AGE-RELATED MORPHOLOGICAL CHANGES OF THYROID GLAND IN CALVES

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Abstract. The thyroid gland of 97 calves between 0 and 6 month old of two main breeds`— Estonian Red (EP) and Estonian Holstein (EH) were investigated. Thyroid gland mass, volume, density dimensions of lobules and follicles, and the height of follicular epithelium were measured. The relationships between these parameters and the age, sex and breed of the calf were analyzed. The relationship between thyroid gland mass and histological structure was investigated. The number and localization of C cells of calves was studied. The results indicate that the growth of the thyroid gland was most intensive during the first postnatal week. There was no obvious relationship between the histological structure and mass of the thyroid gland. C cells were localized intrafolliculary (single cells) and interfolliculary.

Keywords: calves, thyroid gland, C cells, mass.

SKIRTINGO AMŽIAUS VERŠELIŲ SKYDLIAUKĖS MORFOLOGINIAI POKYČIAI

Santrauka. Ištirta dviejų veislių − Estijos žalųjų ir Estijos holšteinų 97 veršelių nuo 0 iki 6 mėn. amžiaus skydliaukės. Išmatuota skydliaukės masė, tūris, skiltelių ir folikulų tankis bei folikulinio epitelio aukštis. Įvertintas santykis tarp šių parametrų ir veršelių amžiaus, lyties ir veislės. Įvertintas santykis tarp skydliaukės masės ir histologinės struktūros, ištirta veršelių skydliaukės C ląstelių skaičius ir lokalizacija. Rezultatai parodė, kad skydliaukė labai sparčiai auga pirmą savaitę po gimimo. Aiškaus patikimumo tarp skydliaukės histologinės struktūros ir masės nenustatyta. C ląstelės lokalizavosi folikulų viduje (pavienės ląstelės) ir tarp folikulų.

Raktažodžiai: veršeliai, skydliaukė, C ląstelės

Introduction. According to Smyth et al. (1996) the pathologies of thyroidal gland in calves account for a considerable part of postnatal diseases. The first indication of the thyroid pathologies is often the increase in mass and dimension of the gland. The interpretation of these signs is relatively complicated. Data about thyroid gland morphometry in calves are scanty. For cows the following relationship between thyroid gland mass/total body mass was established: 5.8±0.06g/100 kg (Kondrahhin, 1989). According to Tehver (1979) the average mass of the thyroid gland of a newborn calf is 10 g.

The report by Smyth et al. (1996) for presents higher values the mass of thyroid gland in newborn calves than these recorded in previous investigations. The autors argue that higher body mass results in a relatively larger thyroid gland and therefore, gland dimensions as an indication of pathology have a limited value. The positive correlation between body mass and the size of thyroid gland was absent and histopathological changes were present in 2.4% of cases. Often glands with normal mass revealed histological abnormalities and vice versa — oversized glands had normal histological structure. Smyth et al.,(1996) report that thyroid glands with histological abnormalities had mass below 30 g in 76% of cases and below 25g – in 69.6% of cases. Hence, the correlation between gland overweight and its pathology is not reliable.

According to Kübar (1981) thyroid gland belongs to organs which have a slow growth rate in the postnatal stage.

There are more data about the presence, localization and number of C cells in thyroid gland in dogs than in calves (Voitkevič, 1963; Stephens, 1985).

The wall of thyroid gland follicles consists of follicular endocrinocytes, which produce thyroxine and triiodothyronine, and parafollicularendocrinocytes, which produce thyrocalcitonin.

Thyroid parafollicular cells or C cells are different from follicular cells ultrastructurally, argyrophilically. They were obviously discriminated from follicular epithelial cells by 300 nm diametric granules and light colour of cytoplasm. Usually the number of C cells is not more than 1% of follicular cells (Voitkevič, 1963; Tehver, 1979). The parafollicular cells were recognized among the follicular epithelial cells and in the interstitial regions, but never protruded into the follicular lumen. Interfollicular C cells are often in groups, and formed follicles contain only C cells (Voitkevič, 1963; Tehver, 1979; Jubb et al., 1996).

Idelman (1963) found that the C cells are rarely parafolliculary (unless calves), usually localizing intra- or subepithelially. In bovine and swine C cells are bigger. According to Capen et al., (1967) the C cells are intra-, epi- and parafolliculary. Intrafolliculary localized C cells (in calves about 10 cells) are under the basal membrane.

