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Structural and Ultrastructural Study of GH, PRL and SMT Cells in Male Goat by Immunocytochemical Methods

M.A. Gómez[a] B. Garcés-Abadías[a] A. Muñoz[b] F. Vásquez[d] J. Serrano[c] A. Bernabé[a]

[a]Histología y Anatomía Patológica, [b]Genética, [c]Biología Animal, Facultad de Veterinaria, Universidad de Murcia, España; [d]Histología, Facultad de Veterinaria, Universidad de Rosario, Argentina

Key Words

Prolactin cell · Growth hormone cell · Somatomammotrope cell · Immunocytochemical techniques · Goat

Abstract

The structural and ultrastructural characteristics of adenohypophyseal growth hormone (GH)-, prolactin (PRL)- and GH-PRL (SMT)-secreting cells were studied using immunocytochemical techniques in two normal and one lactating male goat. SMT cells were found in both types of males, which showed the same characteristics as those reported for the female. PRL and SMT cells were more frequent in the lactating male, which reflects their greater galactopoietic activity. GH cells did not seem to influence this process significantly.

Abbreviations used in this paper

GH	growth hormone
PRL	prolactin
SMT	somatomammotrope

Introduction

Previous papers have established the structural and ultrastructural characteristics of different adenohypophyseal cells in young and adult female goat using histochemical and immunocytochemical techniques [Gómez, 1987; Navarro, 1987; Gómez et al., 1989; Sánchez et al., 1992, 1993; Seva, 1994]. Using immunofluorescence and immunoelectron microscopy in lactating goats, Sánchez et al. [1994] identified a bihormonal cell with growth hormone (GH) and prolactin (PRL) granules, the so-called somatomammotrope or mammosomatotrope cell (SMT cell). Porter et al. [1991] suggested that PRL and GH cells are functionally interchangeable and that SMT cells are a transitional form. Since this conclusion was mainly based on the increase of the number of PRL cells and the corresponding decrease in GH cells observed during lactation, our comparison of one milker buck with normal bucks was designed to confirm the above-mentioned conclusion. In addition, the study intended to identify and characterize SMT cells in the male goat, since this cell type has previously been studied only in the female.

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Dr. Miguel Angel Gómez Sánchez
Dpto. Anatomía y Anatomía Patológica Comparadas y Tecnología de los Alimentos
Histología y Anatomía Patológica, Fac. Veterinaria, Universidad de Murcia
Apdo Correos 4021, E–30071 Murcia (Spain)
Tel. +34 68 364705, Fax +34 68 364147, E-Mail magomez@fcu.um.es

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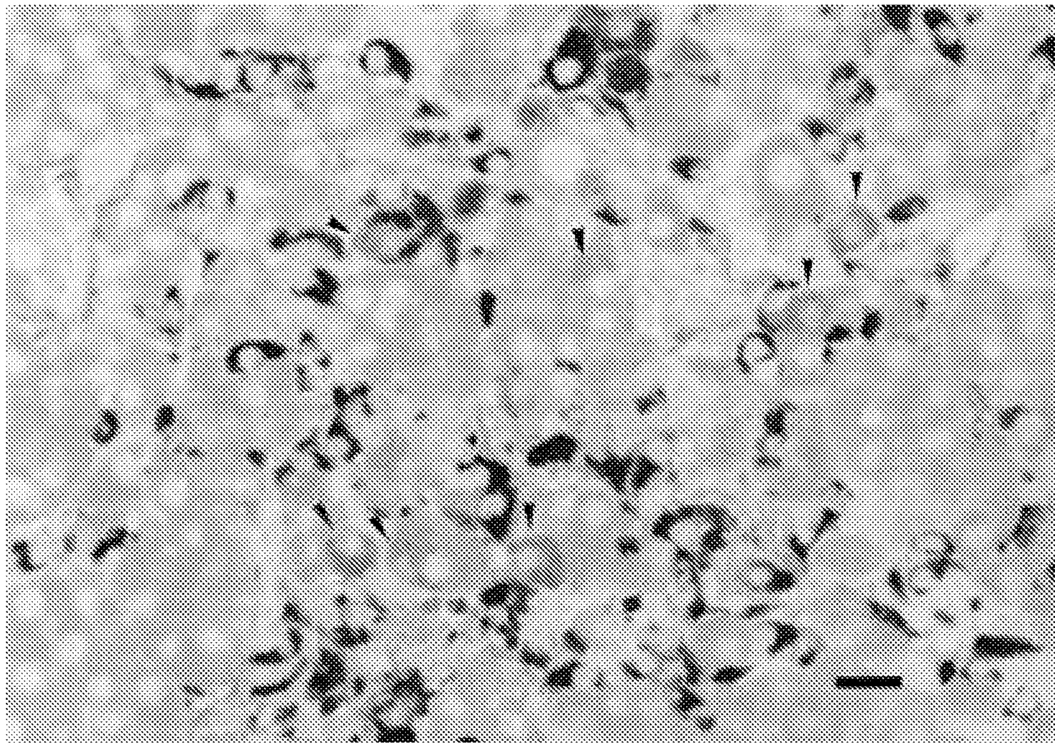

