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Influence of maternal rats' hypothyroidism on morpho-functional peculiarities and glycome of progeny skin

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Abstract

We studied the influence of maternal hypothyroidism on progeny skin morphogenesis by means of histological, histochemical and lectin–histochemical methods. Hypothyroid conditions in rats were achieved by daily food supplementation with antithyroid drug Mercazolil. The experiment was conducted on 10 control and 10 hypothyroid rats, which delivered 70 and 46 offsprings, respectively. We discovered that maternal hypothyroidism induces the accumulation of mast cells (MCs) in the skin of progeny on the 1st, 10th and 20th postnatal days, with decrease of these cell's count returning to control level on 40th postnatal day. These results indicate that offsprings developing under conditions of maternal hypothyroidism are a risk group for changes in immune status and the occurrence of allergic reactions. The *stratum corneum* of epidermis, its lipid barrier as well as pilosebaceous units, in both control and experimental group animals, at the early stages of postnatal ontogenesis are enriched with carbohydrate determinants of *α*DMan, *β*DGal, *β*DGal(1–3)DGalNAc, *α*LFuc, *α*DGalNAc, *α*DGlcNAc, Neu5Ac. *Galanthus nivalis* agglutinin (GNA) is a selective histochemical marker of MCs, while *Lactarius torminosus* fungus agglutinin (LTFA) is a selective label of Langerhans cells. Maternal hypothyroidism resulted in reduction of lectin binding with the structural components of progeny skin and its derivatives. We speculate that alterations in glycoconjugate processing and degradation sequences have an impact on the cell signaling, formation of adhesive contacts, cellular proliferation and differentiation. The lectin set we used clearly demonstrated specific labeling of cellular subpopulations, monitoring glycoconjugates processing and degradation under physiological and pathological conditions in all skin components.

Keywords: skin, Mercazolil, hypothyroid rats' progeny, lectin histochemistry.

Introduction

Thyroid hormones (THs) play crucial role in the differentiation of fetal epidermis, barrier formation, hair growth, wound healing, keratinocyte proliferation and keratin gene expression enhancing the activity of enzymes of the cholesterol sulfate cycle and influencing the development of lamellar granules [1, 2]. Cultures of epidermal keratinocytes from newborn and adult rats showed reverse triiodothyronine (rT3) conversion, suggesting that deiodinases are expressed in the skin. Various THs target genes were also identified in the skin, including *K5*, *K6*, *K14*, *K15*, *K16* and *K17* keratins, which confirms the key role of THs in the endocrine regulation of keratin expression and their influence on keratinocytes proliferation and differentiation (Figure 1).

Over past decades, the frequency of hypothyroidism in general population increased from 0.5% to 2.0%, and in the population group after 50–55 years, mainly women, up to 6–8%. At the same time, frequency of hypothyroidism subclinical form increased up to 12–18% [3–5]. The most common skin symptoms in hypothyroidism are dry skin, hair loss, swelling of the face, hands and feet, itching, decreased sweating, etc. [6]. Several research groups [7, 8] reported that maternal hypothyroidism induces hypothyroidism in the offspring. Moreover, maternal hypothyroidism is accompanied by pregnancy prolongation and decrease in fetal weight [9, 10]. According to data of Klosinska *et al.* [11], fetal development strongly depends on maternal thyroid status until the end of the first trimester. Since the hypothalamus–pituitary–thyroid axis is not sufficiently developed until the end of pregnancy, premature babies have a disorder called hypothyroxinemia of prematurity, which leads to numerous unfavorable consequences (Figure 2).

THs are necessary for the adequate acquisition of total body weight, for the development of brain and somatic tissues of the fetus from the early stages of pregnancy; they affect the synthesis of other hormones and regulate histogenesis of tissues, ensure the activation of thermogenesis, gluconeogenesis, pulmonary gas exchange, cardiac adaptation at birth [12]. In primary hypothyroidism oxidative reactions, oxygen utilization by tissues is inhibited, the activity of various enzyme systems, gas exchange, and basic metabolism reduced. Acidic glycosaminoglycans are deposited in the heart, lungs, kidneys, serous cavities and all layers of the skin [13, 14]. Thyroid hormone receptors (THRs) are found in the nuclei of keratinocytes, fibroblasts, and structural components of pilosebaceous units [15]. However, current knowledge concerning the influence of maternal THs disbalance on the progeny skin and its derivatives morphogenesis is far from complete.

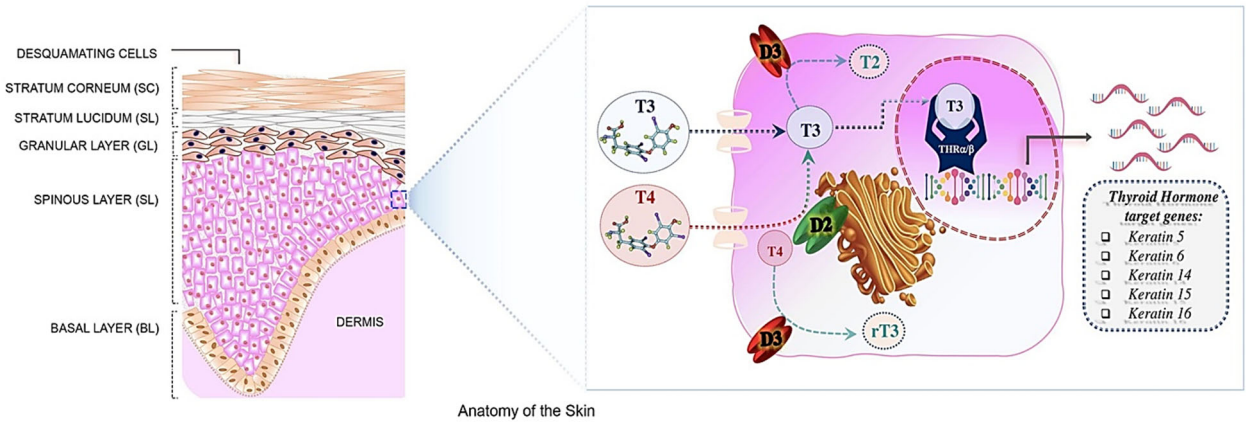

Figure 1 – ***Skin microanatomy and THs signaling. The THs signal is regulated in the skin by transport of T3 and T4 across the plasma membrane and enzymatic activation or inactivation, catalyzed by D2 and D3 deiodinases. The binding of T3 to THRs regulates the expression of the corresponding genes in K5, K6, K14, K15 and K16 keratinocytes, and confirms that THR is a key endocrine regulator that affects the proliferation and differentiation of keratinocytes (according to Mancino et al., 2021 [2]). T2: 3,5-Diiodo-L-thyronine; T3: Triiodothyronine; rT3: Reverse T3; T4: Thyroxine; TH: Thyroid hormone; THR: TH receptor.***

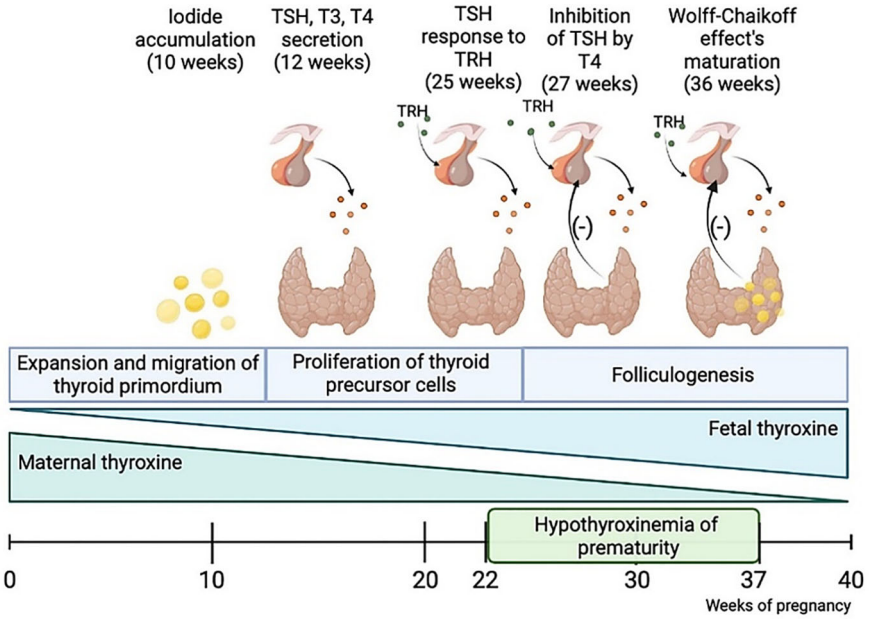

Figure 2 – ***Diagram of relationship between the thyroid gland and the hypothalamic–pituitary system during fetal development (according to Klosinska et al., 2022 [11]). Created with BioRender.com. T3: Triiodothyronine; T4: Thyroxine; TRH: Thyrotropin-releasing hormone; TSH: Thyroid-stimulating hormone.***