The purpose of the present investigation was to characterize the morphometry of thyroid gland during postnatal development and to determine the C cells localization and their number in thyroid gland of calves.

Material and methods. The morphometry and histology of thyroid gland of 97 slaughtered, perished or stillborn from 0 to 6 month old calves of two main breeds – Estonian red (EP) and Estonian Holstein (EH) – were investigated. The distribution of material by age, sex and breed is given in Table 1.

Table 1. **The distribution of material by age, sex and breed**

Age (months)	Sex		Breed		Total
	Female	Male	EP*	EH*	
0—1	8	8	8	8	16
1—2	9	10	10	9	19
2—3	8	12	9	11	20
3—4	7	10	9	8	17
4—5	4	7	6	5	11
5—6	-	14	8	6	14
					97

* EP – Estonian red breed; EH – Estonian Holstein breed

For morphometry and histological study the thyroid gland was removed and cleaned from adherent tissue remnants, weighed and measured. The dimensions were measured separately for left and right lobes. Gland volume was determined by replaced amount of water in a graduated cylinder.

Specimens for light microscopy (LM) were taken from the center and edge of both lobes. The material for LM was fixed in buffered neutral formaldehyde solution (10%), and postfixed in Rossman fixative. Specimens were dehydrated in ethanol, embedded in paraffin and sections of 5 to 6 μm thickness were deparaffinized in xylene, stained with haematoxyline eosin and periodic-acid schiff technique (PAS). An "Olympus" light microscope was used for viewing and photography. Morphological measurements were taken with scale and grid reticles.

Results. Data in Table 2 indicate that the mean mass and volume of thyroid gland were increasing depending on the age. The mean mass of thyroid gland of 0-1 month old calves was 10.3 g (from 9.3 g to 11.5 g); 1-2 month old – 11.1 g (from 9.9 g to 12.7 g); 2-3 month old – 11.5 g (from 10.8 g to 12.3 g); 3-4 month old – 11.6 g (from 11.2 g to 12.3 g); 4-5 month old – 12.0 g (from 11.5 g to 12.9 g); 5-6 month old – 12.8 g (from 12.5 g to 13.1 g). The mean volume of thyroid gland of 0-1 month old calves was 10.1 cm^3; 1-2 month old – 10.9 cm^3; 2-3 month old – 11.2 cm^3; 3-4 month old – 11.4 cm^3; 4-5 month old – 11.6 g; 5-6 month old – 12.2 cm^3. It was found that the first postnatal month is characterized by a slow increase in mass (from 9.3 g to 11.1 g) and volume (from 8.5 cm^3 to 10.9 cm^3) of thyroid gland. The density of the gland was relatively stable. The right lobe dimensions were bigger than those of the left lobe. Mean height of epithelial cells were 5.8 to 8,3 μm. Highest epithelial cells were in 0-1 month old calves and lowest – in 5-6 month old calves. The C cells number is about 4.5 to 10. Parafollicular cells localized interfolliculary, some cells were intrafolliculary (between of follicularendocrinocytes, but not protruded into the follicular lumen) (Fig. 3, 6).

Discussion and conclusions. In his review Tehver (1971) stressed a greater variability of the weight of the thyroid gland in comparison with the weight of the other endocrine organs. The mass and volume of thyroid gland depend on animal breed, body weight, nutrition, physiological status, age, season of the year, geographical region, etc. In adult domestic animals the thyroid gland mass is quite variable. Changes in the mass and size of the gland are more pronounced in males (Križenecky, 1932). Sexual differences in the ratio of thyroid gland weight to total body weight in bovines are minimal. The mean gland weight for 67 bulls was 42.0 g, and for 171 cows – 32.59 g, and the ratio of thyroid gland weight – to total body weight 0.0692 and 0.0693, respectively (Krupski, 1921).

Slow increase in thyroid gland mass occurs in animals during the first 4 to 5 years (Tehver, 1971). Gland structure in older animals reveals typically larger follicles, denser colloid and a greater proportion of connective tissue (Davõdova, 1970).

Height growth of epithelial cells is the fastest in first postnatal week, whereas this parameter stabilizes later. Relative changes in follicle dimensions are lower than changes in gland mass.

In all histological specimens we found simultaneously active and inactive follicles and the lobules.

