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From the Department of Anatomy and Embryology and the Laboratory of Electron Microscopy, College of Veterinary Medicine, Helsinki, and the Electron Microscope Laboratory, University of Helsinki, Finland.

# ON THE PARAFOLLICULAR CELLS IN THE THYROID GLAND OF THE BOVINE FOETUS

By

*L.-A. Lindberg* and *S. Talanti*[*]

It is now known that the epithelium of the thyroid is composed of 2 components, the follicular cells and the parafollicular or light cells. It has been claimed that the parafollicular cells appear to be the source of thyrocalcitonin (*Foster et al.* 1964, *Matsuzawa* 1966 and *Pearse* 1966).

The parafollicular cells differ greatly from the follicular epithelial cells with respect to their cytology and cytochemistry. For the selective demonstration of the parafollicular cells there are now several histological and histochemical methods available (see for references *Pearse* 1966, *Bussolati & Pearse* 1967). Also the ultrastructure of the parafollicular cells differs clearly from that of the follicular epithelial cells (e.g. *Pearse* 1966).

The morphology of the parafollicular cells has been examined mainly in thyroids of adult animals. Embryologic examinations have mainly concerned the relationship between the parafollicular cells and the ultimobranchial body (*Pearse & Carvalheira* 1967). In the literature, some information on the parafollicular cells of newborn and young animals can be found, for example in the classic papers of *Nonidez* (1931). According to these, the structure and the staining characteristics of the cells differ from those of the adult animal. The parafollicular cells of the bovine foetus have not been systematically studied, although the parafollicular cells in the cow have been studied, especially to solve

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their possible role in the aetiology and pathogenesis of puerperal paresis (*Capen & Young* 1967).

The purpose of this study was to examine the characteristics and occurrence of the parafollicular cells in the bovine foetus, using some common selective and histochemical staining methods and electron microscopy.

## MATERIAL AND METHODS

The material for light microscopic studies consisted of 117 foetuses from healthy cows. The thyroids were removed, cut up and fixed in 10 % formalin, Bouin's fluid, 70 % aethyl alcohol or in absolute alcohol. Selected blocks were frozen with dry ice without previous fixation, and cryostate sections were prepared for the enzyme cytochemical technique used.

Argyrophilia was demonstrated by Cajal's silver method (*Nonidez* 1931). The cytochemical methods used were the Luxol fast blue-technique for phospholipids (*Pearse* 1960) and *Wattenberg & Leong*'s (1960) technique for demonstration of α-glycerophosphate dehydrogenase. Moreover, a method suggested by *Solcia & Sampietro* (1968) was used.

In the electron microscopic studies, thyroid samples from 7 bovine foetuses were used. The foetuses were taken from cows as soon as possible after they had been slaughtered. Due to the slaughter-house routine the time that elapsed between the killing of the cows and putting the samples in the fixative was about 20—30 min.

Small pieces of the thyroid glands were fixed in 2.5 % glutar aldehyde buffered with 0.1 M phosphate buffer for 2 hrs. at 4°C and pH 7.2. After fixation the samples were rinsed in a buffer containing 0.2 M sucrose for 24 hrs. at 4°C. Postfixation was carried out in 1 % osmium tetroxide buffered with phosphate buffer for 1 hr. at 4°C. After dehydration in aethyl alcohol the tissue pieces were embedded in Epon (*Luft* 1961). Sections were cut on an LKB ultrotome. They were stained with uranyl acetate (*Brody* 1959) at room temperature, with lead citrate (*Reynolds* 1963) or double stained with uranyl acetate and lead citrate. The sections were examined in an Akashi Tronscope and a Philips EM 200 electron microscope.

Sections about 1.5 μ were also cut with the ultrotome and stained with 1 % Toluidine blue for examination with light microscope.

The age of the foetuses used in this study could not be ascertained but was estimated by using the table of *Richter & Götze* (1950). According to these authors, the calculation of age is based upon the body lengths. The body lengths of the foetuses of the material used for light microscopy varied between 6 and 67 cm, and the estimated age between 2 and 9 foetal months. The body lengths of the foetuses used for the electron microscopic studies varied from 8 to 72 cm, corresponding to an age from 2 to 9 foetal months.

