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Peculiarities of skin glycome in progeny of hypothyroid female rats during pre- and postnatal ontogenesis according to lectin histochemistry data

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SUMMARY

The morphogenesis of the skin in the progeny of hypothyroid female rats in pre- and postnatal ontogenesis was studied using general morphology and lectin histochemistry methods. The experiment was conducted on 20 female Wistar rats weighing 180-200 g, which were divided into two groups: control (10), and experimental (10), from which were obtained 70 and 46 offsprings, respectively. Experimental hypothyroidism was induced by daily food supplementation with antithyroid drug mercazolil at the rate 5 mg/kg of animal body weight. After the second week of the experiment, females in the estrus stage were paired with males. Skin samples from the back of experimental and control groups progeny rats were taken on the embryonic day 16, and on the postnatal days 1, 10, 20 and 40. Skin samples were fixed in 4% neutral formalin and embedded in paraffin. 5-7 µm thick sections were stained with hematoxylin and eosin. Carbohydrate determinants were detected using a set of lectin-peroxidase conjugates as follows: Con A, PNA, HPA, WGA, SNA, LABA and LTFA. Maternal hypothyroidism, alongside increased total body weight of progeny animals, induced hypodermal thickening, enhanced activity of granular cells layer and of keratinization processing. Rearrangement of lectin receptor sites included reduced reactivity of PNA, SNA and WGA, which play an important role in the formation of adhesive contacts between keratinocytes, and of DGal(β1- 3)DGalNAc and βDGal carbohydrate determinants in the sebaceous gland secretory products, which regulate proliferation and differentiation of cells of hair follicles.

Key words: Skin – Mercazolil – Hypothyroid rats progeny – Lectin histochemistry

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INTRODUCTION

Over the past decades, the frequency of hypothyroidism in the general population worldwide has increased from 0.5% to 2.0%, while in the subpopulation aged over 50, predominantly of female gender, grew up to 6-8%. At the same time, the frequency of subclinical hypothyroidism form increased up to 12-18% (Taylor et al., 2018; Mendes et al., 2019; Voloshyn et al., 2020). It was also estimated that maternal hypothyroidism during pregnancy and lactation leads to hypothyroidism of progeny (Lucaccioni et al., 2020; Božinovski et al., 2023). Moreover, maternal hypothyroidism conditions prolongation of pregnancy associated with fetal weight decrease in the embryonic and early postnatal periods of life (Strus et al., 2013; Lutsyk et al., 2018; Shegedin et al., 2020).

It is well known that thyroid hormones regulate proliferation and differentiation of epidermal cells and significantly affect skin homeostasis. The most common dermatological symptoms in hypothyroidism include skin desiccation, hair loss, swelling of the face, itching skin of hands and feet, and decreased sweating (Jamwal et al., 2013). Under the influence of thyroid hormones, from the very early stages of pregnancy, while the fetus is gaining weight, its brain and somatic tissues develop. Thyroid hormones affect synthesis of other hormones and regulate histogenesis of tissues, ensure the activation of such physiological processes as thermogenesis, gluconeogenesis, pulmonary gas exchange, and cardiac adaptation at birth (Forhead et al., 2014). A summary of thyroid influences on the developing embryo and fetus include: neurocytes, neuroglia and synaptic development, cardiomyocytes maturation and cardiovascular system development, type II pneumocytes development and surfactant synthesis, bone, muscles and skin development, body weight and length at birth, activation of thermogenesis in brown adipose tissue at birth, and activation of gluconeogenesis at birth (Klosinska et al., 2022).

With a deficiency of thyroid hormones, which are necessary for almost every cell normal function, severe changes develop in all organs and systems. Under primary hypothyroidism, all types of metabolic processes, in particular oxygen utilization by tissues, oxidative reactions, and the activity of various enzymatic systems, are inhibited. In addition, gas exchange and basic metabolism are reduced. Acidic glycosaminoglycans are deposited in the heart, lungs, kidneys, serous cavities, and all layers of the skin. These bind water, further inducing edematous conditions (Repetska, 2019; Pankiv, 2020). Receptors of thyroid hormones were identified in the nuclei of keratinocytes, in fibroblasts, structural components of hair follicles and sebaceous glands, which indicate an active role of these hormones in the metabolic pathways in all integumental constituents (Silva et al., 2020). However, current knowledge on the influence of maternal thyroid hormone imbalance on the development of skin and its derivatives of their progeny is incomplete in many ways.

Lectins are widely used as tools in histochemical studies mapping glycosylation in cells and tissues (Gabius et al., 2014; Pusam et al., 2021; Varki et al., 2022; Brooks, 2023). It is generally accepted that carbohydrate determinants – oligosaccharidic chains of cell and tissue glycoconjugates – play a key role in the processes of morphogenesis, providing intercellular and cell-to-matrix interactions. Changes in the carbohydrate repertoire of the cell membrane can lead to irreversible consequences in the process of embryogenesis, development of lysosomal storage diseases, and malignant transformation in the postnatal period (Antonyuk, 2005; Schnider et al., 2023). Rearrangement of lectin receptor sites in the cell membranes, cytoplasmatic and nuclear compartments can reflect the level of cellular functional activity, cellular ability to migrate, phagocytic capabilities, the onset of irreversible changes and apoptosis (Brooks, 2023).

The principle of lectin-receptor interaction is based on the complementarity of the lectin active center and mono- or disaccharide glycoconjugate residue (as a rule, terminal or subterminal), available for binding to it. The degree of affinity is determined by the number of places of mutual attachment of the carbohydrate determinant to the active center of the lectin molecule (Brooks et al., 2006; Gabius, 2014; Varki et al., 2022). Considering all the above, general morphology and lectin histochemistry investigation of the skin of progeny of hypothyroid females is relevant. The aim of the present investigation is to study the influence of maternal hypothyroidism on the morphogenic differences in rat offspring skin in pre- and postnatal ontogenesis.

MATERIALS AND METHODS

The study was accomplished on 20 female Wistar rats, weighing 180-200 g, which were divided into two groups: control (10 rats), and experimental (10 rats), from which offspring were obtained in the count 70 and 46, respectively. The difference in the number of offspring between the control and experimental groups is among the chief outcomes of female hypothyroidism, resulting in decreased fertility. The mechanism of this phenomenon is described elsewhere (Lutsyk and Sogomonyan, 2012; Shegedin et al., 2017). Animals were kept in vivarium conditions with compliance of sanitary and hygienic standards and diet. The experiment was conducted after getting the approval of the bioethics committee of Danylo Halytsky Lviv National Medical University for experiments on animals (protocols № 1 of January 31, 2018, and № 4 of April 15, 2024).