In a process of filling with colloids, small follicles merged forming macrofollicles (Fig. 1-5). In the process of gland activation, macrofollicles decreased and large macrofollicles decreased. The thyroid gland containing mainly macrofoll icles, was relatively inactive, whereas presence of a greater number of microfollicles results in a higher thyroid hormone production (Fig. 1).

In further investigations of the postnatal growth of the thyroid gland in two breeds of cows (EP and EH) more emphasis should be placed on interrelationships with the other endocrine organs. The anterior lobe of the pituitary gland and its β-basophilic cells, producing thyreotropic hormone, which regulate the development, structure and function of the thyroid gland, deserve special attention.

Conclusions:

1. The mean mass of the thyroid gland in newborn calves of the breed Estonian Holstein (EH) was more than in Estonian red (EP) (9.3 g and 8.5 g, respectively).
2. The growth of the thyroid gland was most intensive during the first postnatal week.
3. The thyroid gland mass growth mean value was 0.5 g per month.
4. There were no significant differences in mass, volume and lobes dimensions of the thyroid gland between the breeds and sexes.
5. There was no obvious relationship between the histological structure and mass of the thyroid gland.
6. C cells were localized intrafolliculary (single cells) and interfolliculary.

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Table 2. **Thyroid gland morphometry**

Number of calves	Breed	Sex	Mean weight g	Mean volume cm^3	Mean density g/cm^3	Dimensions - LxBxH cm		Mean height of epithelial cell (μm)	Number of C cells
						Right lobe	Left lobe		
Calves 0—1 months									
4	EH	F	11.0	10.8	1.01	4.2x2.8x0.8	4.1x2.2x0.8	8.0	6
4	EH	M	10.4	10.4	1.00	3.7x2.7x0.7	4.1x2.6x0.7	8.3	5
Mean for EH			10.7	10.6	1.01	3.9x2.8x0.8	4.1x2.4x0.7	8.1	5.5
4	EP	F	10.0	9.8	1.00	3.7x2.7x0.8	4.3x2.6x0.8	8.4	8
4	EP	M	9.9	9.7	1.02	3.6x3.0x0.7	3.9x3.0x0.7	8.5	7
Mean for EP			10.0	9.75	1.01	3.6x2.8x0.8	4.1x2.8x0.8	8.4	7.5
Mean for female F			10.5	10.1	1.03	3.9x2.8x0.8	4.2x2.4x0.8	8.2	7
Mean for male M			10.1	10.0	1.01	3.6x2.8x0.7	4.0x2.8x0.7	8.4	6
Mean for 0 - 1 months old			10.3	10.1	1.02	3.7x2.8x0.8	4.1x2.6x0.7	8.3	6.5
Calves 1—2 months									
4	EH	F	10.1	9.6	1.05	3.8x2.9x1.2	3.9x2.8x1.1	8.1	8
5	EH	M	12.4	12.3	1.01	3.7x2.8x1.2	4.3x2.7x1.3	7.9	9
Mean for EH			11.2	10.95	1.02	3.8x2.8x1.2	4.1x2.8x1.2	8.0	8.5