Literature survey figured out a lack of publications characterizing changes in the carbohydrate determinants in within the structural components of progeny skin, developed on the background of maternal hypothyroidism, although the glycome of cells plays an important role in the fundamental life processes. Glycoconjugates ensure mutual recognition and interaction of cells with their micro-environment, which is manifested by contact inhibition of proliferation, differentiation and morphogenesis of organs, interaction of cells with infectious agents, initiation of apoptosis, etc. [16]. Among modern tools of morphological research, lectin histochemistry methods play an important role.

Lectins possess the unique properties of selective binding to terminal mono- or disaccharide determinants of organ and tissue glycopolymers, allowing to obtain relevant information about their cytotopography and significance in the realization of embryo and morphogenesis, their role in dynamics of various physiological processes, as well as in the development of various forms of pathology [17, 18]. With the use of lectins, it is possible to selectively identify certain cellular subpopulations, organelles associated with the processing of particular glycopolymers, as well as areas of the plasma membrane involved in different mechanisms of cellular activity [19, 20]. Structure of typical lectin receptors are presented in Figure 3. Previously conducted studies of other organs (adrenal glands, testes, kidneys, uterus, lungs, etc.) under the conditions of thyroid gland dysfunction revealed prominent modifications of glycopolymers in both mature and developing organ systems [10, 21–24].

Aim

With regard to the abovementioned, the aim of present investigation was directed towards the evaluation of maternal hypothyroidism impact on morpho-functional characteristics of progeny skin.

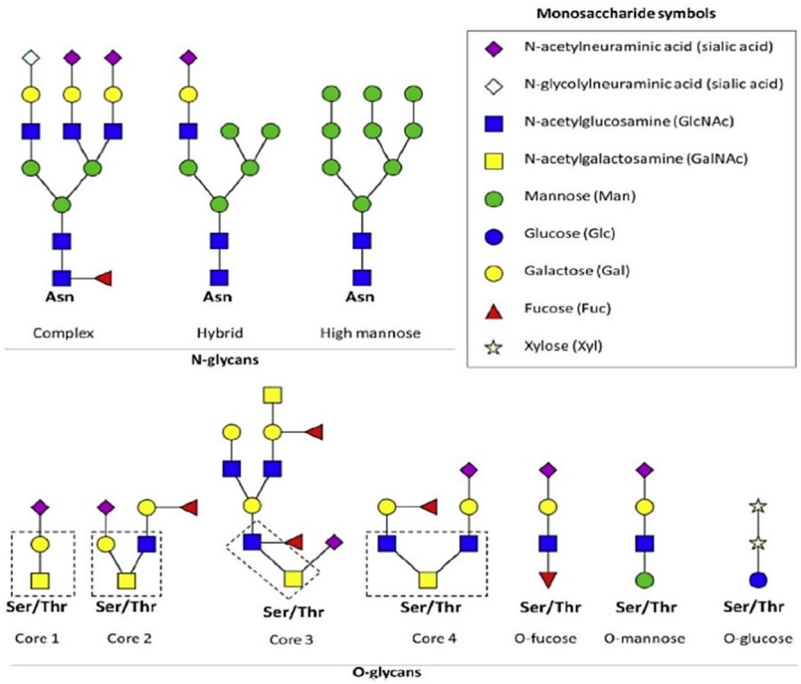

Figure 3 – ***Examples of N- and O-glycan structures that are linked to an asparagine (Asn) residue and to a serine (Ser) or threonine (Thr) residue, respectively. The most common symbolic nomenclature of monosaccharides is presented (according to Aizpurua-Olaizola et al., 2018 [18]).***

Materials and Methods

Investigation was carried out in the Vivarium of Danylo Halytsky Lviv National Medical University, Ukraine, on 20 female Wistar rats, weighing 180–200 g. Animals were divided into two groups: control (n=10) and experimental (n=10). Experimental group animals received antithyroid drug Mercazolil (1-methyl-2-mercaptoimidazole; Zdorovia, Kharkiv, Ukraine) added to daily food compliance at a dose of 5 mg/kg body weight for two weeks. Females were checked for their estrous cycle, and in the presence of estrus, they were mated to males. After 4–5 hours, vaginal smears were checked for the presence of spermatozoa. The presence of spermatozoa in vaginal smears was considered the beginning of pregnancy. During the experiment, in total 116 offspring were received from both groups of rats, including 70 from control and 46 from experimental animals. The offspring were weighed on the 1st, 10th, 20th, and 40th day. While working with animals, requirements of *General Ethical Principles of Experiments Animal* (Ukraine, 2001), harmonized with the recommendations of *European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes* (Strasbourg, 1986), Law of Ukraine No. 3447-IV of February 21, 2006, *On the Protection of Animals from Cruelty*, were followed thoroughly.

Before sampling histological material, animals were removed from the experiment using diethyl ether narcosis overdose. The thyroid glands of maternal rats, as well as the skin samples taken from the back side of their offsprings were fixed in 4% neutral buffered formalin or 1% glutaraldehyde and embedded in paraffin or Araldite/Epon plastic wax. For general morphology, 5–7 μm paraffin or 1 μm plastic sections were stained with Hematoxylin–Eosin (HE) or Toluidine Blue (TB), as described elsewhere [25]. Skin samples were taken from the back of the offsprings of experimental and control group rats on 1st, 10th, 20th, and 40th postnatal days. Thyroid function was controlled by estimation of triiodothyronine (T3) and thyroxine (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 rats' thyroid glands micromorphology, as well as by weighing offsprings at the appropriate terms of development.

Skin glycoconjugates were detected by Lectin–Peroxidase technique using a set of eight lectins with different carbohydrate specificity (Table 1) [26].

Table 1 – ***Used lectins and their carbohydrate specificity***

Lectin	Source of lectin	Its carbohydrate specificity
Con A	*Canavalia ensiformis*	αMan > DGal
GNA	*Galanthus nivalis*	αDMan
PNA	*Arachis hypogaea* (Peanut agglutinin)	βDGal < βDGal(1–3) DGalNAc
HPA	*Helix pomatia*	αDGalNAc
SNA	*Sambucus nigra*	Neu5Ac(α2–6)DGal
LABA	*Laburnum anagyroides* bark	αLFuc
WGA	*Triticum vulgare* (Wheat germ agglutinin)	αDGlcNAc > Neu5Ac
LTFA	*Lactarius torminosus* (Fungus agglutinin)	βDGal(1–3)DGalNAc

International lectin terminology (left column) as a rule is based on the abbreviated names of sources from which lectins are purified (presented in the second column); Last letter 'A': Abbreviation of word 'Agglutinin'. Con A: Concanavalin A; αMan: Alpha-mannose; DGal: D-Galactose; αDMan: Alpha-D-mannose; βDGal: Beta-D-galactose; DGalNAc: *N*-acetyl-D-galactosamine; αDGalNAc: *N*-acetyl-alpha-D-galactosamine; Neu5Ac: *N*-acetyl-neuraminic acid; αLFuc: Alpha-L-fucose; αDGlcNAc: *N*-acetyl-alpha-D-glucosamine.