## RESULTS

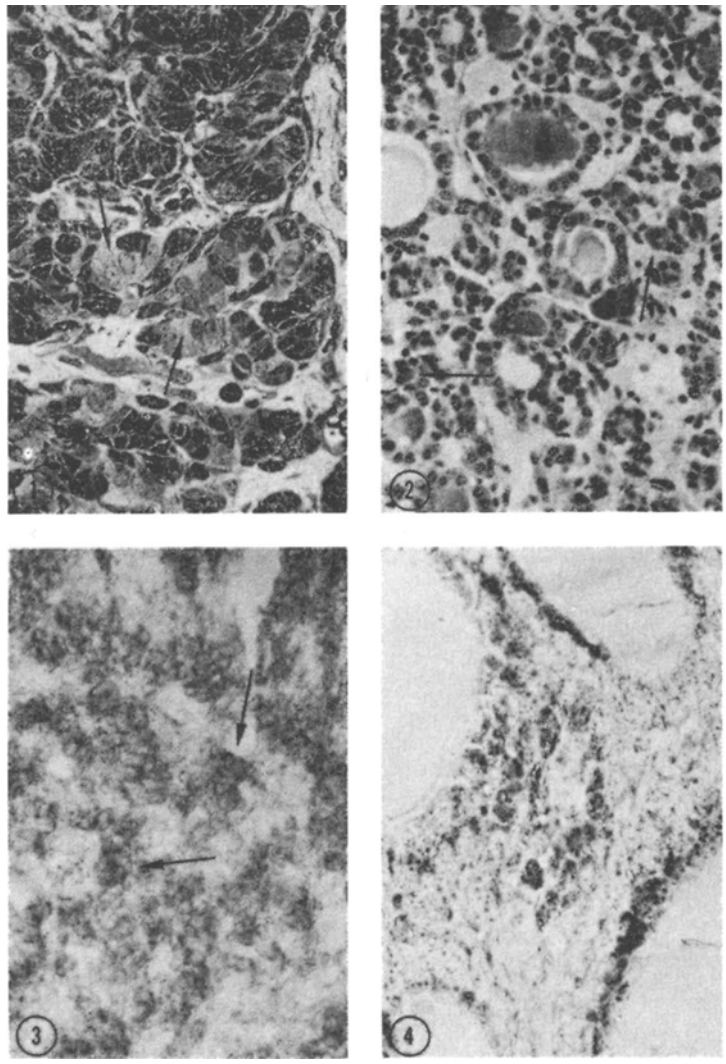
### *Light microscopy*

In the youngest foetuses, the morphology of the thyroid was far from being finally formed. The follicular cells appeared in groups or strings, and no follicles could yet be seen. Only when foetuses had reached a length of 25 cm, the follicles were typically developed. The parafollicular cells appeared often in the youngest foetuses in compact groups (Fig. 1). Only when the follicles had developed, the distribution of the parafollicular cells was comparable to that in adult animals.

In the whole series of foetuses, it was noticed that the argyrophilia of the parafollicular cells was quite weak. The distinguishment between the parafollicular and the follicular cells seemed to be difficult when based only on the argyrophilia (Fig. 2). By histochemical methods, the Luxol fast blue-method and the α-glycerophosphate dehydrogenase reaction, the parafollicular cells could be demonstrated even in the youngest foetuses. However, the intensity of the reaction seemed to be quite low (Fig. 3). By the method of *Solcia & Sampietro* (1968), which presumably stains the secretory granules and 5-hydroxytryptamine selectively in the thyroid parafollicular cells, positive cells could be clearly demonstrated, especially in more developed foetuses (Fig. 4). In the youngest foetuses the reaction was very weak.

### *Electron microscopy*

Parafollicular cells could be seen in all samples from the foetuses examined. They were generally located in small groups or solitary amongst the follicular cells (Figs. 5 and 6). They were often seen near blood capillaries of the interfollicular tissue (Fig. 5), but they never reached the follicle lumen in those foetuses where follicles had already been formed.