Experimental hypothyroidism was induced by antithyroid drug mercazolil (1-methyl-2-mercaptoimidazole, Zdorovia, Kharkiv, Ukraine), which was added daily in powdered form to the rats' food allowance at a rate 5 mg/kg of body weight for 14 days. After the second week of the experiment, females in the estrus stage were mated to males. From the moment of dated pregnancy, on prenatal day 16th and on the postnatal days 1st, 10th, 20th and 40th, skin samples were taken from the back of the offspring of experimental and control group rats. Thyroid function was controlled by estimation of T3 and T4 hormones in the blood serum by radioimmunological method using standard kits in the radioisotope laboratory of Lviv regional clinical hospital, by the investigation of maternal thyroid glands micromorphology, as well as weighing offspring at the appropriate periods of their development. Before sampling histological material, animals were removed from the experiment using diethyl ether narcosis overdose. Thyroid glands of maternal rats, as well as the skin samples taken from the back side of their offspring, were fixed in 4% neutral formalin and embedded in paraffin. For general morphology, 5-7 µm thick tissue sections were stained with hematoxylin and eosin as described elsewhere (Horalsky et al., 2015).

Skin glycoconjugates were detected by lectin-peroxidase technique using a set of seven lectins with different carbohydrate specificities (Table 1). All lectins were purified and their peroxidase conjugates prepared by Professor V.O. Antonyuk (Lectinotest, Lviv, Ukraine). Carbohydrate determinants were visualized according to the lectin-peroxidase-diaminobenzidine staining protocol (Brooks, 2006; Lutsyk et al., 2018). In detail, deparafinized sections were incubated 20 min in methanol containing 0.3% H_2O_2 to block activity of endogenous peroxidase; through graded ethanol brought to phosphate buffered saline (PBS pH 7,4), rinsed in three portions of PBS (5 min each), and incubated 45 min with lectin-peroxidase conjugate (dilution 50-75 µg/ml in PBS) in a moist chamber at room temperature. Lectin receptor sites were visualized in PBS, containing 0.05% diaminobenzidine (Sigma, St. Louis, USA)

Table 1. Used lectins and their carbohydrate specificity.

Lectin name	Sourse of lectin	Its carbohydrate specificity
Con A	Canavalia ensiformis	αDMan > DGlc
PNA	Arachis hypogaea	βDGal, DGal(β1-3)DGalNAc
HPA	Helix pomatia	αDGalNAc
SNA	Sambucus nigra	Neu5Ac(α2-6)DGal/DGalNAc
LABA	Laburnum anagyroides	αLFuc
WGA	Triticum vulgare	αDGlcNAc > Neu5Ac
LTFA	Lactarius torminosus fungus	DGal(*β*1-3)DGalNAc

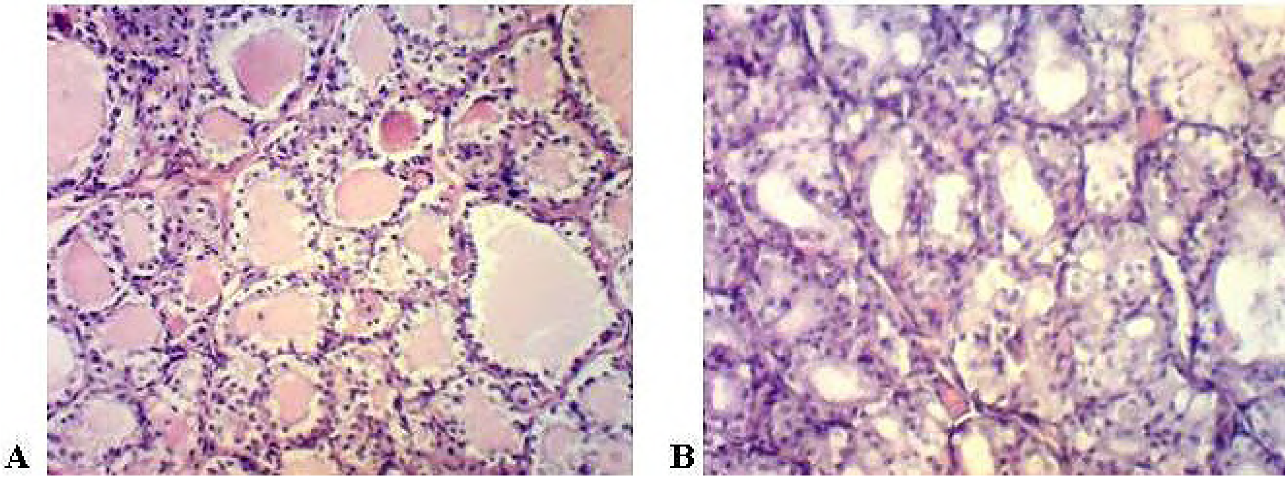

Fig. 1.- Thyroid gland of control and experimental group rats. Staining with hematoxylin and eosin. ×150. **A** – control; **B** – experimental hypothyroidism.

and 0.15% H_2O_2. Thereafter, slides were twice washed in distilled water, and after dehydration they mounted in balsam.

The specificity of histochemical reactions was controlled by: (1) omitting lectin-peroxidase from the staining protocol; and (2) pre-incubation of tissue sections prior to lectin labeling for 60 min in 1% HIO_4 (Reanal, Budapest, Hungary) for oxidative damage of carbohydrate determinants. In both cases, the staining results were negative. Microscopical investigation and photography of the obtained slides were carried out using a microscope "Swift Instruments International", equipped with a digital camera "Echoo-Imager 502 000" using the computer program "TopView 3.2". Statistical processing of T3 and T4 levels evaluation were carried out using Microsoft Office Excel 2003 and Statistica.6 (USA) with the definition of the average value "M" and average error "m" and a sample size "n"=20 (10 of control group and 10 of experimental group rats). Three levels of significance were used: $p<0.05$ – statistically significant, $p<0.01$ and $p<0.001$ – both values highly statistically significant (very strong evidence). For the evaluation of lectin binding, two investigators performed the analysis independently, blinded to lectin type. Binding intensity was represented in the semiquantitative scale as follows: strong, positive, and negative.

