8 ± 5.9 μm (±SD) in the dry period, 95.5 ± 5.5 μm at 28 d after calving, and 104.2 ± 5.3 μm at 56 d postpartum. The percentages of anagen hair follicles during these 3 periods were 41.4 ± 3.4, 18.5 ± 3.4, and 32.3 ± 3.3%, respectively. The corresponding sebaceous gland sizes were 8,362.0 ± 707.6, 7,800.0 ± 831.4, and 9,186.8 ± 962.6 μm^2, respectively. Hair follicle width was positively correlated with percentage of anagen hair follicles. The thickness of epidermal and proliferation rate of epidermal cell were also correlated. However, the hair follicle width, sebaceous gland size and cell proliferation rate, and thickness and proliferation rate of epidermal cells did not show any marked changes.
Key words: dairy cow, skin, morphology, periparturient

Short Communication

Skin of malnourished animals and humans shows signs of atrophy (Mathur and Doe, 1976; Thavaraj and Sesikeran, 1989). Malnourished beef cattle show follicular atrophy and decreased epidermal thickness (Uesaka and Yahata, 1955; Haaland et al., 1977). The nutritional state of hair follicles determines hair growth in sheep (Ryder, 1973), and it has also been suggested that hair thickness is influenced by the nutritional state of the hair follicles in humans and rats (Sims, 1968; Salas et al., 1995). The sebaceous glands of humans respond to prolonged caloric deprivation with diminished sebum secretion and altered sebum composition (Pochi et al., 1970). Undernourished animals have a poor hair coat (Miyamoto et al., 1941; Nielsen et al., 1975; Watson, 1998; Kirby et al., 2009); thus, changes in the hair coat may indicate an animal's nutritional status. Hair growth is a cyclical process that varies during the different follicular phases; hair is produced during the anagen phase and hair growth stops during the telogen phase. Sebum, which gives hair its luster, is secreted by the sebaceous glands. Identification of early changes to the hair coat requires histological examination. Changes in the hair coat may reflect underlying changes in the skin (Landaeta-Hernández et al., 2011). Modern dairy cows produce a large quantity of milk with inadequate energy intake during early lactation, and thus are in negative energy balance during the postpartum period (Berglund and Danell, 1987; Butler, 2003). It is hypothesized that hair follicle size and cycle phase, sebaceous gland size and cell proliferation, and epidermal thickness and cell proliferation in cows show temporal changes during the periparturient period. The aims of this study were, therefore, to identify changes in the skin morphology of dairy cows during the peripartum period.

The study protocol and all procedures were conducted in accordance with the guidelines of the Ethics Committee for the Care and Use of Laboratory Animals for Research of Niigata University, Japan. This experiment was conducted at the Field Center for Sustainable Agriculture and Forestry of Niigata University. Seven multiparous Holstein cows that calved between June and December 2014 were used. The mean ± standard deviation of lactation number was 3.4 ± 1.3. The average milk yield per cow was 8,700 kg/305 d. The herd was managed in a tiestall shed and fed concentrate and forage separately. In the dry period, cows were given 2.0 kg/d of concentrate, 1.0 kg/d of beat pulp pellets,

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and haylage ad libitum. After parturition, the amount of concentrate given was increased by 1 kg every week up to an appropriate volume for each cow depending on its milk yield and BW. The study period consisted of the close-up period (3 wk before parturition) to early lactation (3 wk after parturition), during which time the energy balance decreased drastically and then recovered (Chagas et al., 2007; Bicalho et al., 2017). Over the experimental period, skin and blood samples were collected 3 times, at 28 d before the expected calving date, and 28 and 56 d after parturition. To indicate the energy metabolism of cows, the serum concentrations of fatty acids were measured using a discrete-type clinical chemistry automated analyzer (TBA-120FR, Toshiba Medical Systems, Tochigi, Japan) with NEFA-HR (Wako Pure Chemical Industries, Osaka, Japan). Body condition scores were measured at the same time. One diclofenac tablet containing 25 mg of diclofenac sodium (Bolabomine 25 mg, Tsuruhara Pharmaceutical, Osaka, Japan) was administered per 100 kg of BW 30 min before skin sample collection. For ease of collection, the shoulder is the best position because it is easy to immobilize and take a piece of skin. Two skin biopsies were collected from the left shoulder of each cow using an 8-mm dermatome (Biopsy Punches, Kai Industries, Tokyo, Japan); these biopsies were later used for histological and immunohistochemical analyses.