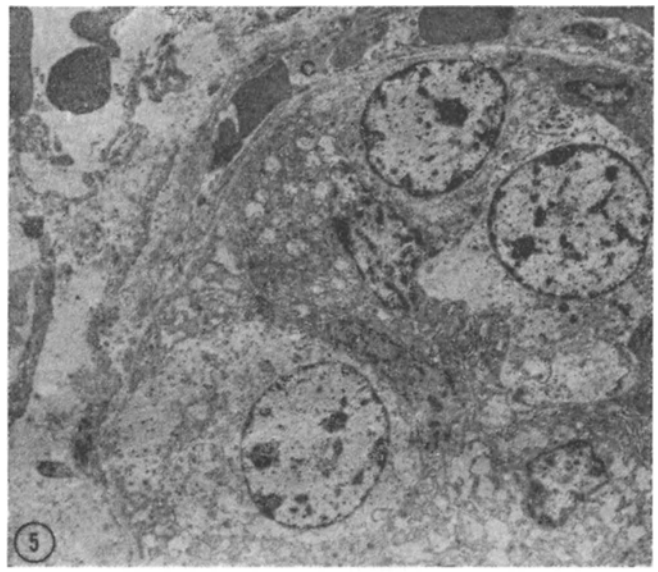



F i g u r e 1. Light micrograph of an Epon embedded and Toluidine blue stained 2 μ section from the thyroid of an 8-cm foetus. Parafollicular cells (arrows) can be seen in groups amongst the follicular cells. × 340.

F i g u r e 2. Light micrograph of the thyroid of a 51-cm foetus. Cells with weak argyrophilia (arrows) can be seen. × 225.

F i g u r e 3. Light micrograph of the thyroid of a 22-cm foetus showing α-glycerophosphate dehydrogenase activity (arrows). × 225.

F i g u r e 4. Light micrograph of a section from the thyroid of a 45-cm foetus stained according to the Solcia-Sampietro method. × 675.



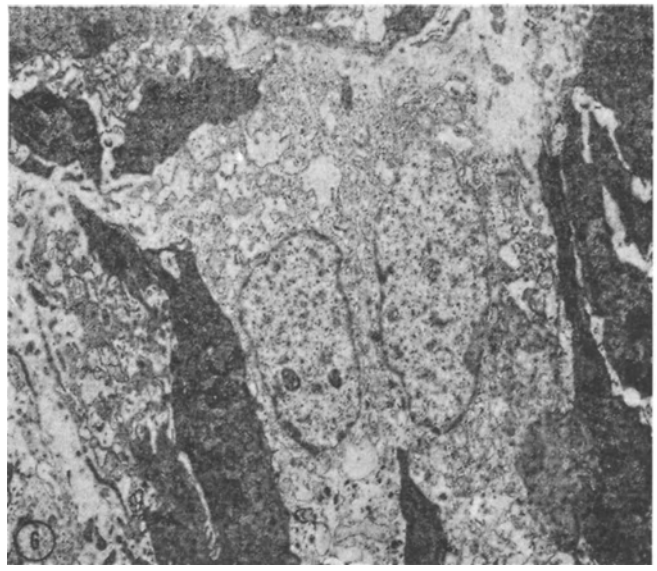


Figure 5. Electron micrograph of the thyroid of a 26-cm foetus showing 3 parafollicular cells with rounded nuclei very close to a blood capillary (c). × 3,100.

Figure 6. Electron micrograph of the thyroid of an 8-cm foetus showing 2 parafollicular cells with rather irregular nuclei. × 3,500.

In the 8-cm foetus, with an estimated age of about 2 months, groups of follicle cells could be seen clumped together starting the formation of follicles. Moreover, some cells of lighter appearance and with a developing granulation, regarded as parafollicular cells, could be seen close to the follicle cells. All cells, including the parafollicular cells, had an irregular and "embryonic" appearance (Figs. 6 and 7).

However, in the 25-cm foetus with an estimated age about 4 months, and in the older foetuses examined, formed follicles and follicle cells containing colloid could be seen.

The parafollicular cells were generally rounded and ovoid, although sometimes more or less process-like extensions could be seen (Figs. 5 and 8). The cytoplasm itself had a rather light, unstained appearance. The nuclei were quite large, regular and rounded except in the 8-cm foetus where the nuclei were irregular (Fig. 6).

The most characteristic components of the parafollicular cells, the secretory granules, were rounded, mostly highly electron dense and surrounded by a membrane. Their diameter was 1000—1500 Å, but in the 8-cm foetus it was somewhat less, about 900—1300 Å. They had a clear, although not very distinct, zone of low density between the matrix and the surrounding membrane, as described previously by *Ekholm & Ericson* (1968) (Fig. 9). In 1 sample, 2 granules surrounded by a membrane seemed to be outside the cell (Fig. 10). At that site, a part of the plasma membrane seemed to have disappeared. Generally, the amount of granules in the parafollicular cells studied was not very great, although it varied between cells. No variation between foetuses of different ages could be observed in this respect, except in the 8-cm foetus in which the amount was smaller than in the other foetuses. The granules were not uniformly distributed in the cytoplasm, but showed a distinct polarity, mostly towards the interfollicular tissue and blood capillaries (Figs. 5, 8 and 11). In some cells they could be seen arranged in long lines along the plasma membrane.