Fig. 1. Large clusters of PRL cells (brown), isolated GH cells (blue) and scarce SMT cells (greyish-brown, arrowheads). Immunocytochemical double technique, lactating buck goat. Bar = 25 μm.

Table 1. Diameter, area and number (± SD) of GH, PRL and SMT cells in normal and lactating buck goat

	Diameter, μm		Area, μm^2		Number		Percentage	
	normal	lactating	normal	lactating	normal	lactating	normal	lactating
GH	12.31±2.9	10.53±2.6	63.83±21.1	47.73±17.5	25.32±7.9	19.81±5.99	42.2	30.01
PRL	14.7±3.8	13.36±3.6	85.03±32.2	71.68±27.3	31.59±8	35.65±7.2	51.64	59.41
SMT	14.11±2.7	13.87±2.5	95.25±49.6	88.31±23.8	0.69±0.07	3.68±1.3	1.14	6.13

Materials and Methods

The adenohypophysis of three adult Murciano-Granadina male goats, one with a fully functional udder, were fixed by carotid perfusion with 4% paraformaldehyde and 0.5% glutaraldehyde in phosphate-buffered saline 0.12 *M*, pH 7.4. The gland was sectioned in two halves along the sagittal plane, one half being used for light microscopy and the other for electron microscopy. Necropsy confirmed that the buck milker had no hepatic, adrenal, or gonadal morphological alterations.

Light Microscopy

The sagittal half of each gland was washed in 0.2 *M*, pH 7.2 cacodylate buffer with 1% sucrose for 2 h. It was then refixed by immersion in Gerad's liquid [Gabe, 1968] for 24 h and embedded in poliwax (Difco). Ten sagittal serial sections (3–4 μm) were mounted on slides. The paraffin was removed from the sections, which were then hydrated before the fixative sublimate was removed with Lugol and blanched with 5% sodium hyposulfite. Avidin-biotin-peroxidase complex and biotin-alkaline phosphatase-peroxidase-antiperoxidase techniques were used for simple and double immunostaining, respectively. The sections were incubated at 30°C with rabbit antisheep GH or rabbit antisheep PRL (UCB Bioproducts, Belgium) sera, at 1:1,000 dilution in Tris-buffered saline both antisera with the simple

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technique and at 1:500 for rabbit anti-PRL serum with the double technique. With the latter technique, the thin sections were treated with paraformaldehyde vapor for 1 h at 60°C after the first immunostaining according to the method described by Wang and Larsson [1985].