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–3,3'-Diaminobenzidine (DAB) staining protocol [17, 24]. In detail, deparaffinized sections were incubated 20 minutes in methanol containing 0.3% hydrogen peroxide (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 (five minutes each), and incubated 45 minutes with Lectin–Peroxidase conjugate (50–75 μg/mL dilution in PBS) in a moist chamber at room temperature. Lectin receptor sites were visualized in PBS, containing 0.05% DAB (Sigma, St. Louis, MO, USA) and 0.15% H_2O_2. Thereafter, slides were twice washed in distilled water, and after dehydration 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 minutes in 1% periodic acid (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 Swift Instruments International microscope, equipped with a Echoo-Imager 502 000 digital camera, using the TopView 3.2 computer program. Statistical processing of the obtained data was carried out using Microsoft Office Excel 2003 and STATISTICA.6 (USA), with the definition of mean (M) and the average error (m). Three levels of confidence were used in the work: * – $p<0.05$; ** – $p<0.01$; *** – $p<0.001$.

Results

Macroscopically, the thyroid glands of the experimental group rats were 2–3 times larger compared to the control group animals. Light microscopic observation revealed significant changes in thyroid follicles micromorphology, namely, enhanced height of thyrocytes, which instead of cuboidal acquired columnar shape, disorganization of colloid or its complete absence in the majority of follicles, accompanied with thyrocytes hyperplasia and pronounced thyroid gland hyperemia (Figure 4, A and B). These morphological findings apparently encompassing hypothyroid status of maternal rats were supplemented with the results of TH measurements in the blood serum: T3 level decreased from 1.16±0.11 nmol/L in control group rats to 1.04±0.13 nmol/L ($p<0.01$) in experimental animals, while T4 decrease was even more significant – from 50.00±3.39 nmol/L in control group to 40.67±3.60 nmol/L ($p<0.01$) in experimental group rats.

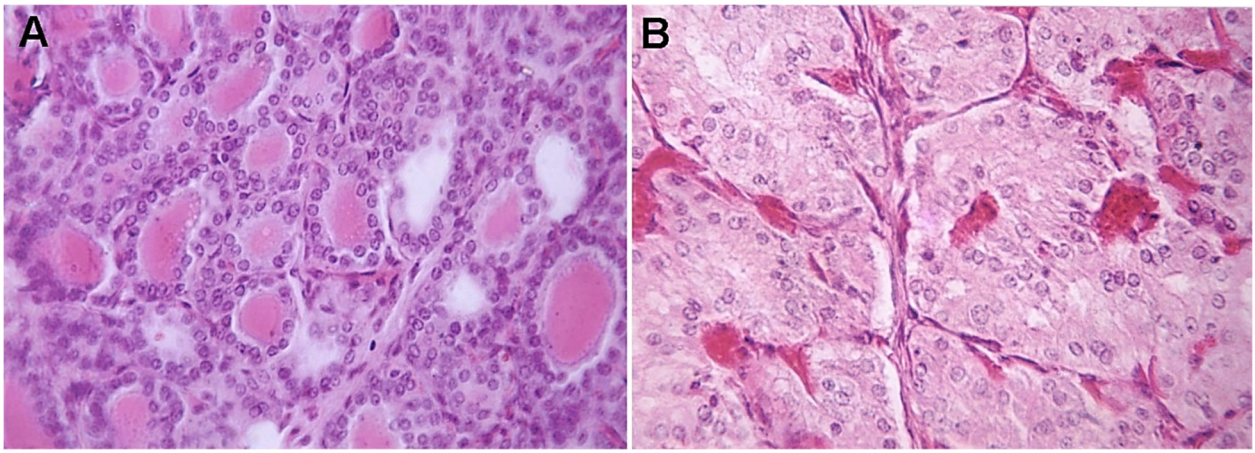

Figure 4 – ***Thyroid glands micromorphology of control (A) and experimental (B) hypothyroid female rats. Hematoxylin–Eosin (HE) staining, ×400.***

General morphology studies of the progeny skin on the 1st postnatal day demonstrated presence of clearly visible epidermis and dermis, the former exposing a well-defined four layered structure, including basal, spinous, granular and horny layers. The dermis consists of two layers – papillary and reticular, the latter decorated by numerous fibroblasts and fine fibrils – products of these cells' synthetic activity. Hair follicles of two types are also identified: those with larger diameter represent covering, guard or integumentary hair, the smaller ones belong to underhaired, or downy hair. At this developmental stage, the subcutaneous adipose tissue is weakly expressed and represented by small groups of adipocytes (Figure 5A). In the skin of experimental animals (Figure 5B), the spinous and granular layers of epidermis, as well as two types of hair follicles are more distinctly visible compared to the control group rats. This observation apparently indicates a more active keratohyalin synthesis in epidermal and hair follicle cells on the background of maternal hypothyroidism, since the progeny natural feeding during late prenatal and early postnatal period of ontogenesis completely depends on the maternal organism metabolism including both – maternal blood plasma before birth and maternal milk in the post parturition period.

On the 10th day of postnatal development, in the skin of control group progeny rats it was detected the enhanced density of different diameter hair follicles located between the hypodermis and the reticular layer of dermis. It is noteworthy that at this developmental stage the hair of offspring rats has structure similar to that of sexually mature rats. On the interphase of papillary and reticular layers of dermis there appeared first sebaceous glands, the secretory portions of which formed by an oval-round shaped sebocytes, exposing oxyphilic cytoplasm and eccentrically located nuclei. However, sebaceous gland ducts, composed of elongated basophilic cells, open directly into the space

surrounding hair shaft. The number of small diameter hair follicles (of downy hairs) increased significantly. The dermis of the skin is characterized by a well-defined microcirculatory bed filled with formed blood elements. The papillary layer of dermis is better developed compared to the newborn rats. The reticular layer exposes bundles of fibrils having different orientations in space.

On the 40th postnatal day, epidermis of control group rats is represented by four layers: *stratum basalis* – single row of highly prismatic cells; *stratum spinosum* – 2–3 rows of cells; *stratum granulosum* – one row of cells with distinct cytoplasmatic granularity; *stratum corneum* (*SC*) – several rows of cornified cells. The papillary layer of the dermis is formed by loose connective tissue with a large number of cellular elements, among which fibroblasts predominate. The reticular layer is composed of multi-directional bundles of collagen and elastic fibers. Hypodermis is not continuous, composed of adipocytes forming small groups. There are a large number of different diameter hair follicles in the dermis, some of which begin from 10th postnatal day supplemented with sebaceous glands. However, the number of pilosebaceous units increased significantly up to 40th postnatal day (Figure 5C). On the 20th postnatal day, hypothyroidism affected progeny rats, alongside an increase of total body weight, demonstrate hypodermis thickening. In the epidermis of this group offspring, it was documented enhanced thickness of spinous and granular cell layers, the latter overloaded with keratohyalin granules apparently encompassing adverse influence of the maternal hypothyroidism affecting metabolic pathways. It is possible that documented trend of keratinization strengthening is one of the signs of a compensatory adaptive nature directed towards protection of the skin from damaging factors. In the skin of hypothyroid rat offsprings on the 40th day of postnatal development, blood vessels of the microcirculatory bed are filled with hyper-aggregates of formed blood elements. Clearly visible hair follicles at various stages of differentiation demonstrate greater or lesser expression of the outer epithelial root sheath layers (Figure 5D).