Skin samples were fixed in a 10% buffered-formalin solution. After fixation with 10% buffer-formalin solution, skin samples were divided pieces 3 mm × 4 mm × 2 mm of size and embedded in paraffin wax or frozen in optimal cutting temperature compound (Sakura Finetek Japan, Tokyo, Japan). Fifteen 5-μm-thick paraffin-wax-embedded sections were stained with hematoxylin and eosin (**HE**). Five 10-μm-thick cryosections from 15 frozen optimal cutting temperature samples were stained with Oil Red O to allow observation of the location of lipids within the tissue and to analyze its size. After general light microscopy analysis, the morphometry of each skin sample was analyzed as described below.

Immunohistochemical detection of proliferating cell nuclear antigen (**PCNA**), a marker of cell proliferation (Mokrý and Němecek, 1995), was performed using a Vectastain ABC-AP standard kit (Vector Laboratories, Burlingame, CA), in accordance with the manufacturer's protocol. In brief, the paraffin sections described above were reacted with a primary mouse anti-PCNA antibody (Abcam, Cambridge, UK) and a secondary biotinylated goat anti-mouse IgG antibody (Vector Laboratories) at dilutions of 1/16,000 and 1/200, respectively. The reaction was visualized using ImmPACT Vector Red Alkaline Phosphatase Substrate (Vector Laboratories). Proliferating cells showed red staining in their nuclei, and the cell proliferation was represented as the ratio (%) of PCNA-positive cells to total cells counted.

The hair follicle width and activity (percentage of follicles in the anagen phase), sebaceous gland size and activity (cell proliferation rate), and epidermal thickness and activity (epidermal cell proliferation rate) were examined as skin morphological parameters. Three to 5 skin sections were chosen randomly from each paraffin block. To assess hair follicle width, hair follicle phase, and epidermal thickness, skin sections stained with HE were used. To measure sebaceous gland size, Oil Red O-stained sections were used. Longitudinal (vertical) sections were used for analyzing hair follicle width, sebaceous gland size and activity, and epidermal thickness and activity, whereas transverse (horizontal) sections were used for investigating hair follicle phase. Five sections were selected randomly and 3 images (magnification × 100) were selected randomly for each section and the skin morphological parameters were assessed using image analysis software (Nikon NIS-Elements Documentation Ver. 3.13, Nikon Corporation, Tokyo, Japan). The numbers of hair follicles in the anagen and telogen phases of the hair cycle were counted and the anagen:telogen ratio was calculated. Exogen follicles were considered as those with features of both anagen and telogen phases. Hair cycle stages were identified using the method described by Innerå et al. (2013) and Kim et al. (2014). Hair follicles in the anagen phase were defined as having a well-defined inner root sheath (Figure 1a). Hair follicles in the telogen phase were defined as having an inner root sheath that appeared as a red amorphous keratin mass (Figure 1b). Hair follicles with characteristics of both anagen and telogen phases were observed (Figure 1c); these follicles were considered to be in the exogen phase, the phase during which a new hair shaft grows before the resting shaft has been shed (Stenn et al., 1998). Epidermal thickness was defined as the distance of the stratum basale and the stratum granulosum. The thickness of each skin section was calculated as the mean of 30 distances randomly measured. Stratum corneum, rete pegs, and hair follicles were excluded from the measurements. To measure the proliferation rate of sebaceous gland cells, 3 randomly selected, paraffin-embedded skin sections per cow were used. The sebocytes in the sebaceous glands were counted. All sebaceous glands on each slide were observed. In addition, to evaluate the epidermal cell proliferation rate, 5 randomly selected skin sections were used. For each skin section, between 200 and 300 epidermal keratinocytes were counted.

Data were saved in Microsoft Excel 2010 (Microsoft Corp., Redmond, WA). Statistical analyses were conducted using JMP (Japanese version 4.4, SAS Institute