Electron Microscopy
The remaining half of each gland was divided into three portions: anterior, medium and posterior. Samples were taken from the central and peripheral regions of each of the three portions, postfixed in 1% OsO_4 cacodylate buffer, dehydrated in graded alcohol and propylene oxide, and embedded in Epon. The immunocolloidal gold complex method was performed on one side of nickel grids. Ultrathin sections were etched by floating on saturated aqueous sodium metaperiodate for 30 min at room temperature before immunostaining. These were incubated at 30°C with rabbit antisheep GH serum diluted 1:2,500 in Tris-buffered saline, or rabbit antisheep PRL serum (UCB Bioproducts, Belgium) diluted 1:5,000 in the simple and double techniques. In the double technique, the grids were treated for 1 h at 60°C with paraformadehyde vapor after the first immunostaining according to the method of Wang and Larsson [1985]. Sections were incubated at room temperature for 1 h with 10-nm goat antirabbit IgG colloidal gold and 20-nm goat antirabbit IgG colloidal gold (Biocell, UK) diluted 1:40 as the secondary antibody. Grids were routinely contrasted with uranyl acetate and lead citrate.

In order to confirm the specificity of the immunostaining, control tests were carried out by substitution of the specific antisera for normal rabbit serum or phosphate-buffered saline, and using the specific antiserum adsorbed with the corresponding homologous hormone (0.1 ml anti-GH ovine 1:100 with 10 µg GH ovine or 0.1 ml anti-PRL ovine 1:1,500 with 10 µg PRL ovine, UCB Bioproducts, Belgium).

Morphometry
Sampling for morphometric analysis was carried out randomly according to Aherne and Dunnill's formula [1982], the sample being considered satisfactory when the standard deviation was 5% smaller than the arithmetical mean.

The numerical densities of the GH, PRL and SMT cells were calculated in five 10,000-μm^2 fields per section. The fields were chosen randomly from ten sections of pars distalis, separated from each other by 30 µm. A total of 50 fields per animal were evaluated.

The other parameters (cell area and diameter by light microscopy, numerical density of the secretory granule and granular diameter by electron microscopy) were calculated with an Image Analyzer Computer (IMCO 10 Kontron Bildanalyse) using the software of Microm Image processing (Microm, Spain). Twenty immunoreactive cells of each animal were chosen randomly from the different sections separated from each other by 50 µm, in order to determine the area and the cellular diameter. The micrographs of twenty whole GH and PRL cells from all 3 animals and five SMT cells from the buck milker were analyzed at a final magnification of 14,000× in order to determine the size of 20 secretory granules per hormone. The secretory granules were classified according to their diameter into three classes [see Kurosumi, 1991], small (<350 nm), medium (small and large granules) and large (>500 nm). Cells were classified according to the size of the secretory granules into type I cells with 70% of large secretory granules, type II cells with 70% of medium secretory granules, and type III cells with 70% of small secretory granules.

Results

GH Cells
Light microscopy showed the GH cells to be distributed through the whole pars distalis in small clusters or isolated (fig. 1) but predominating in the caudoventral region. The numerical density was higher in the normal males than in the lactating buck goat (table 1). The shape varied and the cells were smaller in the lactating buck goat than in the two normal males (table 1).

Under electron microscopy, GH cells (fig. 2–4) showed round and very electrodense secretory granules throughout the cytoplasm. The mean size of the secretory granules was 370 ± 87 nm (167–753 nm) in normal males, and 441 ± 80 nm (282–703 nm) in the lactating buck goat. Cell types I and II predominated in the lactating buck goat (table 2). There were no ultrastructural differences between the cytoplasmic vacuolar system, which was poorly developed in all the animals.

PRL Cells
Light microscopy showed the PRL cells to be distributed through the whole pars distalis except for the rostrocaudodorsal band. The numerical density was greater in the lactating buck goat than in normal males (table 1). This cell type usually formed large clusters or cords (fig. 1), sometimes mixed with GH cells. The shape varied (oval, cup, triangular, cylindrical) although elongated forms predominated. Cell size (area and diameter) was larger in normal males (table 1).

By electron microscopy spherical and very electrodense PRL secretory granules appeared evenly distributed through the whole cytoplasm (fig. 5, 6). Their mean size was 493 ± 12 nm (171–850 nm) in normal males and 590 ± 12 nm (249–1,054 nm) in the lactating buck goat. PRL cell type I (fig. 5) predominated in the lactating buck goat, whereas PRL cell type II (fig. 6) predominated in normal males (table 2). The rough endoplasmic reticulum appeared somewhat more developed in the lactating buck goat. Multiple exocytosis (fig. 5) was more common in PRL cells than in GH cells although there were no differences in this characteristic between the PRL cells of normal males and the lactating buck goat.