RESULTS

Thyroid glands of experimental group rats at the time of their removal from experiment (days 30, 36, 46, 56 and 76 since the beginning of mercazolil feeding, which correspond to progeny days 16 prenatal, 1, 10, 20 and 40 postnatal) were approximately 2-3 times larger compared to the control group animals. Microscopically cuboidal thyroid follicular epithelium under the experimental conditions acquired a cylindrical shape, with the follicular lumen devoid of colloid (Fig. 1). Moreover, thyrocytes demonstrated signs of hyperplasia in combination with hyperemia of the organ in total. The thyroid glands as a whole increased its weight and size due to the enhanced, but ineffective cellular activity, hyperemia and edema. These morphological changes in the thyroid glands were accompanied by a decreased content of thyroid hormones in blood serum (T3: control = 1.16±0.11 nmol/l, experiment = 1.04±0.13 nmol/l, $p<0.01$; T4: control = 50.00±3.39 nmol/l, experiment = 40.67±3.6 nmol/l, $p<0.01$).

General morphology studies of progeny skin on embryonic day 16 revealed that the epidermis of both control and experimental animals consists of 1-2 layers of highly prismatic epithelial cells with visible figures of mitosis, and dermis composed of embryonic connective tissue. It is noteworthy that, at this term of development, no epithelial combs or dermal papillae are visible. a microcirculatory bed is filled with formed blood elements, among which predominate normoblasts with oxyphilic cytoplasm and basophilic nuclei. The hypodermis is absent (Fig. 2A, B). On postnatal day one, the skin of both control and experimental group animals begins to acquire a

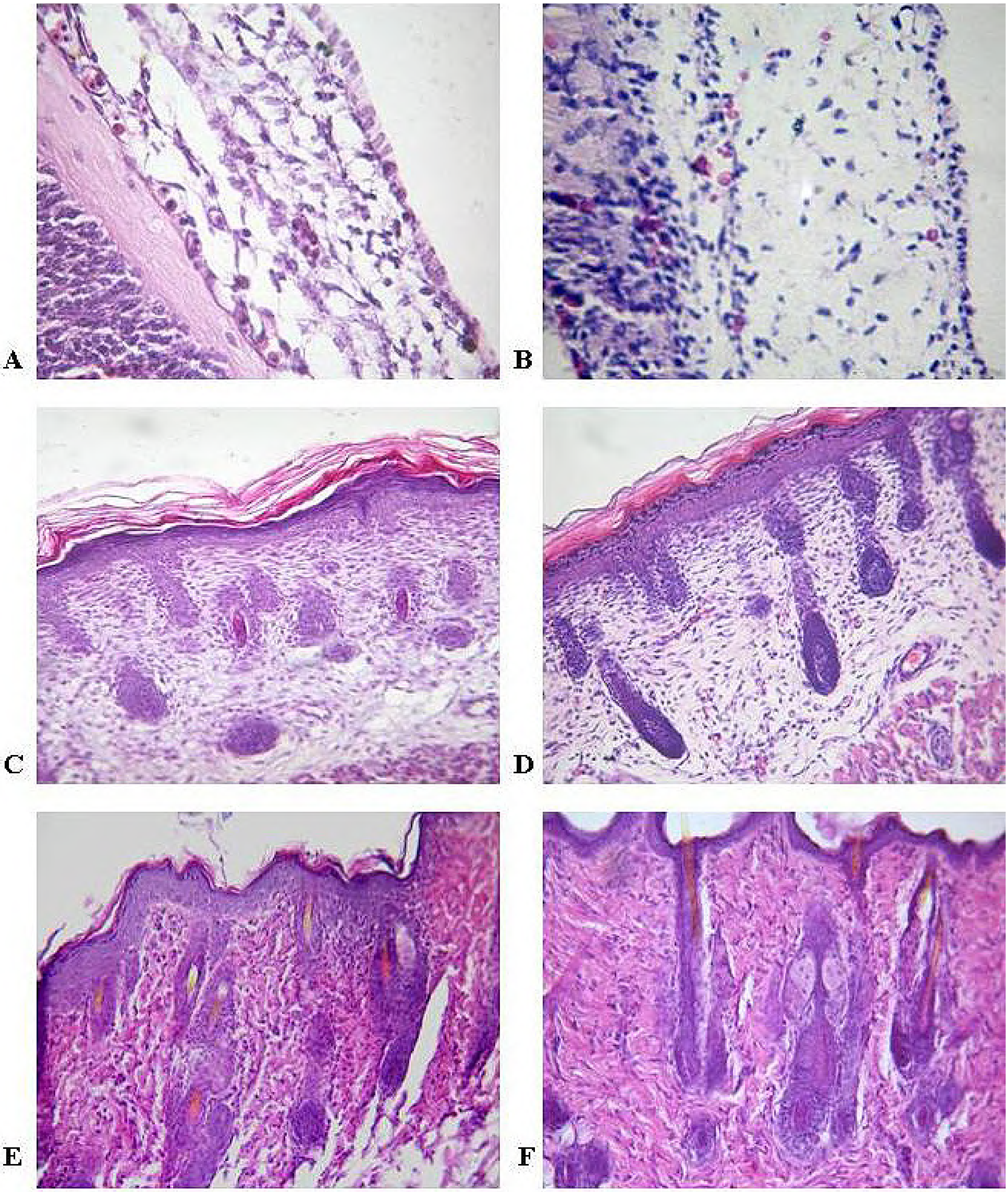

Fig. 2.- Skin of a progeny rats on the subsequent stages of morphogenesis. Hematoxylin and eosin stain. **A** – control, embryonic day 16, ×400. **B** – experiment, embryonic day 16, ×400. **C** – control, postnatal day one, ×200. **D** – experiment, postnatal day one, ×200. **E** – control, postnatal day 40, ×200. **F** – experiment, postnatal day 40, ×200.

definitive structure. The epidermis consists of well-defined four layers, including basal, spinous, granular and horny. The dermis is differentiated into two layers – papillary and reticular, the latter enriched with fibrous structures and hair follicles of two types: the larger follicles belong to covering hairs, the smaller – to downy hairs (Fig. 2C). At this time of development, subcutaneous adipose tissue is weakly expressed. In the skin of experimental group animals, the granular layer of the epidermis is overexpressed, with intense cytoplasmic granularity compared to controls, while the number of hair follicles is reduced (Fig. 2D).