5	EP	F	11.2	10.9	1.02	4.0x2.7x1.1	4.1x2.9x0.9	7.8	10
5	EP	M	10.6	10.4	1.01	3.8x2.8x0.8	3.8x2.8x0.9	8.0	8
Mean for EP			10.9	10.7	1.01	3.9x2.8x0.9	3.9x2.9x0.9	7.9	9
Mean for female F			11.8	11.6	1.02	3.9x2.8x1.2	4.0x2.8x1.0	8.0	9
Mean for male M			10.4	10.3	1.01	3.8x2.8x1.0	4.0x2.8x1.1	8.0	8.5
Mean for 1 - 2 months old			11.1	10.9	1.02	3.9x2.8x1.1	4.0x2.8x1.1	8.0	8.8
Calves 2—3 months									
5	EH	F	11.0	10.8	1.02	3.7x2.6x0.9	3.8x2.9x0.9	7.4	10
6	EH	M	12.0	11.9	1.01	3.8x2.9x0.9	3.9x3.0x0.9	7.6	9
Mean for EH			11.5	11.4	1.01	3.7x2.7x0.9	3.8x2.9x0.9	7.5	9.5
4	EP	F	11.2	11.1	1.01	3.8x2.5x0.9	3.9x3.0x0.9	7.3	10
4	EP	M	11.9	11.3	1.05	3.8x3.0x0.8	4.0x3.1x1.0	7.5	11
Mean for EP			11.5	11.2	1.03	3.8x2.7x0.8	3.9x3.0x0.9	7.4	10.5
Mean for female F			11.1	10.9	1.02	3.7x2.5x0.9	3.8x2.9x0.9	7.4	10
Mean for male M			11.9	11.6	1.03	3.8x2.9x0.8	3.9x3.0x0.9	7.6	10
Mean for 2—3 months old			11.5	11.2	1.03	3.7x2.7x0.8	3.8x2.9x0.9	7.5	10
Calves 3—4 months									
3	EH	F	11.4	11.2	1.02	3.8x4.0x1.0	3.9x4.1x1.1	6.8	8
4	EH	M	11.6	11.4	1.02	4.0x4.1x1.0	4.1x4.2x1.0	7.2	7
Mean for EH			11.5	11.3	1.02	3.9x4.0x1.0	4.0x4.1x1.0	7.0	7.5
4	EP	F	11.8	11.6	1.02	4.0x3.5x0.9	4.1x3.7x0.7	7.0	10
6	EP	M	11.9	11.7	1.02	4.2x3.7x1.0	4.4x3.9x0.9	7.2	5
Mean for EP			11.8	11.7	1.01	4.1x3.5x0.9	4.2x3.8x0.8	7.1	7.5
Mean for female F			11.6	11.4	1.02	3.9x3.7x0.9	4.0x3.9x0.9	6.9	9
Mean for male M			11.7	11.5	1.02	4.0x3.8x1.0	4.1x3.9x0.9	7.2	6
Mean for 3 - 4 months old			11.6	11.4	1.02	3.9x3.7x0.9	4.0x3.9x0.9	7.1	7.5
Calves 4—5 months									
2	EH	F	11.9	11.0	1.08	4.0x3.1x1.1	4.1x3.2x0.9	6.7	9
3	EH	M	12.6	12.4	1.02	4.6x3.7x1.0	4.8x3.9x1.0	6.7	8
Mean for EH			12.2	11.7	1.04	4.3x3.4x1.0	4.4x3.5x0.9	6.7	8.5
2	EP	F	11.8	11.6	1.02	4.3x3.2x0.9	4.4x3.4x1.0	6.6	7
4	EP	M	12.1	11.7	1.03	4.2x3.1x0.8	4.5x3.6x0.9	6.8	6
Mean for EP			11.9	11.6	1.03	4.2x3.1x0.8	4.4x3.5x0.9	6.7	6.5
Mean for female F			11.8	11.3	1.04	4.1x3.1x1.0	4.2x3.3x0.9	6.7	5
Mean for male M			12.3	12.0	1.03	4.4x3.4x0.9	4.6x3.7x0.9	6.8	7
Mean for 4 - 5 months old			12.0	11.6	1.03	4.2x3.2x0.9	4.4x3.5x0.9	6.7	6
Calves 5—6 months									
0	EH	F							
6	EH	M	12.7	12.4	1.02	4.7x3.0x1.0	4.8x3.2x1.2	5.8	4
Mean for EH			12.7	12.4	1.02	4.7x3.0x1.0	4.8x3.2x1.2	5.8	4
0	EP	F							
8	EP	M	12.9	12.0	1.08	4.6x3.1x0.9	4.9x3.7x1.0	5.7	5
Mean for EP			12.9	12.0	1.08	4.6x3.1x0.9	4.9x3.7x1.0	5.7	5
Mean for female F									
Mean for male M			12.8	12.2	1.05	4.7x3.1x1.0	4.9x3.5x1.1	5.8	4.5
Mean for 5 - 6 months old			12.8	12.2	1.05	4.6x3.0x0.9	4.8x3.4x1.1	5.8	4.5