SMT Cells
With light microscopy the cytoplasm of these cells appeared greyish-brown with alkaline phosphatase-peroxidase-antiperoxidase (fig. 1). SMT cells were located in the rostral region near wide lumina capillaries, where they formed small clusters or were isolated. The numeric den-

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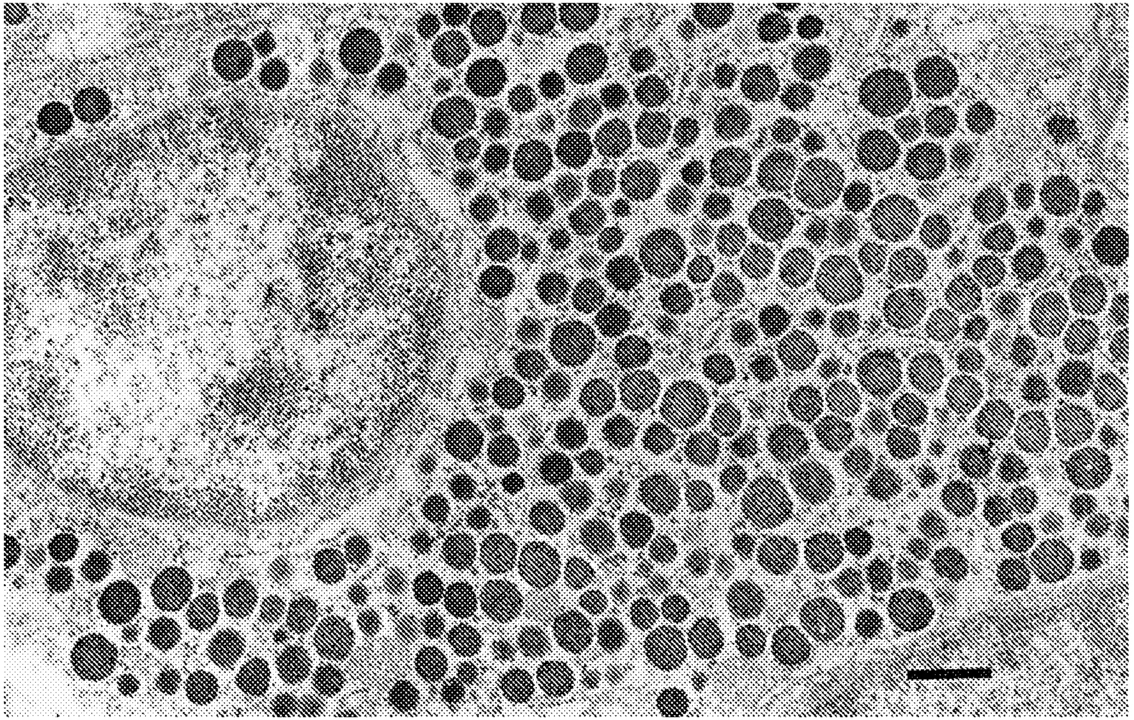

Fig. 2. GH cell type I. Lactating buck goat. Bar = 1 μm.

