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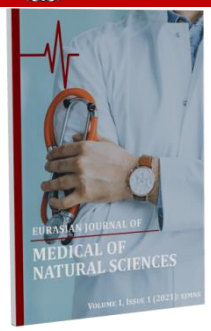

FEATURES OF MORPHOLOGICAL FORMATION OF KNEE JOINT ELEMENTS IN OFFSPRING DEVELOPED UNDER THE INFLUENCE OF EXPERIMENTAL HYPOTHYROIDISM

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ABSTRACT

Hypothyroidism, and the exact presence of thyroid hormone in the blood in low concentrations leads to the fact that the metabolism in the body gets out of control, as a result of which pathological changes of varying degrees develop. Hypothyroidism is caused by several factors: injuries of a gland, tumors or cystic diseases, disorders of the pituitary-hypothalamic system, defects in the development of the gland, surgical interventions errors, long-term use of antithyroid drugs, etc. The situation with hypothyroidism is common among population, and the deficiency is common in our region. This article describes the morphometric changes in the knee system in early ontogenesis, which was obtained in the study of offspring born from a mother with experimental hypothyroidism during pregnancy. The model of experimental hypothyroidism and the results of the early study show morphofunctional changes in the elements of the knee joint and the generation of this disease.

Relevance. Thyroid diseases occupy a high place in the world among endocrine diseases in terms of morbidity. According to the World Health Organization (WHO), currently more than 665 million people worldwide suffer from endemic goiter and other thyroid diseases. 1.5 billion people suffer from iodine deficiency diseases. Hypothyroidism syndrome is most often characterized by a violation of the production of endogenous TG. The most common type of hypothyroidism, which accounts for 99% of all cases, is primary hypothyroidism, which occurs due to insufficient synthesis of TG in the thyroid gland. Primary hypothyroidism accounts for 3.8-4.6% of the population. Secondary hypothyroidism is extremely rare. Knowledge of the functional anatomy of the knee joint elements and ligamentous apparatus is very limited, and the existing one is contradictory. There is no information on the micromorphology of the ligaments and their attachment to the bone, structural and biomechanical mechanisms. Therefore, one of the important problematic issues is the definition of the specified morphological features of the complex of anatomical structures of large joints, determination of its places of least stability, age periods of injury risk, and biomechanics of injury.

A number of targeted scientific studies are being conducted in the world to study the age-gender functional structure of the thyroid gland. In this regard, scientific studies aimed at studying the factors leading to the development of hypothyroidism syndrome in thyroid pathology, especially hypothyroidism during pregnancy, prevention of the negative impact of hypothyroidism on fetal development, including the risk of serious pathological changes in the musculoskeletal system, detection of severe, potentially disabling pathologies of a growing organism, in which morphological changes in the bone and ligament apparatus occur, study of the morphofunctional features of large joints, prevention and treatment of injuries and degenerative-dystrophic changes in this area are of particular scientific and practical importance.

In Uzbekistan, a number of scientists have worked on assessing the morphological parameters of various organs and systems under the influence of hypothyroidism (S.M. Akhmedova, 2017; M.T. Yuldasheva, 2019; Kh.A. Rasulov, 2019, 2023; F.Kh. Azizova, 2023), but the morphological features of the knee joint structures of children born to mothers with hypothyroidism have not been fully studied.

Materials and methods. Forty sexually mature white female laboratory rats weighing 200 ± 17 grams were used for the experiment. The rats selected for the experiment were kept in quarantine for 45 days and provided with direct access to food and water, using a complete diet. (Look at Table 1).

Table 1

Description of the experimental study material

Details of the materials		Control	Experiment 1	Experiment 2
Number of pregnant rats		10	15	15
Administered drug		Physiological solution	Mercazolil	Mercazolil and L-thyroxine
Number of offspring obtained		118	96	109
Number of dead offspring		4	12	5
Number of offring involved in the study		30	60	60
Experimental withdrawal period and number of offspring	Day 7	6	12	12
	Day 14	6	12	12
	Day 21	6	12	12
	Day 30	6	12	12
	Day 45	6	12	12

To determine the amount of thyroid hormones, blood was taken from the tail vein of the mother and pups of rats and analyzed.