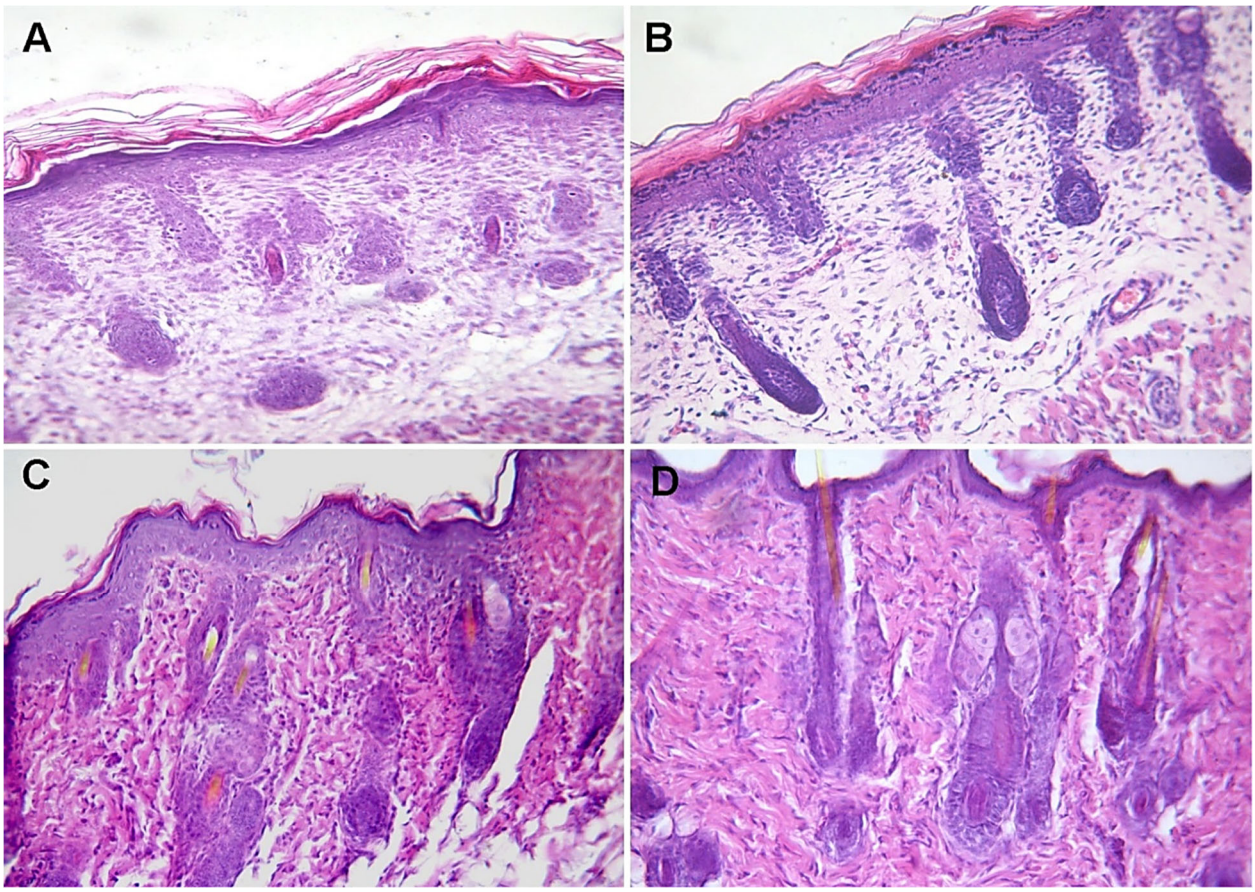

Figure 5 – ***Skin of the offspring of control and hypothyroid female rats on the 1st and 40th days of postnatal development: (A) Control, 1st postnatal day: well-defined epidermis, dermis, different diameters hair follicles; (B) Experiment, 1st postnatal day; (C) Control, postnatal 40th day; (D) Experiment, 40th postnatal day. HE staining: (A–D) ×200.***

In the post-TB staining preparations, on the 1st–40th postnatal days in the skin of both control and experimental animals, mast cells (MCs) were distinctly detected in the dermal reticular layer. Some of these cells, especially with perivascular localization, exposed signs of degranulation (Figure 6, A and B). On the 1st and 10th postnatal days on the background of maternal hypothyroidism, the MC count in the skin increased markedly compared to control samples, apparently indicating the activation of immune or allergic reactions in early postnatal ontogenesis (Table 2). On the other hand, during a lifespan of experimental group rats, a total count of cutaneous MCs decreased constantly from 24.08±0.73 on the 1st postnatal day to 16.16±0.34 on 40th postnatal day, completely adjusting to the control group ratio (16.44±0.36). This observation most likely claims for certain normalization of skin parameters after progeny disjunction from hypothyroid maternal feeding.

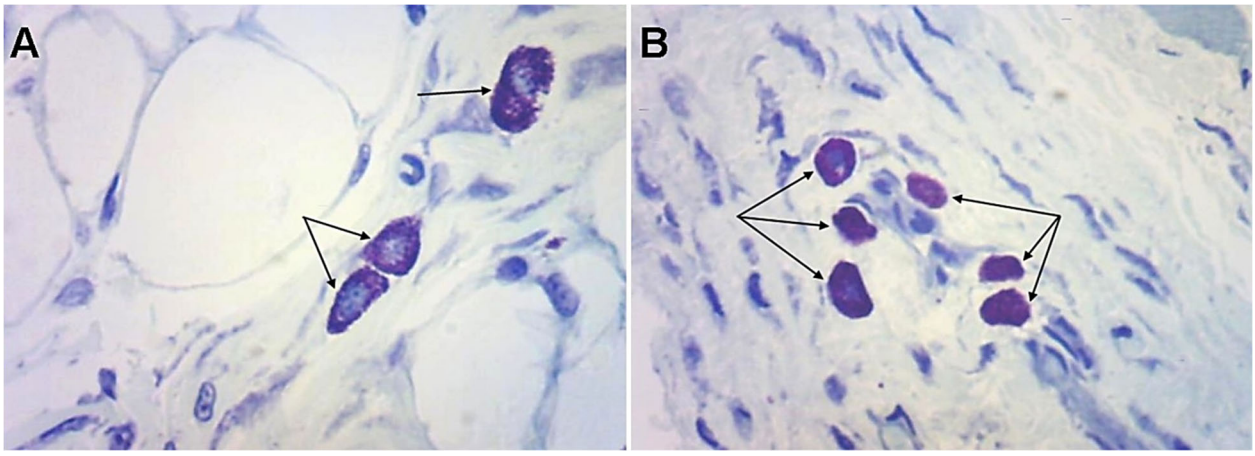

Figure 6 – ***Mast cells (arrows) in the skin of rats on 40th postnatal day: (A) Control; (B) Hypothyroid rat offspring. Semithin sections, Toluidine Blue (TB) staining: (A and B) ×600.***

Table 2 – ***Number of mast cells in the skin of control and hypothyroid progeny rats***

Groups of animals	1st postnatal day		10th postnatal day		20th postnatal day		40th postnatal day	
	M±m	*p*	*M±m*	*p*	*M±m*	*p*	*M±m*	*p*
Control	17.00±1.11		13.08±0.98		14.28±0.13		16.44±0.36	
Experiment	24.08±0.73	<0.001	21.84±0.94	<0.001	18.48±0.39	<0.01	16.16±0.34	0.87

M: Mean; m: Average error.

Lectin histochemistry investigation of semithin sections of the skin of rat progeny revealed that among the applied lectins, mannose-specific *Galanthus nivalis* agglutinin (GNA) exposed the most selective binding to the cutaneous MCs (Figure 7, A and B). On the 1st day of postnatal development, the most intense exposure of receptor sites for all used lectins was observed on the surface and within cytoplasm of keratinocytes of epidermis. Glycopolymers with terminal carbohydrate determinants of αDGalNAc [*Helix pomatia* agglutinin (HPA) labeling] (Figure 8A) and αLFuc [*Laburnum anagyroides* bark (LABA) labeling] were also exposed in the outer epithelial root sheath of hair follicles. The process of medullary substance formation in the covering (guard) hair was accompanied by the accumulation of DGlcNAc residues and sialoglycans identified by the wheat germ agglutinin (WGA). In contrast, HPA lectin receptors were absent both in control and experimental samples (Figure 8, A and B). DGal(β1–3)DGalNAc oligosaccharides [*Lactarius torminosus* fungus agglutinin (LTFA) receptor sites], on the 1st day of postnatal development, were detected in the individual cells, scattered in the epidermal spinous layer, most likely Langerhans cells, the number of which increased at the initial stages of the newborn rats contact with the external environment. On the background of maternal hypothyroidism, the enhanced intensity of αDGlcNAc-specific WGA lectin binding accompanied with simultaneous reduction of *Sambucus nigra* agglutinin (SNA) receptor sites were detected in the cells of covering hairs medullary portion. However, the fibrous structures of hair follicles, nerve fibers and cells of outer epithelial root sheath exposed certain accumulation of sialoglycans.