On the postnatal day 10, a large number of hair follicles with varying diameters, reflecting different stages of maturation, are located between the reticular layer of dermis and hypodermis of control group offsprings. At this stage of development, the hair of offspring rats gain structure similar to that of sexually mature animals. Clearly visible sebaceous glands are seen in the interface between papillary and reticular layer of the dermis, the ducts of which open into the hair root, and secretory units formed by oval-round shape sebocytes with oxyphilic cytoplasm. Nuclei of sebocytes are eccentrically localized, have elongated shape, and are basophilic. The root of covering and downy hair has similar structure in control and experimental group animals. The dermis of the skin is well defined with a large number of microcirculatory vessels filled with formed blood elements. Papillary and reticular layers are well identified, with randomly oriented connective tissue fibers.

On the postnatal days 20-40, the epidermis of both control and experimental group animals is represented by four distinct layers: basal – one row of highly prismatic cells; spinous – 2-3 rows; granular – one row; corneum – several rows of keratinized epitheliocytes. The papillary layer of the dermis is formed of loose connective tissue with a large number of cellular elements, among which fibroblasts predominate. The reticular layer is composed of multidirectional bundles of collagen and elastic fibers. Incontinuous, weakly expressed hypodermis consists of adipocytes forming small groups. The dermis is enriched with a large number of different diameter hair follicles, some of them on the 20 postnatal day decorated with sebaceous glands, the number of which increase up to the postnatal day 40 (Fig. 2E). Maternal hypothyroidism, alongside with enhanced body weight of progeny animals, on the postnatal days 20 and 40, induced thickening of the hypodermis. In the epidermis of experimental group animals, the number of rows in the spinous and granular layers increase apparently due to the intensified keratinization processes. In the skin of experimental group offspring on the postnatal day 40, the microcirculatory bed exposes signs of vasodilation and is overloaded with hyperaggregates of formed blood elements. Depending on the stage of differentiation, hair follicles with greater or lesser extent demonstrate thickening of outer epithelial root sheaths (Fig. 2F).

Investigation of skin glycome with a set of lectins with different carbohydrate specificities revealed presence in both control and hypothyroid group animals on the prenatal day 16 αDGalNAc, αDGlcNAc, βDGal, αLFuc, αDMan and Neu5Ac carbohydrate determinants. In the embryonic vessels of the dermis, formed blood elements were enriched with glycoconjugate determinants αDGalNAc (HPA receptor sites), as well as DGlcNAc and Neu5Ac (WGA receptor sites) (Fig. 3A, B). On the postnatal day one, the most intense exposure of lectin receptor sites was detected on the surface of epidermal keratinocytes, while carbohydrate determinants of αDGalNAc (HPA receptors) (Fig. 3C) and αLFuc (LABA receptors) more selectively decorated outer epithelial root sheaths of the developing hair follicles. The process of medullary substance formation in the covering hairs was accompanied by the accumulation of sialoglycans recognized by WGA and lack of HPA receptors (Fig. 3D).

In the offspring of the experimental group with maternal hypothyroidism, cells of hair medulla showed decreased intensity of WGA and SNA lectins labeling, the affinity of which is directed towards αDGlcNAc and Neu5Ac(α2-6)Gal/GalNAc carbohydrate determinants. These same lectins exposed enhanced reactivity with fibrillary structures of dermal root sheaths, nerve fibers and outer epithelial root sheaths compared to controls. DGal(β1-3)DGalNAc oligosaccharides (LTFA receptor sites) were detected on the postnatal day one within individual cells of the epidermal spinous layer, most likely the cells of Langerhans, the number of which dramatically increased due to the initial contact of newborn rats with the external environment (Fig. 3E, F).

In the skin of control group progeny rats on the postnatal day 10, cells of hair medulla, as well as cells of the outer epithelial root sheaths, exposed selective labelling with SNA lectin (Fig. 4A, C). At the same stage of development, SNA receptor sites were also detected in the sebaceous glands, predominantly on the surface of sebocytes (Fig.