The pups were killed by decapitation on the 7th, 14th, 21-30th and 45th days after birth. Tissues for histological studies were taken from the knee joint structures. The knee joint structures were fixed in 10% formalin solution and paraffin blocks were prepared by dehydration in alcohols of different strengths. Histological preparations of 5-8 μm thickness were prepared from the paraffin blocks and stained with hematoxylin and eosin. For histological studies, the elements of the rat knee joint (distal femoral epiphysis, proximal femoral epiphysis, menisci, synovial bursa, ligaments) were isolated and placed in separate

containers for fixation in 10% neutral formalin buffer. Soft tissue samples isolated from experimental animals were immediately processed according to standard procedures. First, excess formations were excised from the section. The synovial bursa and ligaments required for the study in the sample were excised with scissors and examined at each observation period, and the organometric indices of the joint were assessed in the state of integrity. The obtained sections were dehydrated in increasing percentages of alcohol and fixed in 10% neutralized formalin.

Bone tissue underwent a decalcification stage taking into account the possible bone hardness during the observation periods. Hard (bone) tissues were decalcified using trichloroacetic acid. For decalcification, a 7% aqueous solution of trichloroacetic acid was added to a 20% formalin solution, and the rats were kept in this mixture for 3 to 8 days depending on the periods of ontogenesis. The mixture was refreshed daily at 10:00 AM, and the bone pieces were examined conventionally by needle puncture to assess the degree of decalcification (until the tissue was softened and elastic). The samples were then embedded in paraffin blocks in the frontal position to ensure full exposure of the histological section. Sections of 4-5 μm thickness were removed from the paraffin blocks on a digital microtome and placed on working glass plates, and after deparaffinization, they were stained in several different ways for preparation.

The prepared sections were stained with hematoxylin and eosin and according to Van Gieson for studying the structure of connective tissues, as well as Toluidine blue and Azan for studying connective tissue, cells and its intermediate elements. The prepared micropreparations were viewed under a SARL Zeiss Microscopy GmbH microscope and photographed with an Axio Lab.A1 camera (Germany).

Results. To conduct a comparative scientific analysis in the model of experimental hypothyroidism, the elements of the knee joint (joint-forming bones, articular surfaces, ligaments, joint capsule and auxiliary structures) of the offspring born from control rats at all observation periods were morphologically studied, and the obtained results on their morphological composition are included.

It is known that the congruence of the joint, its differentiation in ontogenesis depend on all the elements of the joint and their harmonious development. Furthermore, in postnatal ontogenesis, it improves in accordance with the increase in the load force falling on the joint. During the observation period of the control group, when the articular surface of the rat knee joint was morphologically studied for histological examination, the control animal had internal differentiation of the hyaline bone in the initial period, normal formation of the hyaline bone corresponds to the observation period.

During this period, symmetrically distributed basophilicity was detected along the border of the basal layer of the articular surface in the animals of the comparison group, and the superficial layer of the joint was represented by thin fibrous structures. In certain areas of the joint surface, the thickness of the fibrous layer and an ordered distribution of the connective tissue structure were observed (see Fig. 1). In the rats of the control group, the cambial layer of chondrocytes was sufficiently thick, and the width of the joint space was observed (Look at Fig. 2).

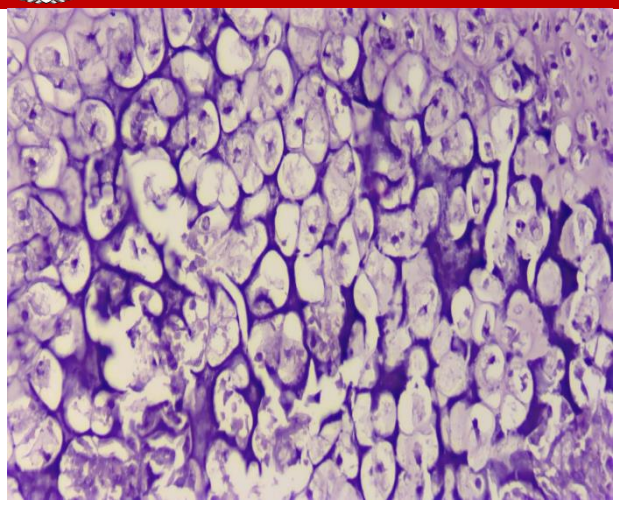

Figure 1. In the control animal, the initial differentiation of the hyaline layer from the inside is characteristic of normal formation. GE., K.400