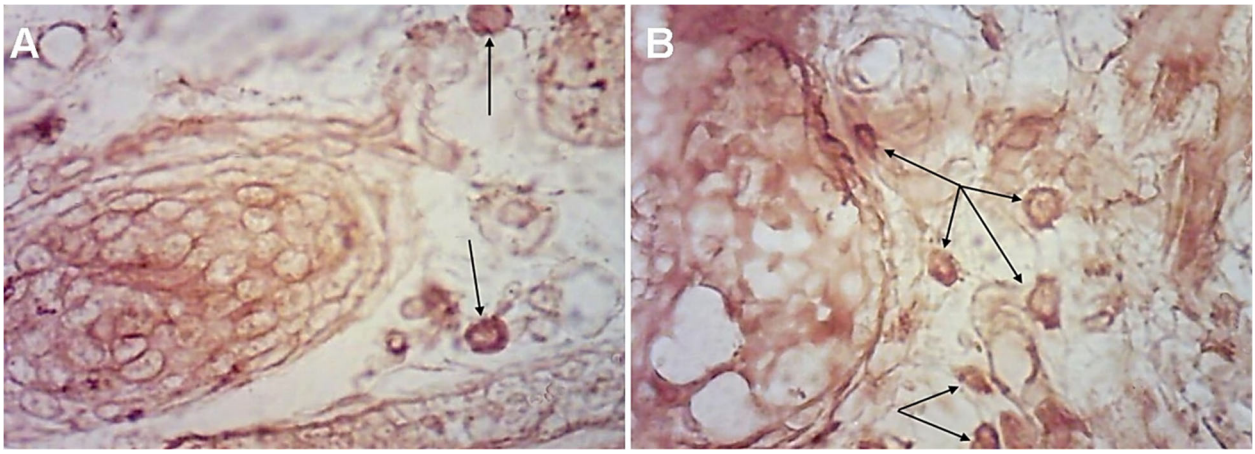

Figure 7 – ***Lectin histochemistry of rat progeny skin on 10th postnatal day: (A) Control; (B) Hypothyroid rat offspring: enhanced count of strong lectin reactive mast cells (arrows). Semithin section, GNA lectin binding: (A and B) ×600. GNA: Galanthus nivalis agglutinin.***

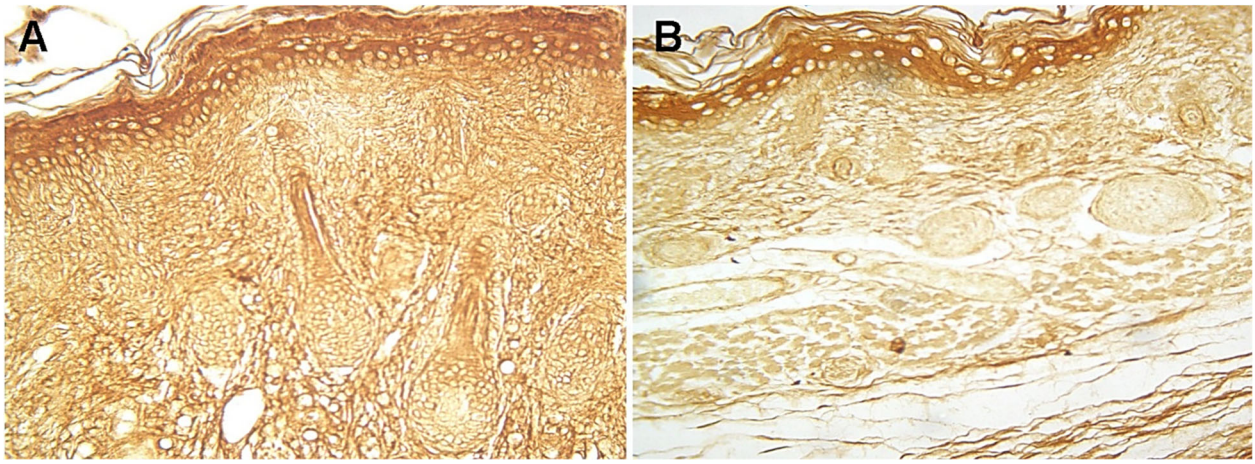

Figure 8 – ***HPA lectin binding (×200) in the skin of a newborn rats: (A) Control; (B) Hypothyroid rat offspring: evidence of decreased lectin reactivity. HPA: Helix pomatia agglutinin.***

On the 10th postnatal day, skin of control group progeny rats expose Neu5Ac(α2–6)Gal/GalNAc carbohydrate determinants, recognized by SNA lectin (Figure 9, A and C), which rather selectively labeled plasma membranes of the cells of hair medulla and of the outer epithelial root sheaths. The same lectin actively reacted with plasma membranes of cells, forming secretory units of sebaceous glands, adjusting to epithelial root sheaths (Figure 9E). In the skin of experimental group animals, a decrease in the intensity of SNA binding was noted (Figure 9, B, D and F). This decrease of sialoglycans exposure may be induced by incomplete glycosylation of carbohydrates in Golgi apparatus due to disorganized metabolic pathways conditioned by the maternal thyroid gland dysfunction. Hair follicles of different diameters demonstrate certain specificity in WGA binding. Namely, in the smaller diameter hair follicles of wool hair, lacking medulla, WGA labeling was restricted to the outer epithelial root sheaths, while in the larger diameter follicles of guard hair WGA receptors were intensely exposed in hair cortex, medulla and cuticle, as well as in the components of hair follicles (Figure 10, A, C and E). On the contrary, the hair roots cuticle of hypothyroid group progeny rats was WGA-negative or exposed only residual signs of this lectin binding (Figure 10, B, D and F).

On the 20th and 40th postnatal days, skin of control group progeny rats exposed strong reactivity with SNA and WGA lectins, the most intense labeling attributed to *SC* and the lipid barrier of epidermis, cells of hair follicles outer epithelial root sheaths, of the sebaceous glands' terminal secretory units, as well as fibroblasts of dermal papillary layers (Figure 11A). The skin of the experimental group animals on the same developmental stage demonstrated similar distribution of SNA and WGA receptor sites. However, hypothyroidism affected rats who lost lectin binding carbohydrates in the hair follicles, while sebaceous gland cells demonstrated enhanced lectin reactivity (Figure 11B). Control group rats on the 20th and 40th postnatal days expressed intense labeling of *SC* keratinocytes, terminal secretory units of sebaceous glands, cells of inner epithelial root sheaths of covering hair, as well as fibroblasts of dermal papillary layer with galactose-specific peanut agglutinin (PNA) and LTFA lectin (Figure 11, C and E). In the skin of the same term experimental group animals, the sebaceous gland cells lost their βDGal and DGal(β1–3)DGalNAc sugar determinants (Figure 11, D and F). Interestingly, PNA and LTFA lectin receptor sites were identified in the hair bulb pigmentocytes.

Glycoconjugates with αDGalNAc, αDMan and αLFuc sugars termini [HPA, concanavalin A (Con A) and LABA lectin receptor sites] on the 20th and 40th postnatal days richly decorated epidermal *SC*, as well as the sebaceous gland cells and nerve fibers within the neurovascular bundles. These same lectins showed similar binding specificity to the structural components of experimental group skin. However, sebocytes under experimental conditions lost αDGalNAc receptors (demonstrate reduced HPA lectin binding), which can be related to a change in the chemical composition of their secretory products due to the hypothyroid developmental status. The process of hair follicle cells differentiation was accompanied by the redistribution of αLFuc carbohydrate determinants (LABA receptor sites) from inner to outer epithelial root sheath.

Discussions

Among the target organs influenced by THs, skin occupies the primary place, since T3 and T4 are involved in the maintenance of skin homeostasis [2, 27]. Therefore, certain dermatological pathologies are associated with alterations of the thyroid status. Namely, alopecia, dermatitis, and vitiligo are associated with thyroiditis, alopecia and eczema often accompany Graves' disease – these few examples illustrate intimate functional relationship between the thyroid gland and skin. Results of our general morphology investigations figured out differences in skin morphogenesis, which developed under the influence of maternal hypothyroidism. In particular, whereas skin of the newborn rats demonstrated practically no difference between control and experimental group animals, on the 10th and 20th postnatal days skin of progeny rats affected by maternal hypothyroidism exposed increased thickness of epidermal spinous and granular cell layers, supplemented by hypodermis thickening and increased total body weight.