The endoplasmic reticulum of the parafollicular cells consisted, especially in the 8-cm foetus, mainly of cisternae of the rough surfaced type. However, vesicles of the smooth surfaced type could also be seen, especially in older foetuses. The bulk of the endoplasmic reticulum was mainly localized to areas prac-

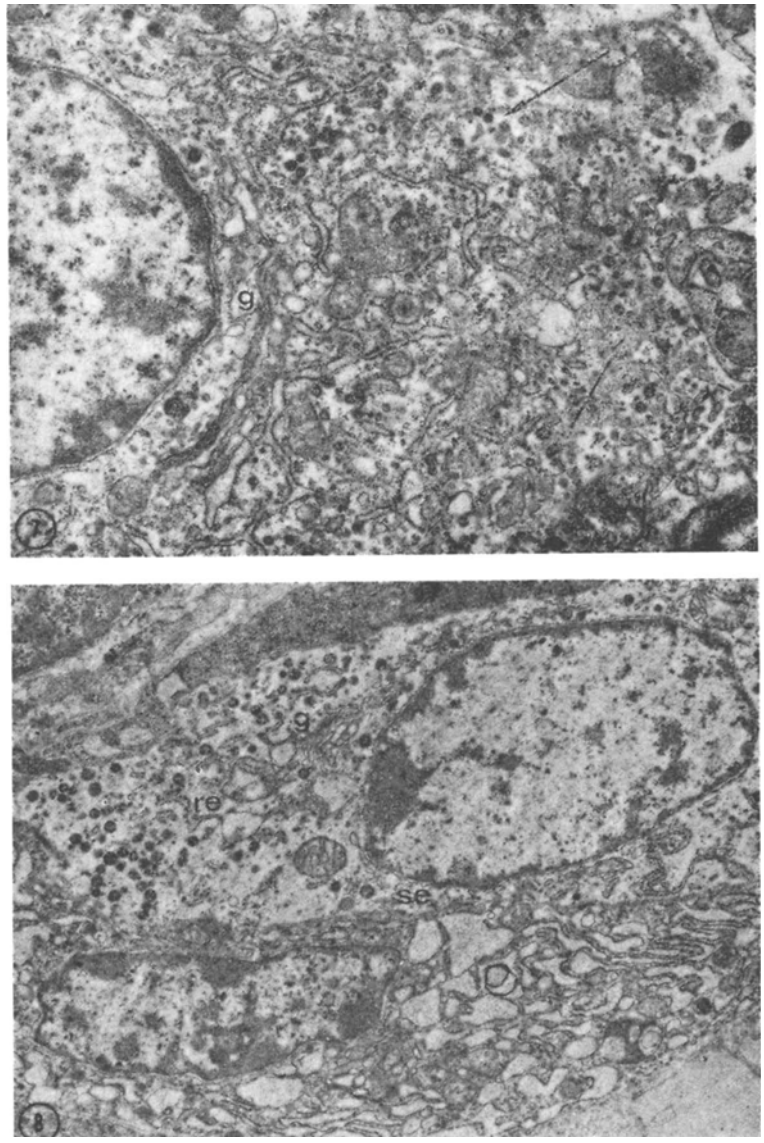
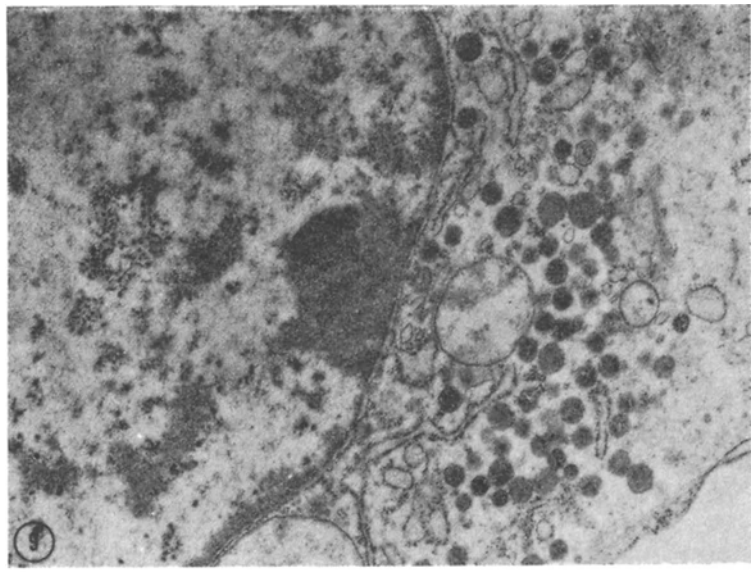