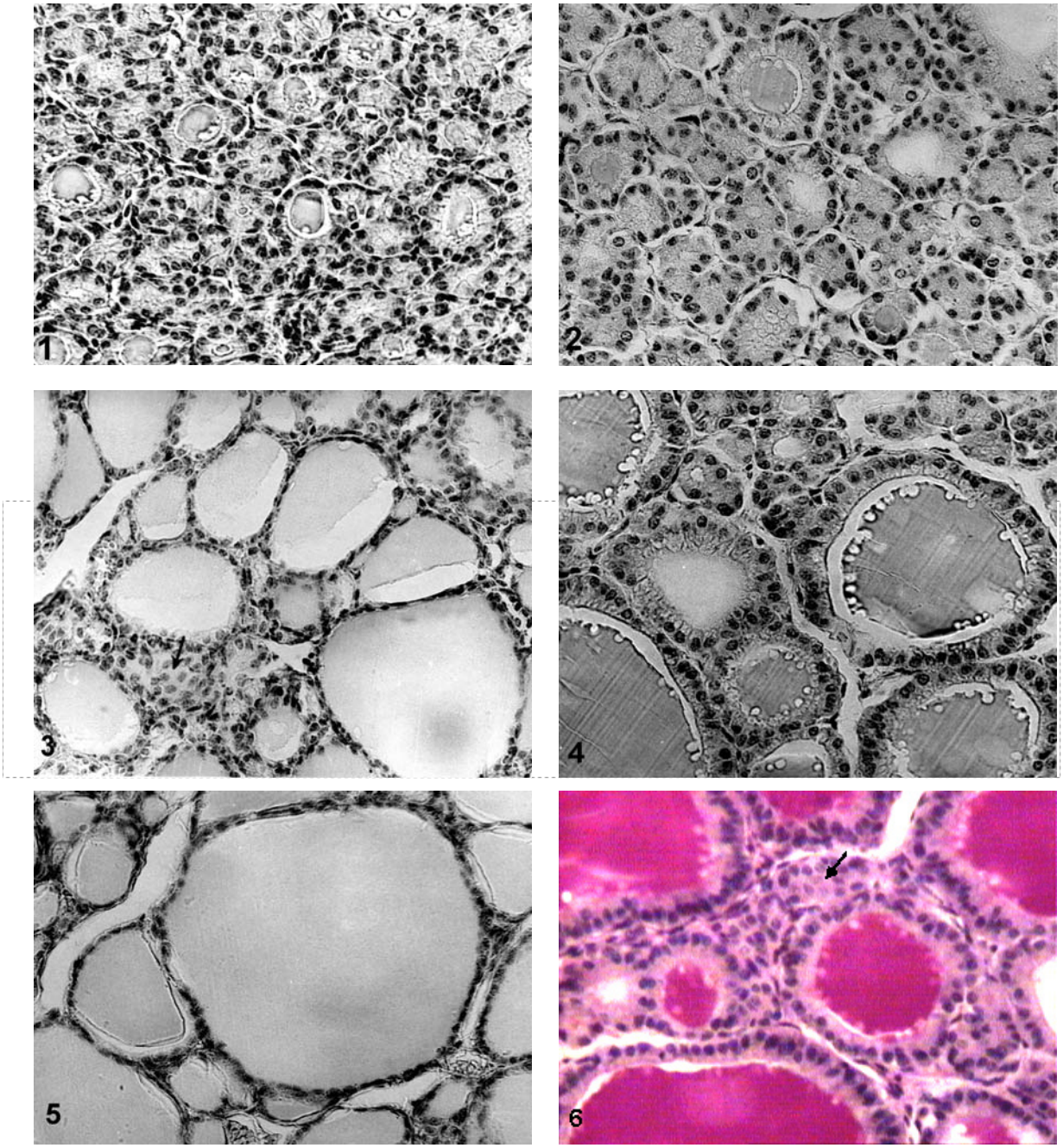

Figure 1. **Thyroid gland of bull calf 7 days old, breed – Estonian Red. Great number of microfollicles filled with colloid reveals functional activity. Haematoxyline-eosine. Magnification 200.**

Figure 2. **Thyroid gland of female calf 14 days old, breed – Estonian Holstein. Active secretional phase. Haematoxyline-eosine. Magnification 200.**

Figure 3. **Thyroid gland of female calf 27 days old, breed – Estonian Holstein. Different stages of the origin of macrofollicles. Arrow indicates C cells. Haematoxyline-eosine. Magnification 200.**

Figure 4. **Thyroid gland of female calf 1.5 months old, breed – Estonian Holstein. Microfollicles in excretiorial phase, in a center – a large follicle. Epitelial cells high, cylindrical. Haematoxyline-eosine. Magnification 200.**

Figure 5. **Thyroid gland of bull calf 2 months old, breed – Estonian Red. Inactive macrofollicle. Follicular epithelium thin, flat; follicle filled dense homogene colloid. Haematoxyline-eosine. Magnification 200.**

Figure 6. **Thyroid gland of bull calf 4 months old, breed – Estonian Red. Interfollicular C cells (arrow). PAS. Magnification 200.**

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