According to Komárek *et al.* [28], the rat hair is differentiated into two types – covering "guard hair" and

downy "underhair". On the 10th postnatal day, the root of covering hair is composed of medulla, cortex and cuticle. Centrally located oxyphilic medulla consists of 2–3 rows of partially keratinized polygonal cells with elongated compact nuclei and trichohyalin granules in cytoplasm. Cortex consists of flat horny scales with the presence of hard keratin granules and air bubbles in the cytoplasm. Our observations are consistent with data of Komárek *et al.* [28]: in our study, hard keratin exposed oxyphilic staining in contrast to basophilic staining of soft keratin, located in the cytoplasm of epidermal granular layer cells. The cuticle consists of a single layer of unstained cells. In the offspring of experimental group rats, the papillary layer of dermis is more pronounced in comparison with the control group animals, and there is also a greater number of hair follicles of small diameter surrounding hair roots without medulla – representing downy hair.

Our observation of the intense development of epidermal granular cell layer in the experimental group progeny rats on the 40th postnatal day apparently indicate the strengthening of keratinization processes, which is among the factors of skin protection from damaging forces and is consistent with results of Božinovski *et al.* [7]. Interestingly, hypothyroidism in women during pregnancy and lactation leads to hypothyroidism in offspring, which in turn changes the intensity of metabolic processes [7, 8].

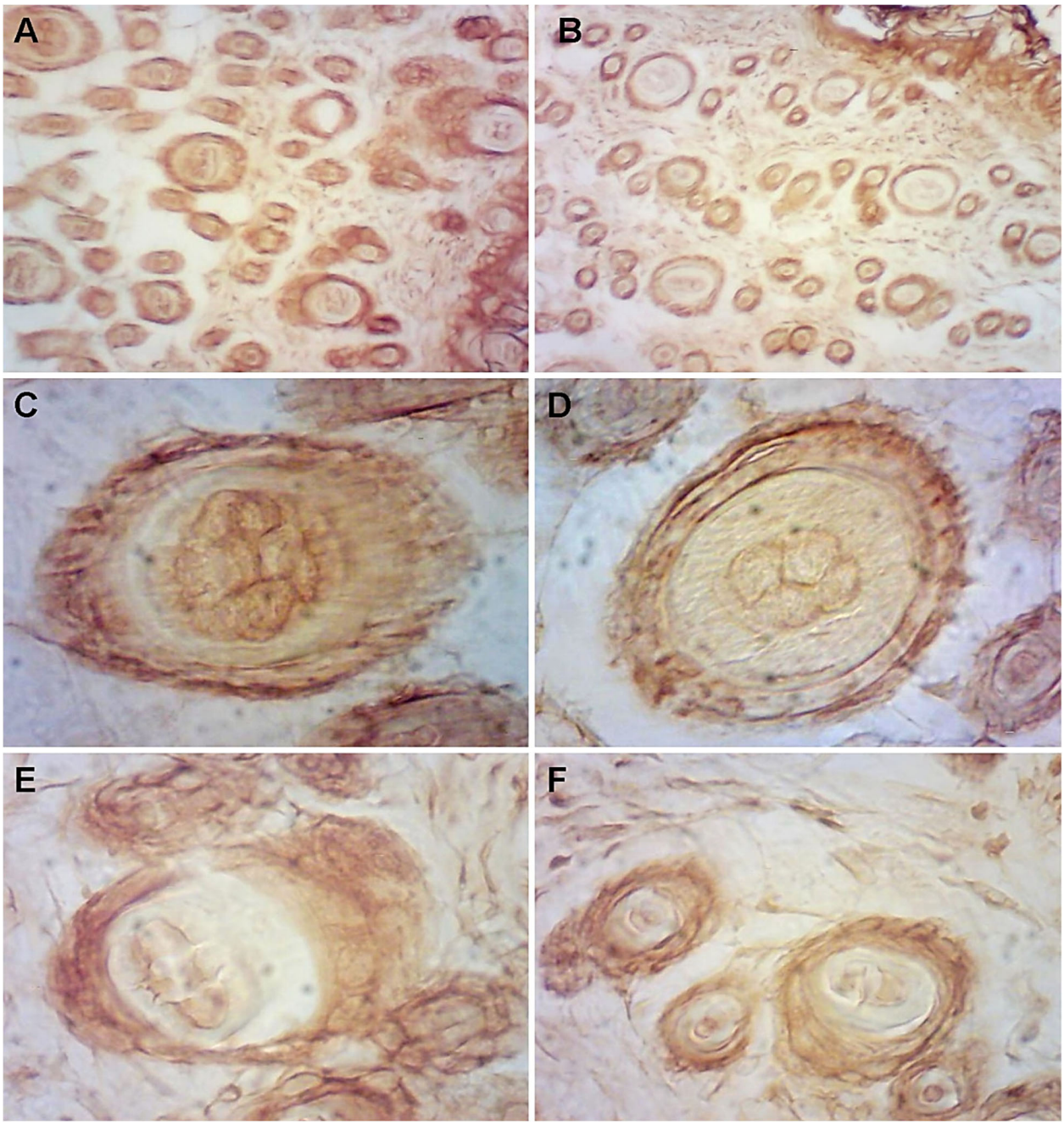

Figure 9 – ***SNA lectin binding to hair follicles of rat progeny on the 10th postnatal day: (A) Control (×150); (B) Experiment: hypothyroid rat offspring (×150); (C) Control: SNA is binding to the cells of outer epithelial root sheath of hair follicles and to the hair medulla cells (×900); (D) Experiment: decreased lectin binding with hair medulla (×900); (E) Control: SNA binding to plasma membranes of sebocytes (×900); (F) Experiment: decrease in SNA binding to the sebaceous gland cells (×900). SNA: Sambucus nigra agglutinin.***