Figure 7. Electron micrograph of the thyroid of an 8-cm foetus showing a part of a parafollicular cell with developing granulation (arrows), Golgi apparatus (g) and the bulk of mostly rough-surfaced endoplasmic reticulum (re). × 13,000.

Figure 8. Electron micrograph of a thyroid parafollicular cell of a 26-cm foetus showing distinct polarity of the secretory granules, Golgi apparatus (g), smooth-surfaced (se) and rough-surfaced (re) endoplasmic reticulum. × 7,700.



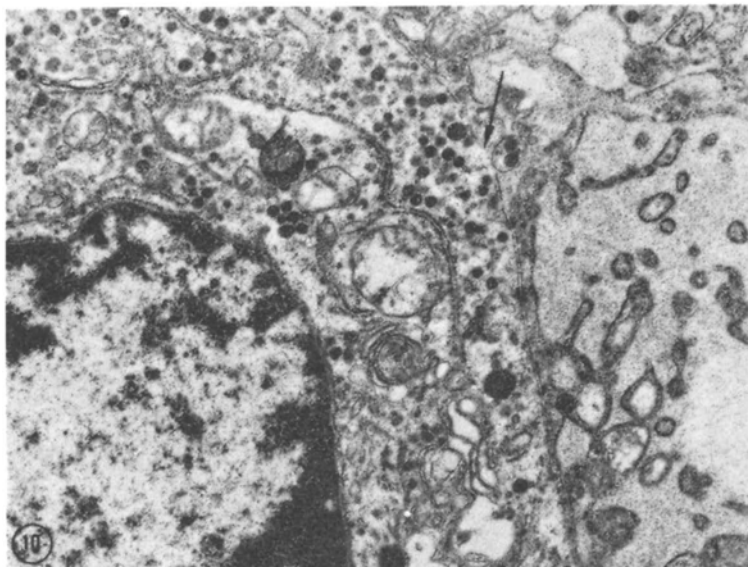


Figure 9. Electron micrograph of the thyroid of a 28-cm foetus. A part of a parafollicular cell with secretory granules surrounded by membranes can be seen. × 17,000.

Figure 10. Electron micrograph of a parafollicular cell of a 28-cm foetus showing 2 secretory granules surrounded by a membrane appearing to be outside the cell. At that site (arrow), a part of the plasma membrane seems to have disappeared. × 13,500.

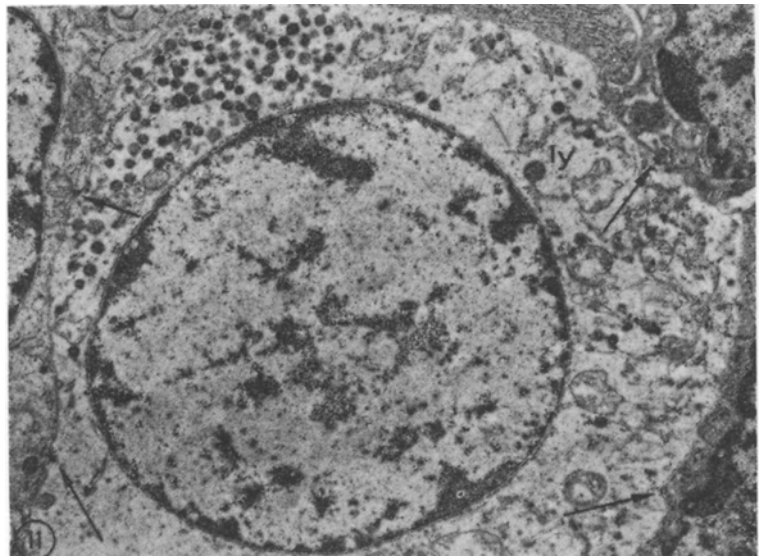



Figure 11. Electron micrograph of a thyroid parafollicular cell of a 26-cm foetus showing polarity of the secretory granules, desmosomes (arrows) and a lysosome (ly). × 7,300.

tically free of granules, although it could also be seen in granulated areas (Figs. 7, 8 and 10).