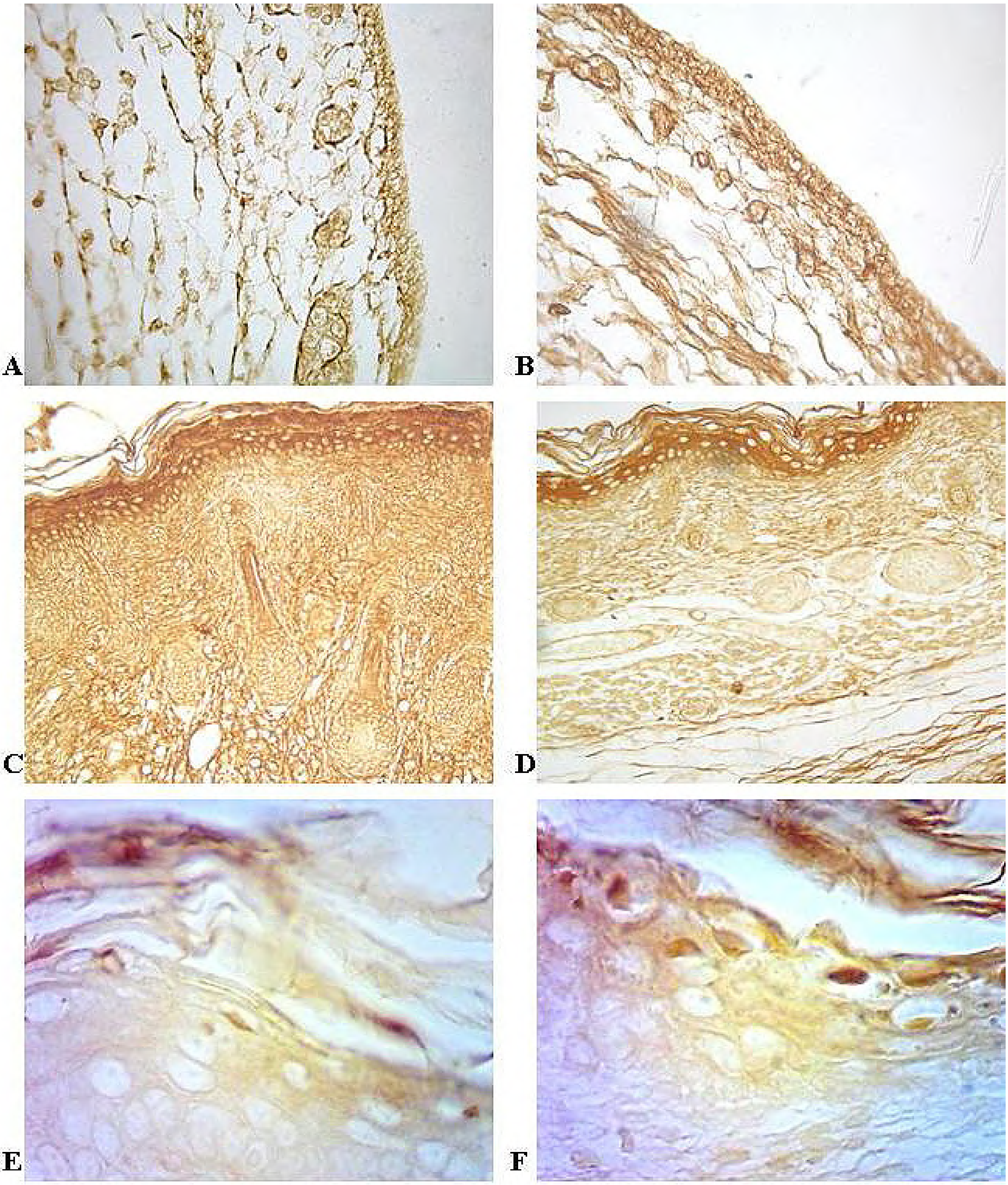

Fig. 3.- Skin of a progeny rats in pre- and early postnatal ontogenesis. **A** – control, embryonic day 16, WGA label. ×400. **B** – experiment, embryonic day 16, WGA label. ×400. **C** – control, postnatal day one, HPA label. ×200. **D** – experiment, postnatal day one, HPA label. ×200. **E** – control, postnatal day one, Langerhans cells in the spinous layer, LTFA label. ×900. **F** – experiment, postnatal day one, Langerhans cells in the spinous layer, LTFA label. ×900.

4E). In the skin of the experimental group animals, a decrease in the SNA reactivity, labeling Neu5Ac(α2-6)Gal/GalNAc carbohydrate determinants, was noted (Fig. 4B, D, F). The hair follicles of different diameters demonstrated differences in WGA reactivity. Namely, in hair follicles of smaller diameter (underhair) lacked medulla, whereas cells of outer epithelial root sheaths were WGA-positive. In the hair follicles of larger diameter (covering hair), WGA receptors are exposed

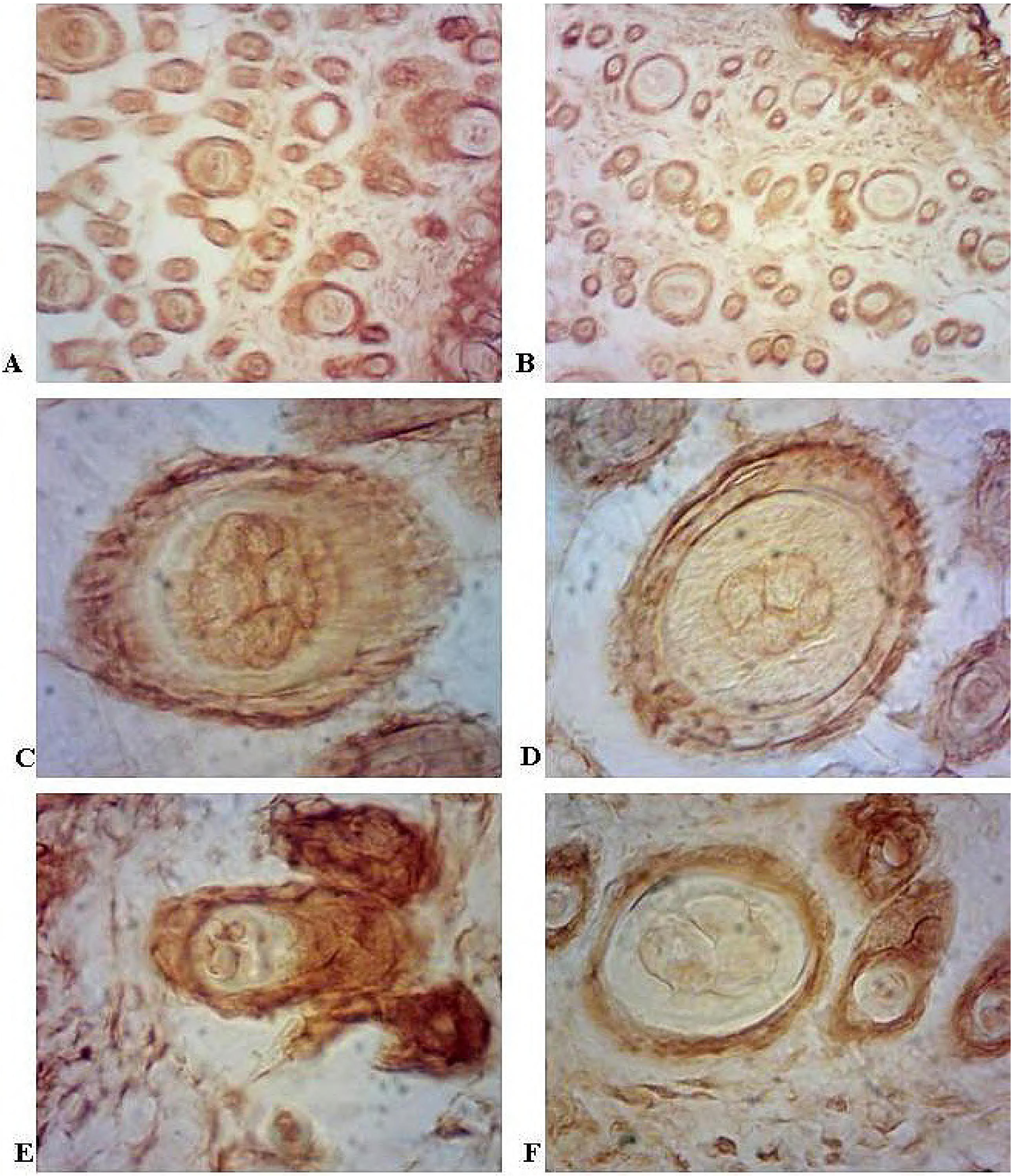

Fig. 4.- Skin of a progeny rats, postnatal day 10, SNA labeling. **A** – control: SNA reactivity of outer epithelial root sheath and hair medulla. ×150. **B** – experiment: decreased lectin labeling. ×150. **C** – control, detail of Fig. 4A. ×900. **D** – experiment: detail of Fig. 4B. ×900. **E** – control: SNA labeling of hair follicle with the adjusting sebaceous glands. ×900. **F** – experiment: decreased lectin labeling in comparison with Fig. 4E. ×900.

with high density in the cortex, medulla and cuticle of hair roots, as well as in the outer epithelial and dermal root sheaths (Fig. 5A, C). On the contrary, in the hair of experimental group progeny rats, the cuticle was labeled weakly, with barely noticeable exposure of WGA receptors (Fig. 5B, D).