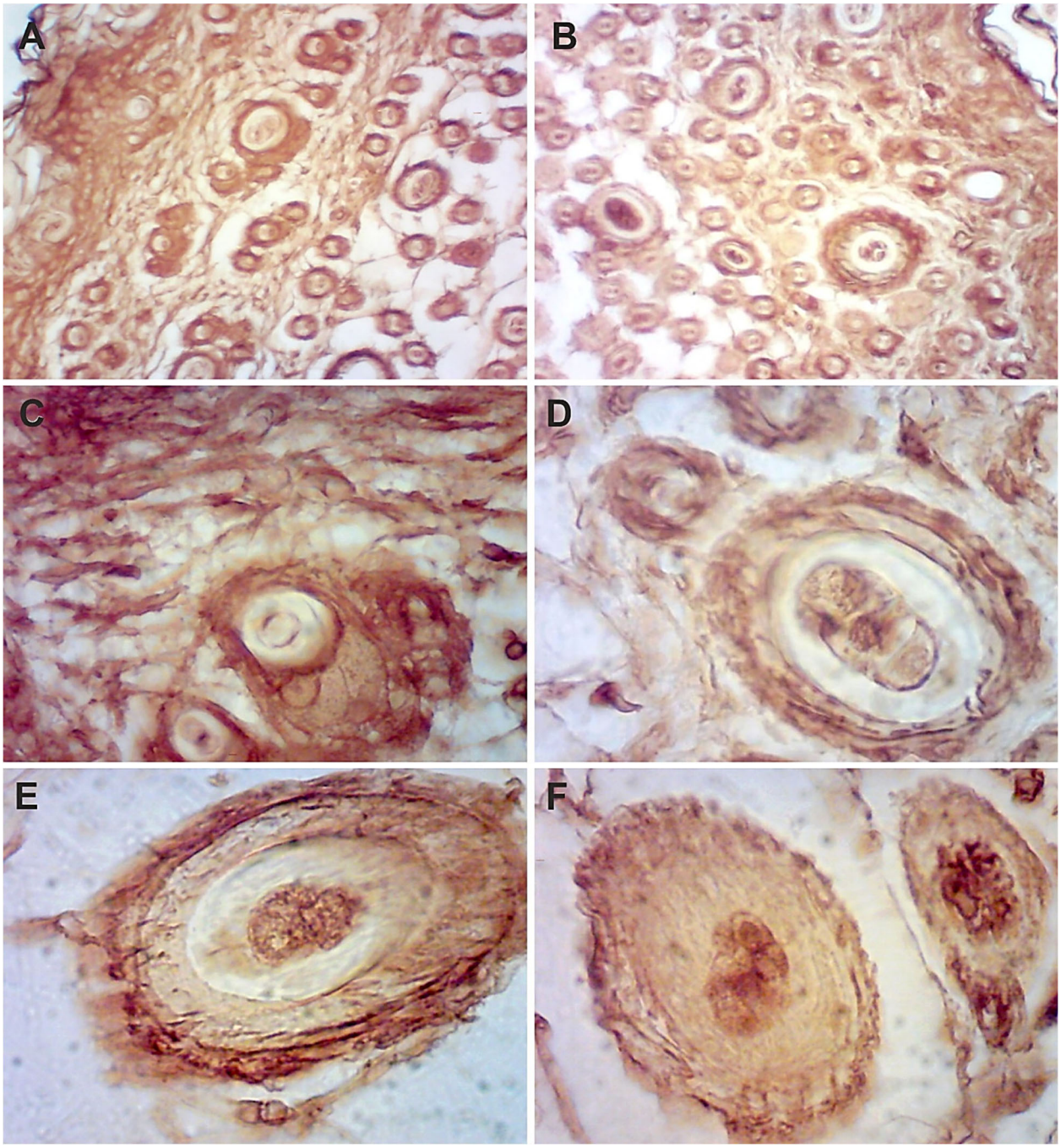

Figure 10 – ***WGA lectin binding with hair follicle constituents of 10th postnatal day rats: (A, C and E) Control (×150, ×600 and ×900, respectively); (B, D and F) Hypothyroid rat offspring: intense WGA reactivity with hair medulla, cuticle and cells of outer epithelial root sheaths; sebaceous gland cells also strong reactive (×150, ×600 and ×900, respectively). WGA: Wheat germ agglutinin.***

The skin is the largest organ of the body, serving as a barrier to the external environment [29]. It is rich in MCs, serving an important source of cytokines, chemokines, and growth factors that play an important role in the skin barrier function [30–32]. MCs possess a crucial role in the maintenance of homeostasis and integrity of the skin barrier by interacting with neighboring non-immune cells, such as fibroblasts, keratinocytes, and endothelial cells [30]. The potential relationships between the thyroid gland and MCs are certainly complex and likely twofold. There is evidence that the effects of T3 can be modulated by MCs, and that MCs can modulate thyroid function. Such interactions are clinically relevant in autoimmune diseases such as rheumatoid arthritis, inflammatory bowel disease or in autoimmune thyroiditis such as chronic urticaria characterized by recurrent episodes [33]. Activation of MCs provokes inflammatory skin reactions in Graves' disease and Hashimoto's disease [34]. The hypothalamus, releasing thyroid-stimulating hormone (TSH), stimulates an increase in the content of T3 in MCs. T3 is stored together with histamine in MC granules or degraded to 3-iodothyronamine (T1AM) and/or 3-iodothyroacetic acid (TA1). T1AM and TA1 come from the circulation or are produced within MCs and induce their degranulation, releasing T3 and histamine, which mediate pain, itch and central effects including neuroprotection/neuroinflammation (Figure 12) [35].

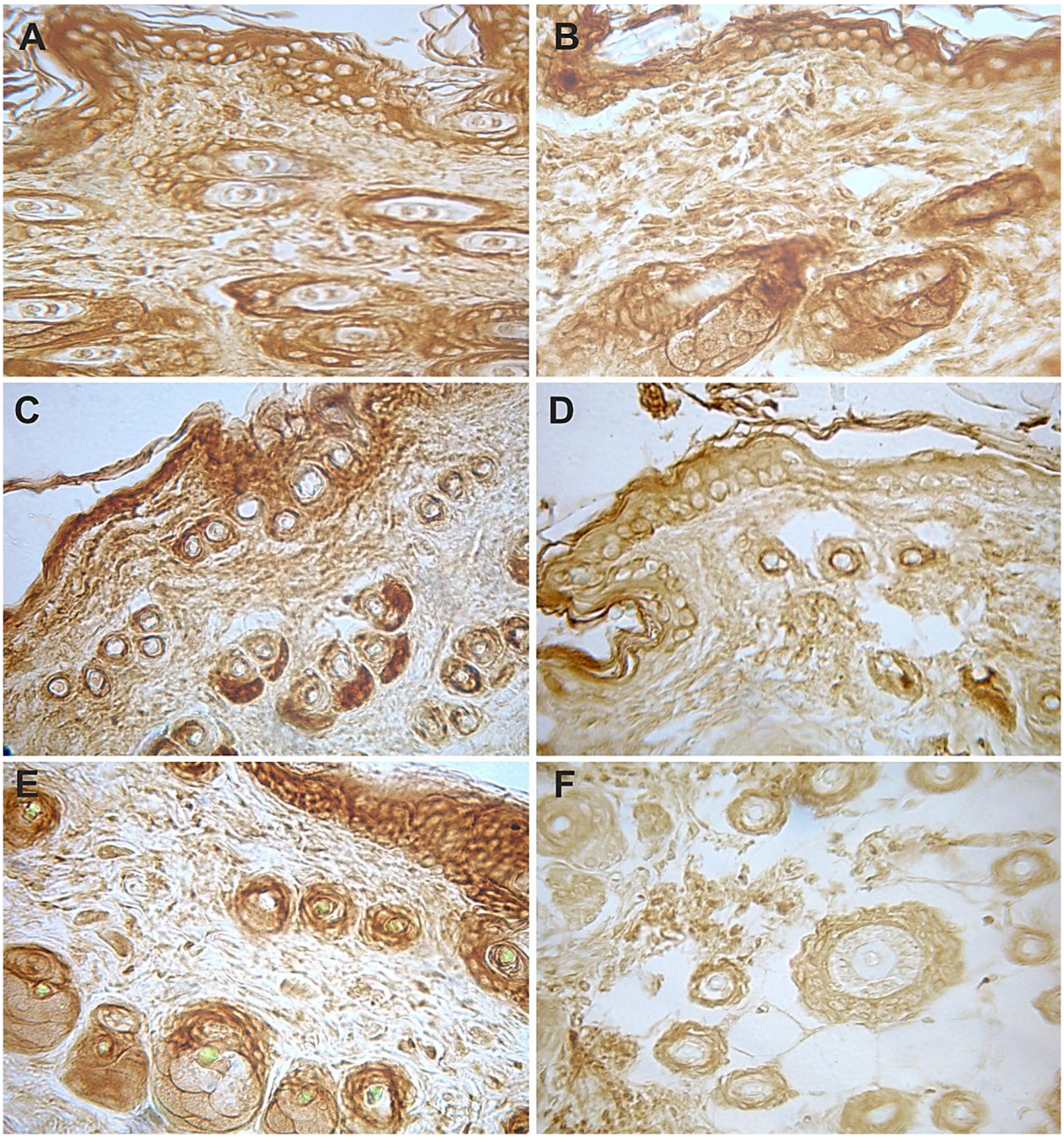

Figure 11 – ***Lectin binding (×400) to skin derivatives of rats on 20th postnatal day: (A) Control, (B) Experimental group rats: in both slides, strong SNA binding to SC of epidermis, to outer epithelial root sheaths of hair follicles, and to the adjusting sebaceous gland cells; (C) Control, (D) Experimental group rats: intense PNA labeling of SC and of sebaceous glands secretory units; significantly reduced sebocytes reactivity in the experimental group rats; (E) Control, (F) Experimental group rats post-LTFA labeling: a significantly decreased exposure of LTFA lectin receptor sites in sebaceous glands alongside with persistent lectin reactivity in SC and outer epithelial root sheaths; dermal fibroblast also demonstrate intense LTFA reactivity – upper left corner in (F) fragment. LTFA: Lactarius torminosus fungus agglutinin; PNA: Peanut agglutinin; SC: Stratum corneum; SNA: Sambucus nigra agglutinin.***

The MCs count in the skin of control group progeny rats revealed these cells highest concentration on the 1st postnatal day, followed by a slight decrease on the 10th day, and their gradual increase until the 40th day (Table 2). Under the influence of maternal hypothyroidism, the number of MCs increased from the 1st to the 20th day, with a gradual decrease up to the 40th day, becoming practically equal to this same index in control group animals. MCs are among the starting effectors of inflammation and the source of a large number of initial mediators of inflammation, such as histamine, serotonin (in small rodents), cysteinyl leukotrienes, prostaglandins (mainly D2), thrombocyte activating factors, numerous enzymes, etc. With this regard, MCs largely determine further events in the mediator cascade and intercellular interactions in the focus of inflammation [29]. MCs as important agonists mediating the immune status as reported by Costela-Ruiz *et al.* [33]. Taking into consideration all the above, a conclusion can be made that the increased number of MCs on the background of maternal hypothyroidism apparently encompass a high risk

of developing allergic reactions in early postnatal period of their offsprings development. Moreover, it is likely that gradual decrease in the MCs count under physiological conditions is an age associated processes of adaptation to the external environment and transition to an independent mode of progeny feeding.