The Golgi apparatus was found in close relationship to the endoplasmic reticulum (Figs. 7 and 8).

The mitochondria of the parafollicular cells were not so well preserved, apparently because of the difficulty in getting the samples into the fixation solution quickly enough. They were rather numerous and distributed randomly over the whole cytoplasm.

Between the parafollicular cells and these and the follicular cells desmosomes were sometimes observed (Fig. 11).

Both in parafollicular and follicular cells some lysosomes could be seen. In parafollicular cells they could be distinguished from the secretory granules by their larger size (Figs. 7, 10 and 11).

## DISCUSSION

As could be expected, the results indicate that there are parafollicular cells in the thyroid of the bovine foetus. They could be found in a great number, often in groups, already in that phase

of the embryological development in which the follicles of the thyroid had not yet developed.

The cytological and the cytochemical characteristics of the foetal parafollicular cells differed only slightly from those in the adult animal. The differences seemed to be more pronounced the younger the foetus was. However, even in the youngest foetuses examined, it was possible to demonstrate the characteristic components of the parafollicular cells, i.e. the cytoplasmic granules. In all foetuses, the parafollicular cells contained the typical cell organelles. Thus the differences in the ultrastructure were not so prominent.

The differences between the results obtained by the histochemical and selective methods comparing young and more developed foetuses were, approximately measured, in the intensity of the reactions. These differences can hardly be caused by technical errors alone. In this work slaughter material was used, and it can be supposed that postmortal changes have been influencing the tissue. However, it can be presumed that these changes would have influenced the entire material in the same way.

On the basis of these results it might be concluded that the function of the parafollicular cells in the bovine starts already in the embryonic period. This is especially indicated by the fact that cytoplasmic granules can be demonstrated. The localization of the granules, regarded as the secretory products of the cell, might also indicate that they are released into the circulation. This could be indicated by the distinct polarity of the granules mostly towards interfollicular tissue and blood capillaries. An interesting observation in this connection is the 2 granules found in 1 sample, apparently outside the parafollicular cell and surrounded by a membrane. This might indicate one possible mechanism for the release of the granules. If the differences in the intensity of the reactions obtained by histochemical or selective methods are real, they may indicate that the secretion is quantitatively less in the foetus than in the adult animal.

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## SUMMARY

The thyroids of 117 bovine foetuses were studied by some histochemical and selective staining methods with respect to the occurrence of parafollicular cells. Furthermore, 7 foetuses were studied by electron microscopy. The length of the foetuses varied from 6 to 72 cm corresponding to an estimated age of about 2 to 9 months. Parafollicular cells were observed in the thyroids of all foetuses examined. The ultrastructure of the parafollicular cells in the foetuses differed very little from that described in adult animals. Histochemically, Luxol fast blue positive phospholipids and α-glycerophosphate dehydrogenase

activity could be observed in the cells. The Solcia-Sampietro method gave positive results in most of the foetuses examined. The reactions observed at light microscopic level were rather faint in the youngest foetuses. The functional aspects of the results are discussed.

## SAMMANFATTNING

*Om förekomsten av parafollikulära celler i sköldkörteln hos nötfoster och deras morfologi.*

Sköldkörtlarna från 117 nötfoster undersöktes med några histokemiska och selektiva färgningsmetoder med avseende på förekomsten av parafollikulära celler. Därutöver undersöktes sköldkörtelvävnad från 7 foster elektronmikroskopiskt. Fostrens längd varierade från 6 till 72 cm, motsvarande en beräknad ålder av 2 till 9 månader. Parafollikulära celler konstaterades i sköldkörtlarna hos alla undersökta foster. De fetala parafollikulära cellernas ultrastruktur skiljde sig mycket litet från den struktur som beskrivits hos fullvuxna djur. Histokemiskt konstaterades i cellerna Luxol fast blue positiva fosfolipider samt α-glyserofosfat dehydrogenas aktivitet. Solcia-Sampietro metoden gav positivt resultat i de flesta undersökta fostren. De ljusmikroskopiska reaktionernas intensitet var relativt svag hos de minsta fostren. De anförda resultatens funktionella betydelse diskuteras.

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