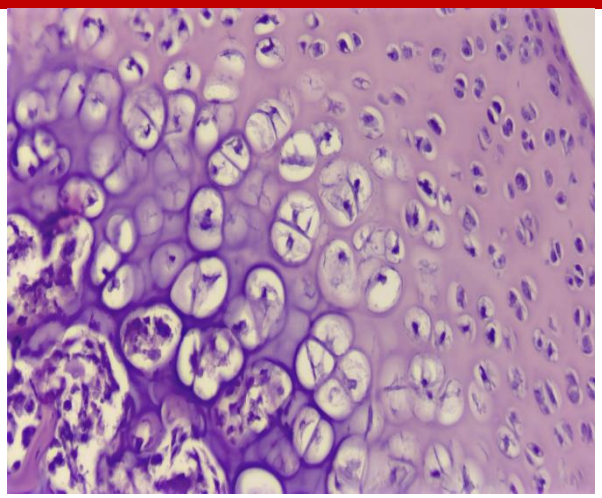

Figure 2. Thickness of the fibrous layer on the surface of the joints and the ordered distribution of the connective tissue structure. GE., K.400

At later observation periods (21-30 days), it was found that the superficial layer of the articular cartilage in all areas of the articular surfaces was covered with lined cells (see Fig. 3).

It was found that vertical rows of round chondrocytes located in the basal and intermediate zones of the articular cartilage formed isogenic groups. In contrast, chondrocytes located in the superficial zones did not form such groups. An even contour of their layers was found on the surface of the articular cartilage (Look at Fig. 4).

In control animals, significant signs of differentiation could be observed in the articular cartilage by day 45. Isogenic groups of round cells located in the hyaline cartilage formed vertical columns. Signs of development, thickening or improvement of the perichondria fibers indicate an improvement in the surfaces forming the joint in accordance with age.

In the joints of newborn animals, the formation process is not complete, the layers of the synovial membrane are not clearly differentiated, and the nipples are not developed. The cells of the synovial membrane are poorly differentiated from each other. The synovial cells of the integumentary layer are mainly rounded and have an average nuclear-cytoplasmic ratio. In the knee joints of rats, the main component of the synovial membrane is the intercellular substance.

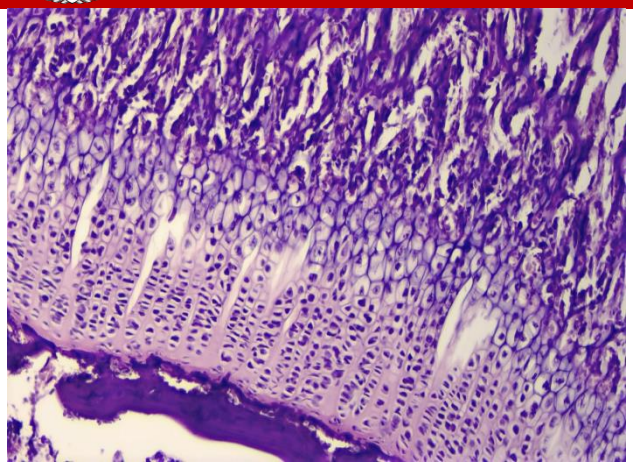

Figure 3. Hyaline proliferative cells - chondrocytes, located in columns in the basal layer. GE. K.400.