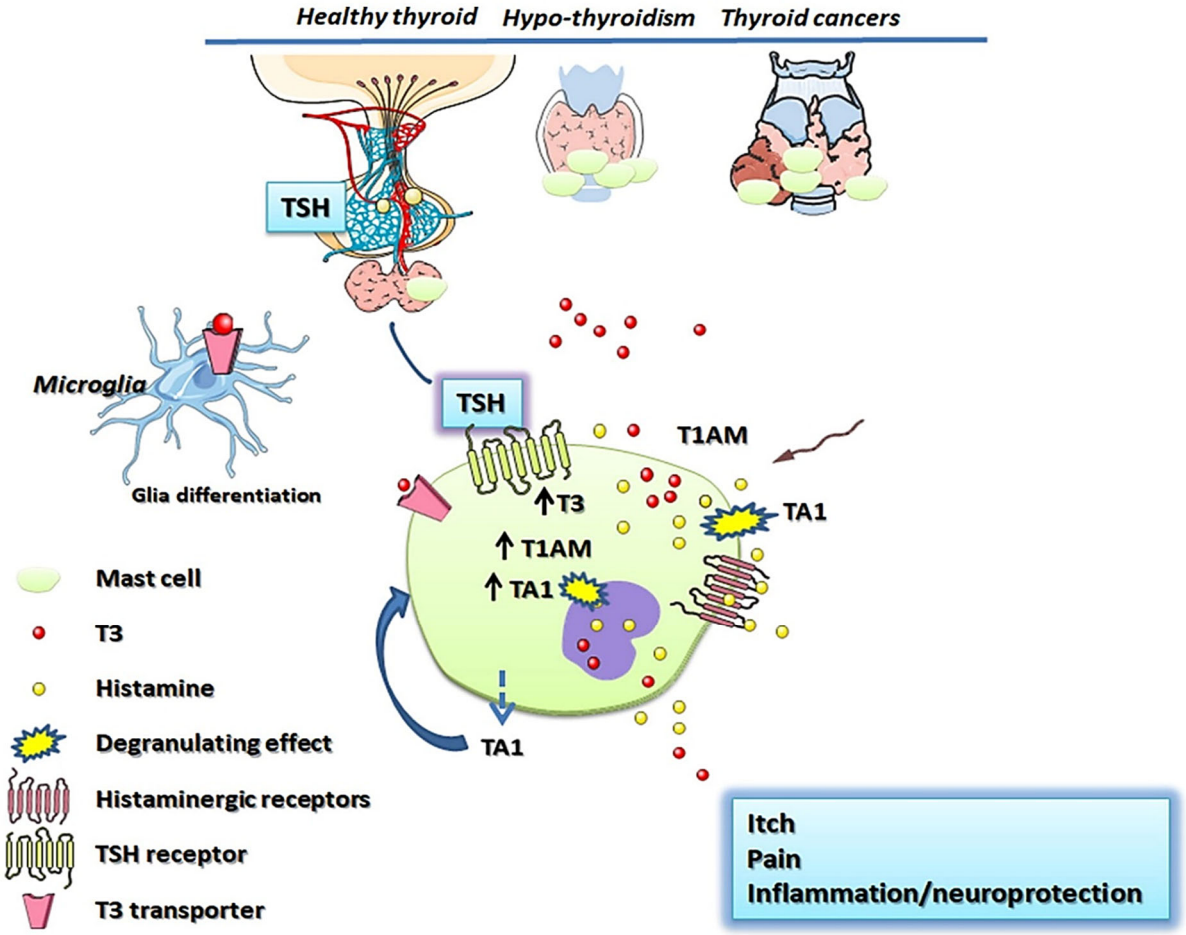

Figure 12 – ***Schematic representation of the relationship between the thyroid gland and mast cells (according to Landucci et al., 2019 [35]). T1AM: 3-Iodothyronamine; T3: Triiodothyronine; TA1: 3-Iodothyroacetic acid; TSH: Thyroid-stimulating hormone.***

Our lectin histochemistry studies demonstrate that maternal hypothyroidism induces rearrangement of carbohydrate determinants in the structural components of the progeny skin. In particular, on the 1st–10th postnatal days, it was documented a certain decrease in the exposure of sialoglycans, which play the role of signaling molecules, affecting growth and differentiation processes in organ tissues [20, 36, 37]. It is also noteworthy that PNA and LTFA lectins on the 20th and 40th postnatal days showed a high affinity to epidermal keratinocytes, especially those located in *SC*, to the secretory units of sebaceous glands, and to the inner epithelial root sheath of the guard hair, depending on the degree of cellular differentiation. Moreover, a reduced content of PNA lectin receptor sites in sebocytes and cells of the inner epithelial root sheaths apparently encompass negative trends in these cells' regeneration capabilities. As a general trend in our lectin histochemistry investigation, we report here a decrease of lectin binding in hypothyroidism affected skin, relating this drift to the incomplete final glycosylation steps in the glycoconjugates processing within Golgi apparatus, subsequently resulting from T3-dependant disturbances of cellular metabolic pathways.

This option is supported by the studies of Pankiv [13] and Repetska [14], who postulated that under primary hypothyroidism all types of metabolic processes, including oxygen utilization and oxidative reactions, gas exchange and basic metabolism are inhibited, accompanied with the reduced activity of various enzyme systems. As a result of such reduction, the acidic glycosaminoglycans are deposited in the heart, lungs, kidneys, serous cavities and all layers of skin, leading to the development of hydropic edema in these organs. It was also reported that hypothyroidism-associated inhibition of enzymatic activity in the sulfate cycle of cholesterol, induce significant changes in the chemical composition of the skin lipid barrier, affects the development of lamellar granules (keratinosomes, or Odland bodies) and contributes to their accumulation [15]. With reference to the lectin related studies, it should be noted that due to the exceptional selectivity of lectin binding it was discovered the existence of keratinocyte progenitor cells and melanocyte progenitor cells as two separate cellular subpopulations, developing from pluripotent stem cells of hair follicle [38]. Moreover, these same authors detected that pluripotent stem cells, located in the bulge of hair follicle can differentiate into nerve cells, glial cells, keratinocytes, smooth muscle cells, cardiac muscle cells, and melanocytes.

Conclusions

Maternal hypothyroidism induces the accumulation of MCs in the skin of progeny on the 1st, 10th and 20th postnatal days, with decrease of these cells count adjusting to control level on 40th postnatal day. Taking this trend into consideration, offsprings developed under maternal hypothyroidism should be treated as a risk group for changes in immune status and the occurrence of allergic reactions. *SC* of epidermis, its lipid barrier as well as pilosebaceous units, in both control and experimental group animals, at the early stages of postnatal ontogenesis are enriched with carbohydrate determinants of αDMan, βDGal, βDGal(1–3)DGalNAc, αLFuc, αDGalNAc, αDGlcNAc, Neu5Ac. GNA lectin can be recommended as a selective histochemical marker of MCs, while LTFA lectin can be used as a selective labeling of Langerhans cells. Development under maternal hypothyroid conditions induced reduction of lectin binding with structural components of their progeny skin and its derivatives, indicating alterations in glycoconjugate processing and degradation sequences, having impact on the cell signaling, formation of adhesive contacts, cellular proliferation and differentiation. Used lectin set can be recommended for the specific labeling of cellular subpopulations, monitoring glycoconjugates processing and degradation under physiological and pathological conditions in the dermo–epidermal layers, pilosebaceous units and their derivatives.

Conflict of interests

The authors declare that they have no conflict of interests.

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