On the postnatal days 20 and 40, the skin of control group progeny rats exposed strong reactivity of all epidermal layers with lectins SNA (Neu5Ac(α2-6) Gal/GalNAc) and WGA (αDGlcNAc>Neu5Ac). The most reactive were cells of the stratum corneum and the epidermal lipid barrier substance (Fig.

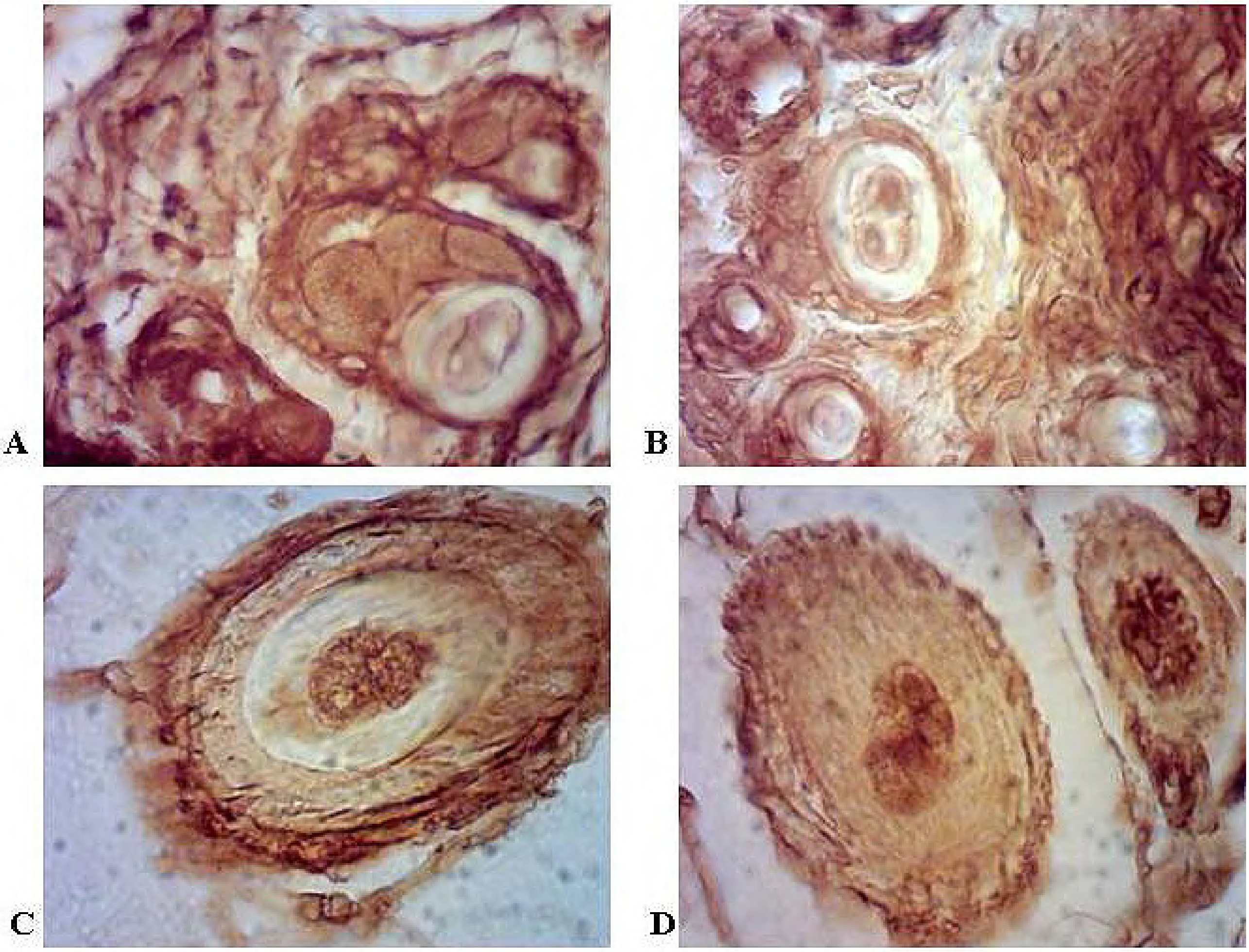

Fig. 5.- Skin of a rat progeny on postnatal day 10, WGA labeling. **A** – control. ×600. **B** – experiment. ×600. **C** – control. ×900. **D** – experiment: decreased WGA reactivity with outer epithelial root sheath and hair medulla. ×900.

6A). Moreover, receptor sites for the same sialo-specific lectins were detected in the hair follicles and within the cells of sebaceous glands secretory cells, as well as in the fibroblasts of the dermal papillary layer. In the skin of experimental group animals on the postnatal days 20 and 40, SNA and WGA receptor sites had a similar localization to that in control group rats (Fig. 6B). In the growth area of hair follicles, the most intense binding was observed with the cells of the inner epithelial root sheaths. Galactose-specific lectins PNA and LTFA on the 20 and 40 days of postnatal development in control group rats demonstrated preferential labeling of stratum corneum keratinocytes, as well as secretory units of sebaceous glands (Fig. 6C, E). Cells of the inner epithelial root sheath demonstrated rather heterogenous reactivity with these lectins, depending on the degree of their maturity. In the papillary layer of the dermis, PNA and LTFA preferentially labeled fibroblast-like cells, dispersed in the ground substance. The skin of the hypothyroid progeny rats on the postnatal days 20 and 40 demonstrated lectin labelling similar to the control group, specifically decreased labeling by PNA and LTFA of the sebocytes (Fig. 6D, F), in association with enhanced labelling with these lectins of hair bulb pigmentocytes.