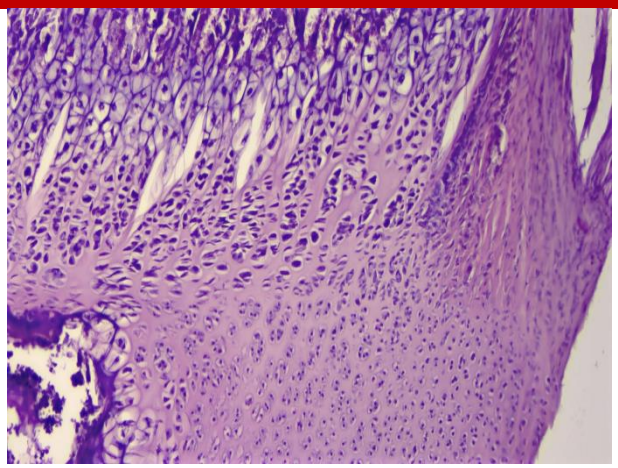

Figure 4. On the surface of the joints, an even contour of the layers of the joints was determined. GE., K.400

To confirm that experimental hypothyroidism was induced in the animals, the amount of triiodothyronine (T3), free thyroxine (T4) and thyroid stimulating hormone (TSH) was determined in the blood of rats on different days of the experiment (Look at Table 2).

Table 2

Hormonal parameters in the blood of rats in the control and experimental groups

Groups	Hormonal content in the blood (M ± m)	Day 7	Day 14	Day 21	Day 30	Day 45
Control group	TTG (mkME/ml)	0,14±0,2	0,2±0,02	0,3±0,04	0,4±0,17	0,5±0,19
	Triiodothyronine (T3)	8,5±0,08	9,4±1,3	9,8±±0,3	10,4±0,3	11,3±0,3
	Thyroxine (free T4) (pmol / l)	12,00±1,4	12,02±1,5	12,03±1.2	13,04±1,4	14,00±1,4
Experimental group	TTG (mkME/ml)	0,18±0,7	0,4±0,03	0,42±0,04	0,44±0,02	0,42±0,05
	Triiodothyronine (T3)	7,7±0,4	5,03±0,9	0,42±0,04	0,44±0,01	0,42±0,03
	Thyroxine (unbound T4) (pmol / l)	10,3 ±0,3	6,01±0,8	0,42±0,04	0,4 ±0,02	0,4 ±0,02

Note: * - P≤0.05, ** - P≤0.01, differences compared to the control group were considered significant.

An analysis of the animals' blood showed that the T3 and T4 hormones in the hypothyroid and control groups of rats did not differ significantly from each other on the 7th day of the experiment. On the 14th day of the experiment, a clear decrease in the T4 indicator and a less pronounced decrease in the T3 indicator were observed. On the 21st day of the experiment, it was found that T3 decreased by one time, and the T4 hormone indicator

decreased by two times. In the blood of 30-day-old rats, the thyroid hormone T3 decreased by one and a half times, and T4 decreased by 4 times. Thus, in experimental hypothyroidism, the analysis of the hormone indicator showed a reliable decrease in the thyroxine hormone (T4) in the blood of rats. The decrease in the T4 hormone was clearly reflected from the 14th day, and by the last day of the experiment, the reliability decreased to 4 times.

The amount of thyroid hormones in the blood is controlled by thyrotropin. A decrease in the amount of T3 and T4 hormones in the blood led to an increase in the TSH hormone. On the 7th day of the experiment, the amount of TSH was the same as in the control group. By the 14th day of the experiment, a gradual increase in TSH was noted, and by the 21st day it was 2 times higher than in the control group.

Conclusion.

In the cartilaginous tissue of the knee joint of the offspring born from pregnant rats in a state of hypothyroidism, histological changes were observed during the observation period, accompanied by an uneven arrangement of chondrocyte differentiation in the outer, middle and inner layers. Among the synovial membrane cells of the offspring of mothers with hypothyroidism, more fibroblasts were observed, mitotic binding cells were observed, and an increase in fat cells accumulating near the vessels along the blood vessels was observed, and a lag in the relief of the nipples from the histological structure of the control animals (corresponding to the previous observation period) was revealed. On the 7th and 14th days of observation, the thickness of the hyaline thickets covering the bone surfaces of the knee joint in the experimental group compared to the control group significantly differed from the comparative values ($R < 0.05$). On the 21st day of observation, this indicator was 260 ± 10.6 μm, and on the 45th day, the thickness of the hyaline collar was 286 ± 12.5 μm, which is 12% lower than the control indicator at the same time.

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