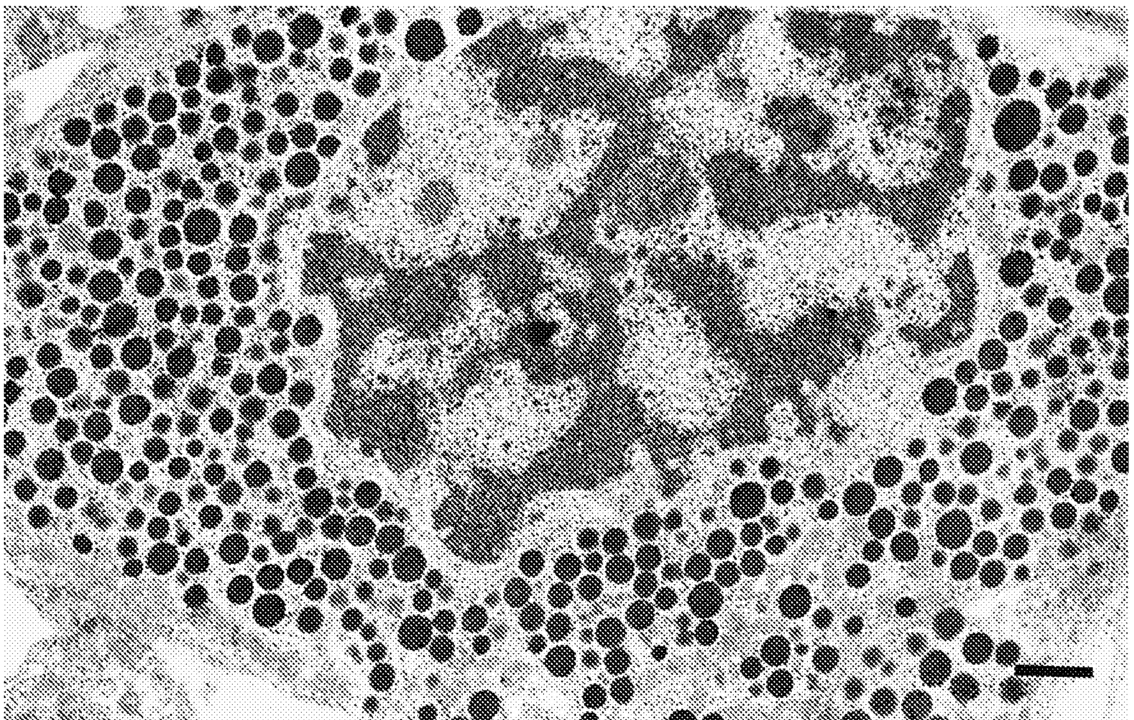

Fig. 3. GH cell type II. Normal male goat. Bar = 1 μm.

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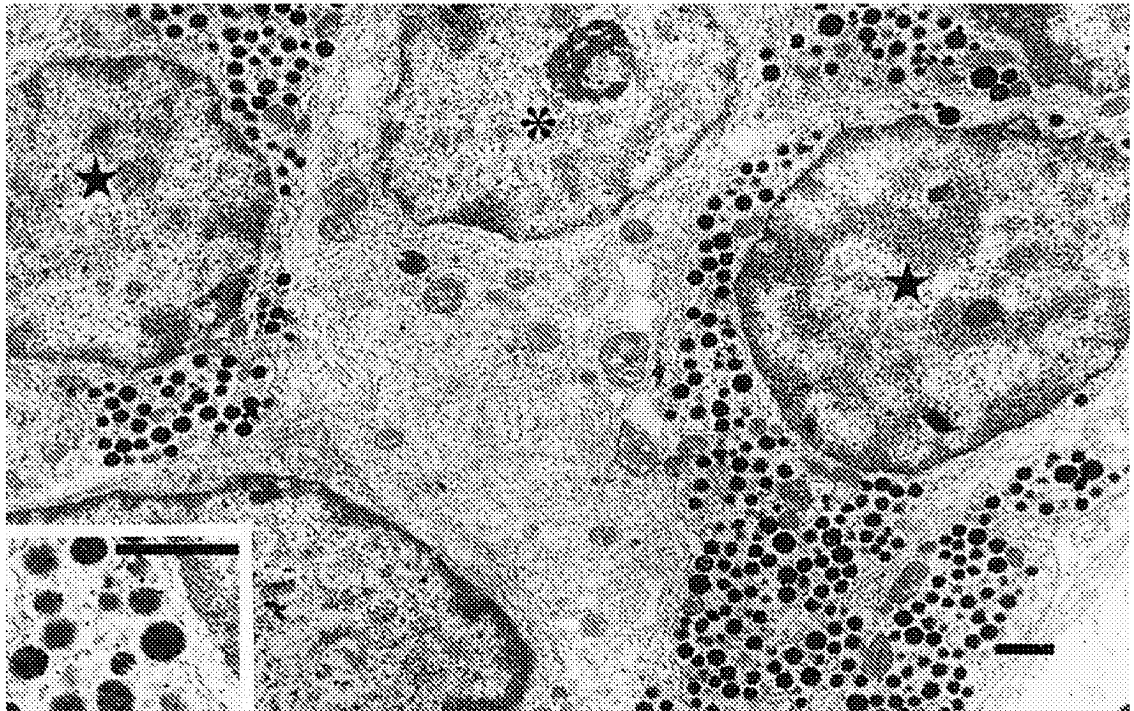