Glycoconjugates with carbohydrate determinants αDGalNAc (HPA receptors), αDMan (Con A receptors) and αLFuc (LABA receptors) on the postnatal days 20 and 40, similarly to the other experimentally used lectins, showed high affinity to the scales of the stratum corneum, as well as to the surface of keratinocytes of other epidermal layers, secretory substance of sebocytes, and nerve fibers of neuro-vascular bundles. No significant differences were detected in these lectins' reactivity with cutaneous structures components of control and experimental groups rats. The only observed difference was decreased labeling of αDGalNAc determinants (HPA receptors) by sebo-

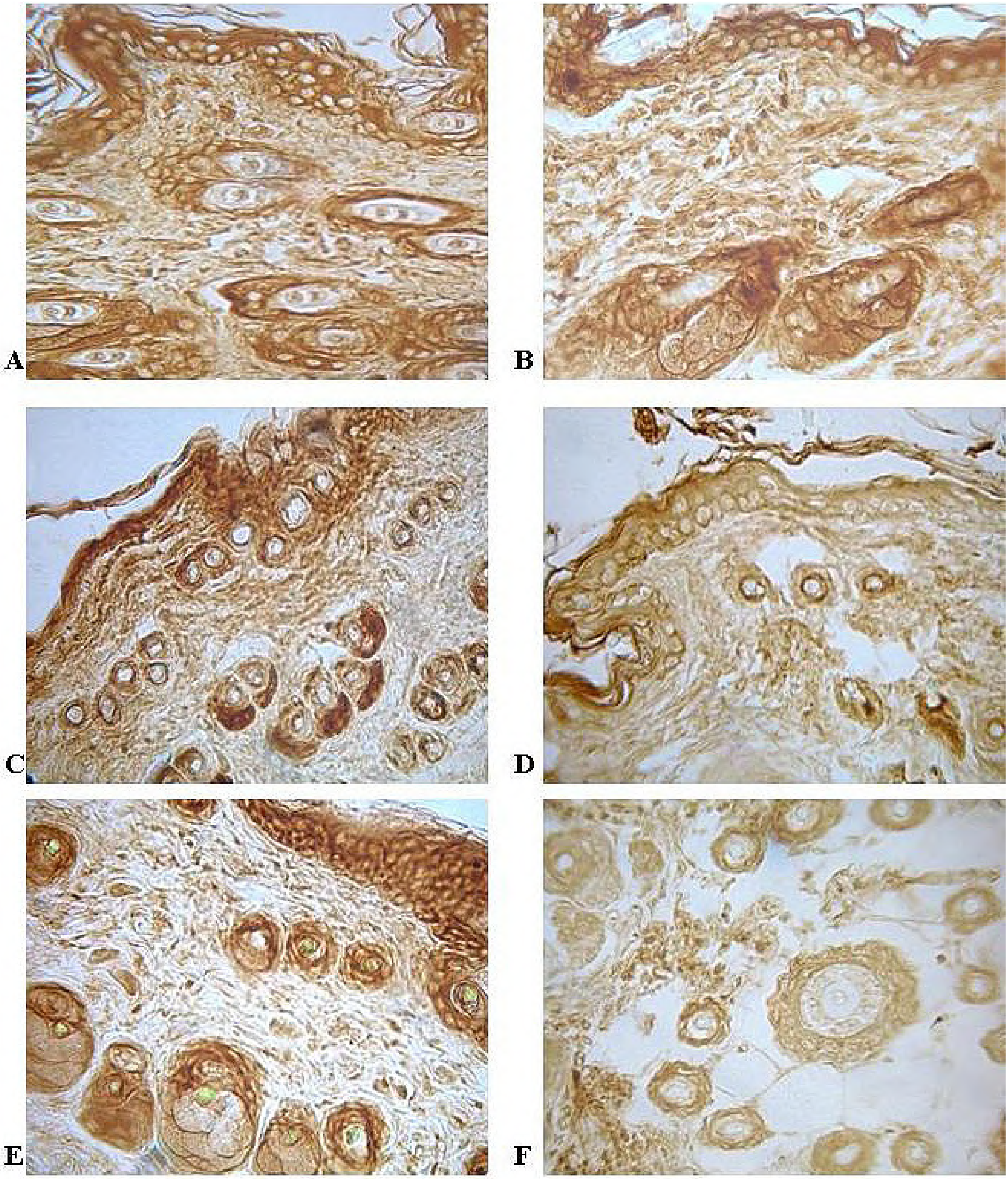

Fig. 6.- Skin of a progeny rats, postnatal day 20. **A** – control: SNA labeling of stratum corneum keratinocytes, cells of outer epithelial root sheaths of hair follicles and of sebaceous glands. ×400. **B** – experiment, no obvious differences in SNA labeling compared with Fig. 6A. ×400. **C** – control: strong PNA reactivity of stratum corneum and secretory portions of sebaceous glands. ×400. **D** – experiment: reduced PNA reactivity of sebaceous glands. ×400. **E** – control: strong binding of LTFA with stratum corneum keratinocytes, cells of outer epithelial root sheaths of hair follicles and with sebaceous glands secretory units. ×400. **F** – experiment: decreased LTFA reactivity of pilo- sebaceous units, very strong lectin binding to the scattered cells (apparently fibroblasts) in dermal connective tissue. ×400.

cytes. The process of hair follicle cells differentiation was accompanied by the redistribution of αL-Fuc containing glycoconjugates (LABA receptors) from the inner to the outer epithelial root sheaths.

DISCUSSION

Skin is among the most important target organs influenced by thyroid hormones, which play a crucial role in the maintenance of skin homeostasis. Therefore, certain skin diseases are closely associated with thyroid gland disturbances. In particular, alopecia, dermatitis, and vitiligo are associated with thyroiditis, while alopecia and eczema are often associated with Graves' disease (Mancino et al., 2020, 2021).

Our findings in this study, based on general morphology and lectin histochemistry investigations, demonstrate significant influence of maternal thyroid imbalance on the morphogenesis of skin and its derivatives in their offsprings. On the postnatal days 10 and 20, the progeny of mercazolil-treated rats had increased body weight, thickening of epidermal spinous and granular layers, and increased number of small diameter hair follicles. In contrast, prenatal-day-16 offspring of hypothyroid rats showed no differences in these gross and microscopic elements compared to their control counterparts.