Fig. 4. GH cell type III (stars). Inset: Gold particles within secretory granules. Normal male goat. Bar = 1 μm.

Table 2. Mean diameter (D) and range (R) of secretory granules of GH and PRL cells classified according to granule size (types I, II, III) in normal and lactating buck goat

	Type I		Type II		Type III	
	normal	lactating	normal	lactating	normal	lactating
GH, %	0	4.4	77.8	95.6	22.2	0
D GH, nm		565±74	393±25	435±70	289±74	
R GH, nm		421–703	208–753	282–699	167–463	
PRL, %	54.5	70.4	36.4	29.6	9.1	0
D PRL, nm	580±99	565±74	469±84	484±100	286±76	
R PRL, nm	371–846	421–703	236–850	249–865	171–454	

Diameter and range are given as mean ± SD (in nm).

sity was greater in the lactating buck goat (table 1). The shape varied and the size was greater in normal males (table 1).

SMT cells were not detected by electron microscopy in normal males perhaps because they are very localized and less abundant than PRL and GH cells. In the lactating buck goat the secretory granules were spherical and very electrodense with a mean diameter of 377 ± 68 nm (185–583 nm) in the case of GH secretory granules, and of 550 ± 12 nm (332–1,048 nm) in the case of PRL secretory granules (fig. 7). The proportion of SMT cells showing a greater number of GH secretory granules was 26.7%, while 46.6% showed a greater number of PRL secretory granules and 26.7% showed the same proportion of types. No secretory granules with immunostaining for both antisera were observed. The rough endoplasmic reticulum

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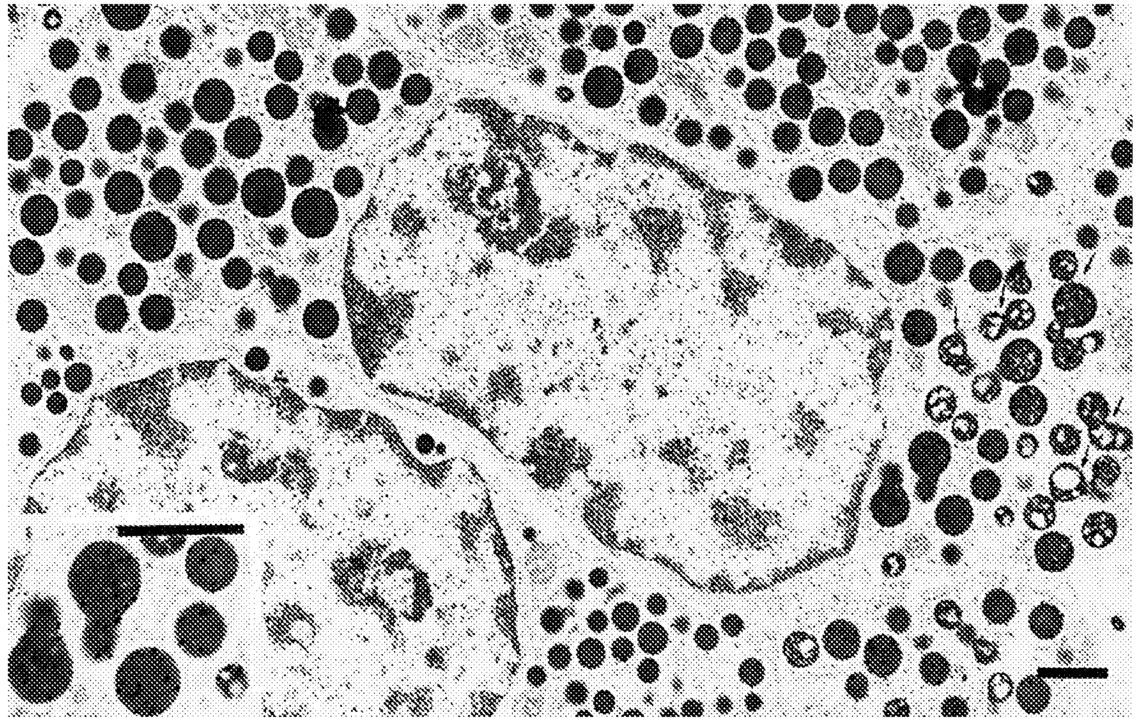