According to Krinke (2000), hair of rats is differentiated into two types – covering "guard hair" and downy "underhair". During development, the root of covering hair is composed of medulla, cortex and cuticle. The medulla, located in the center of the hair root, stains oxyphilic, and consists of two or three rows of partially keratinized polygonal cells with elongated compact nuclei and cytoplasmatic trichohyaline granules. The cortex consists of flat horny scales containing hard keratin granules and air bubbles within the cytoplasm. Our findings are consistent with data of Krinke (2000), reporting oxyphilia of hard keratin in contrast to basophilia of soft keratin, located in the epidermal granular layer. The cuticle is represented by one layer of poorly stained cells. In the progeny of the experimental group rats was observed more pronounced papillary layer of dermis compared to the control group. Also, in the experimental group animals it was documented more numerous small diameter (downy hair) follicles, lacking medulla.

Our findings on the overdeveloped granulosa layer in the epidermis of the experimental group progeny rats on the postnatal day 40 are consistent with data of others (Božinovski et al., 2023), who related these changes to strengthening of keratinization leading to the enhancement of protective mechanisms, preventing mechanical damage of skin. Recent clinical observations of Lucaccioni et al. (2020), who reported the development of transitional hypothyroidism in women during pregnancy and lactation, allow us to speculate that hypothyroid conditioning of both maternal and progeny organisms stimulate protective mechanisms, possibly preventing integumental damage in the fetus during the labor processes.

Carbohydrate residues of plasma membrane glycoproteins and glycolipids form cell surface glycocalyx, which serve as a specific designatory coating of each particular cell, ensuring intercellular recognition and interrelationships, as well as appropriate interaction with the microenvironment (Antonyuk, 2005; Gabius and Kayser, 2014; Varki et al., 2022; Brooks, 2023).

Glycopolimers are also present in the membranes of the Golgi apparatus, the endoplasmic reticulum and the nuclear envelope. Considering our previous findings using lectin histochemistry methods, we conclude that adult rats are more susceptible to thyroid hormone imbalance compared to their progeny or young animals. At the same time, hypothyroid conditions cause certain delay in embryonic processes and early postnatal development (Lutsyk et al., 2012, 2018, 2021; Shegedin et al., 2017, 2019; Strus et al., 2013). This notion is in good correlation with clinical observations by others (Lucacciomi et al., 2020; Klosinska et al. 2022; Božinovski et al. 2023).

Lectin histochemistry studies of the skin and its derivatives in progeny rats developing under the influence of maternal hypothyroidism revealed rearrangement of carbohydrate determinants. In particular, during postnatal days 1-10, we noted a decrease in the expression of sialoglycans, which are believed to play the role of signaling molecules and to affect the processes growth and differentiation of tissue structures (Brooks, 2023). It is also noteworthy that PNA and LTFA lectins on the 20 and 40 days of postnatal development exposed high affinity to the keratinocytes of epidermis, especially to the stratum corneum cells, secretory units of sebaceous glands and cells of the inner epithelial root sheaths of covering hair. However, the detected changes relate to the degree of hair follicles differentiation. In the experimental group animals, a reduction of PNA lectin receptors in sebaceous glands and cells of the internal epithelial root sheath was documented. Such modification of glycopolymers is likely to have a negative impact on regenerative processes that require cell surface interactions.

Previous studies of lectin receptors' rearrangement induced by hypo- and hyperthyroidism in different organs (ovaries, testes, adrenal glands, skin) revealed inhibited masking of DGal determinants of glycoconjugates by LFuc, DGalNAc and Neu5Ac residues (Lutsyk et al., 2012, 2018, 2021; Strus et al., 2013; Shegedin et al., 2017, 2019). We hypothesize that the thyroid imbalance induces changes in the cellular glycome by disturbances in the final glycosylation processing of glycoconjugates in the compartments of the Golgi apparatus. The changes found in the glycome can also be due to the errors in the enzymatic degradation of cell surface glycoconjugates induced by the deficit in thyroid hormones.

Amoh et al. (2017) reported identification of different types of stem cells residing within the skin, including keratinocyte progenitor cells, melanocyte progenitor cells expressed by pluripotent hair follicle stem cells. These same authors also suggest that pluripotent stem cells, located in the region of the bulge of the hair follicle, can differentiate into the nerve cells, glial cells, keratinocytes, smooth muscle cells, cardiac muscle cells, and melanocytes. Repetska (2019) and Pankiv (2020) speculated that in primary hypothyroidism, all types of metabolic processes, e.g. oxygen utilization by tissues, oxidative reactions, the activity of various enzyme systems are inhibited, whereas gas exchange, and basic homeostatic metabolism, are reduced. Acidic glycosaminoglycans, deposited in the heart, lungs, kidneys, serous cavities, are also present in all layers of the skin. According to Silva et al. (2020), hypothyroidism reduces the activity of enzymes in the sulfate cycle of cholesterol and changes the chemical composition of the lipid barrier of the skin, affecting the development of lamellar granules (keratinosomes, Odland`s bodies) and promoting their accumulation.

Reported results of lectin histochemical studies extend the understanding of carbohydrate-related molecular mechanisms of integumental histophysiology. Thyroid hormones are important regulators of the normal condition of the skin. Thus, our results demonstrating the modification of glycoconjugates in all skin components, as well as modification of keratinization processes, could be helpful in the development of new pharmacological agents for the treatment of skin diseases and in cosmetology.

CONCLUSIONS

Maternal hypothyroidism has a significant impact on the morphogenesis of skin and its derivatives in their offsprings, in particular, inducing the reduction of carbohydrate determinants, which play an important role in the formation of adhesive junctions, differentiation and proliferation, and cutaneous morphogenic processes.

In this study of fetal and newborn rat skin, at the early stages of ontogenesis, the stratum corneum of the epidermis and the lipid barrier of both control and experimental group animals expressed reactivity with all used lectins, while at later stages of differentiation, a reduction of PNA binding was detected in the basal and spinous cell layers of experimental group animals. At the same time, we documented seriatim rearrangement of lectin receptors sites in the structural components of both downy and cover hair follicles.

Sebaceous gland cells of experimental, but not control group rats on the postnatal days 20 and 40 were lacking βDGal(1-3)DGalNAc and βDGal carbohydrate determinants. We hypothesize that these differences indicate changes in the composition of sebaceous secretion and negatively affect the processes of differentiation and functional activity of the structural components of the skin as a whole.

Finally, lectin labeling confirmed their role as a useful tool for selective histochemical binding to epidermal macrophages (Langerhans cells – LTFA) and fibroblasts (SNA).

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