Fig. 5. PRL cell type I with multiple exocytosis (arrows). Inset: Gold particles within secretory granules. Normal male goat. Bar = 1 μm.

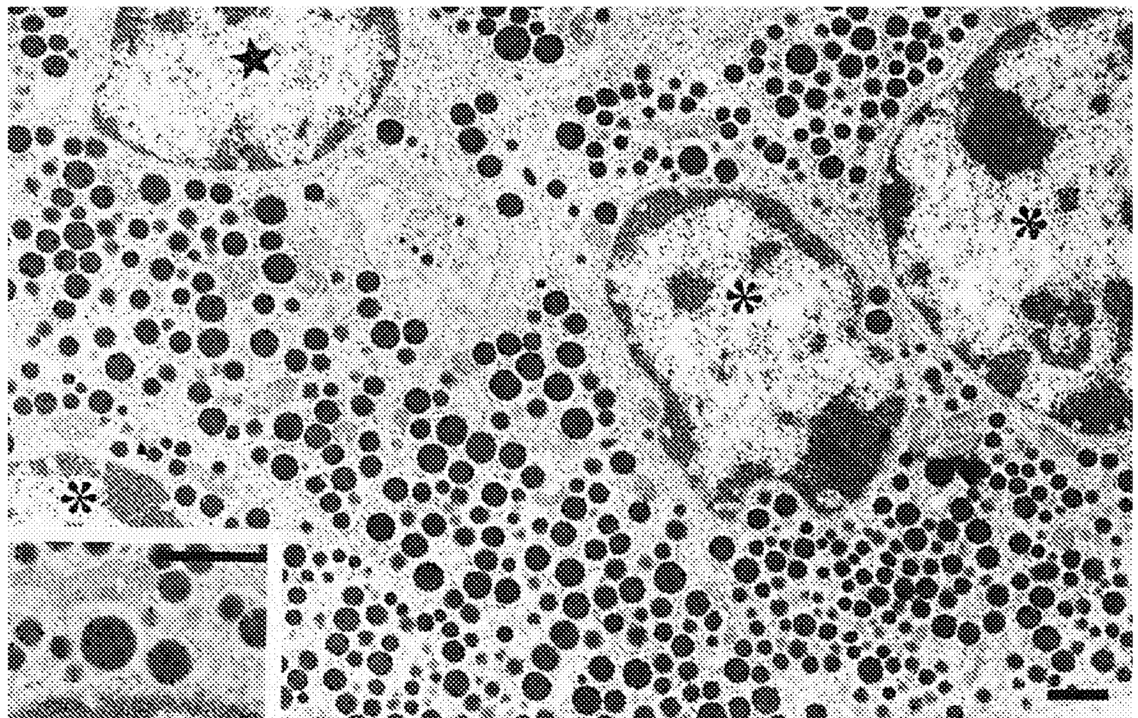

Fig. 6. PRL cell types II (star) and III (asterisks). Inset: Note gold particles within secretory granules. Normal male goat. Bar = 1 μm.

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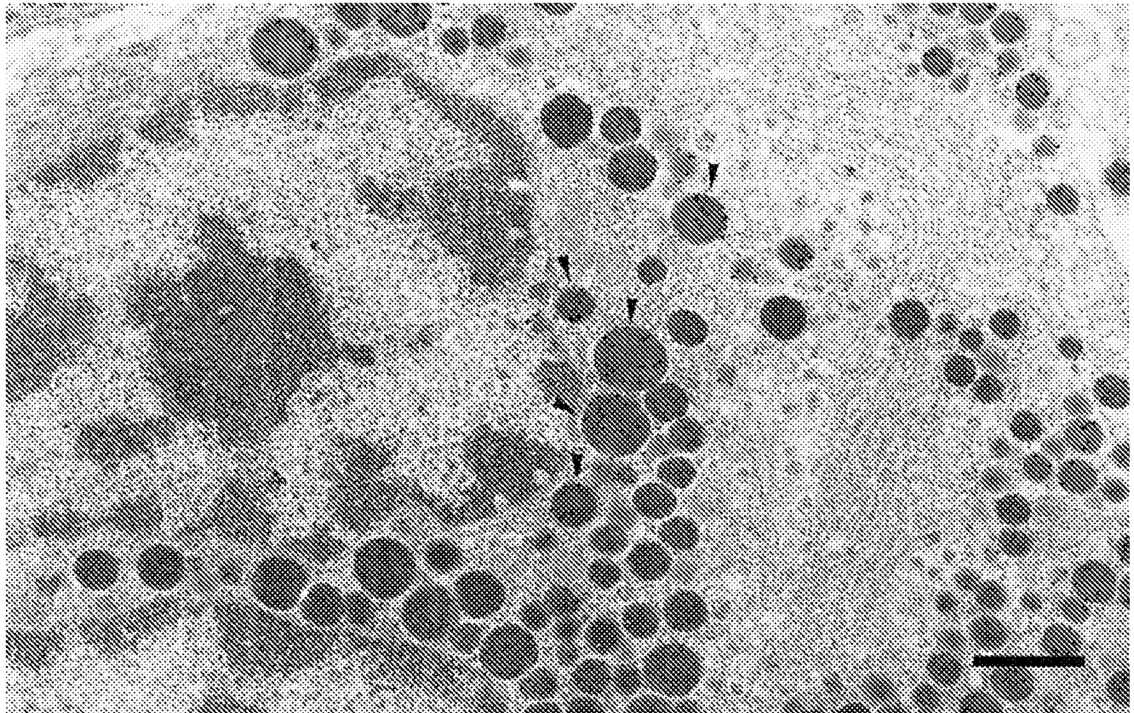

Fig. 7. SMT cell with abundant GH secretory granules (gold particles of 10 nm). PRL secretory granules are identified by gold particles of 20 nm (arrowheads). Lactating buck goat. Bar = 1 µm.

was distributed through the whole cytoplasm as narrow or light-dilated cisterns, and the Golgi complex was poorly developed.

Discussion

SMT cells were found in low numbers in the lactating male, and were even scarcer in the normal males. The morphological characteristics of this cell type were similar to those already known for the female [Sánchez et al., 1994], since they show two kinds of granule, one with PRL and the other with GH.

The most abundant cell types, PRL and GH, could be grouped into classes according to granule size [Kurosumi, 1991], as was reported for the female [Sánchez et al., 1992, 1994]. Type III cells showed morphological signs of functional activity (a high number of secretory granules, scarce lysosomes, lipid droplets), which were not observed in sheep [Gómez-Marín., 1994] and rat [Kurosumi, 1991].

The lactating buck had smaller PRL cells, a higher number of PRL and SMT cells, and larger PRL secretory granules than normal males. These observations reflect the galactopoietic activity of this individual. However, both kinds of males showed the same level of multiple exocytosis in PRL cells, a result that was not expected since this phenomenon is usually related with lactation [Rennels et al., 1983]. On the other hand, the observation that GH cells were less abundant in the lactating male suggests that this cell type is not related with galactopoietic activity. All these results indicate that the adenohypophysis of the lactating buck shows different characteristics with regard to that of normal males. More studies on normal and lactating males are needed to clarify the functional meaning of these adenohypophyseal cell types.

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