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Structural and ultrastructural modifications of adenohypophyseal gonadotropic cells in goat (*Capra hircus*) in anoestrus, gestation and milk production

J.A. Navarro, M.A. Gómez, A. Bernabé, S. Gómez and J. Sánchez
Department of Histology and Pathology, Faculty of Veterinary, University of Murcia, Murcia, Spain

Summary. The structural and ultrastructural modifications of the gonadotropic cells of goats were studied with an immunohistochemical method (peroxidase-antiperoxidase), in anoestrus, gestation and milk production. The cell type which predominates in anoestrus corresponds in its morphological characteristics to the classic FSH cells, and has two populations of homogeneous and electrodense secretory granules (141 - 244 nm and 244 - 400 nm in diameter), rough endoplasmic reticulum of flat cisternae and many large-sized lysosomes. During gestation secretory granules show a characteristic reduction in size and are less abundant; lysosomes are also more scarce and the endoplasmic reticulum shows a high development; dilated and intercommunicated cisternae show a slight electrondense content, characteristic of typical LH cells. During milk production the cells show an increase in the number of secretory granules which are still small, and an increase in the number of lysosomes which appear as in anoestrus.

Key words: Gonadotropic Cells, Adenohypophysis, Ultrastructure, Immunohistochemistry, Goat

Introduction

As has already been suggested in the rat (Barrnet et al., 1956) secretion of the two gonadotropic hormones FSH and LH takes place in the same cell type; this was subsequently corroborated by numerous studies using immunocytochemical techniques on this species, on pig (Batten and Hopkins, 1978; Dacheux, 1978), and sheep (Gross, 1984; Gross et al., 1984). In the goat one type of gonadotrope cells (GN cells) in adult males have been described (Shirasawa et al., 1985) and two in kids (Gómez et al., 1987). The aim of this paper is to identify GN cells of adult female goats in anoestrus, pregnancy and milk production, using the superimposition of immunomarked semithin sections adjacent to ultrathin sections, and study their changes through those reproductive stages.

Offprint requests to: Dr. J. A. Navarro Cámara, Histología y Anatomía Patológica, Facultad de Veterinaria, Universidad de Murcia, Aptdo. Correos 4021, E-30071 Murcia, Spain

Materials and methods

Materials and methods are described in an earlier paper about the adrenocorticotropic hormone cells (Navarro et al., 1991) using the *Pars distalis* of adenohypophysis of 15 female goats *(Capra hircus)*, three or five years old distributed in three groups of 5 animals each (anoestrus, gestation and milk production). The pituitary gland was quickly removed after euthanasia with Penthotal.

The sagittal half of each gland was fixed in sublimate formalin (Gerad's liquid) (Gabe, 1968), embedded in polywax (Difco) and sectioned at 4 - 5 μm thick to study its structure, while the other half was divided into transversal portions (anterior, media and posterior) and fixed in 5% glutaraldehyde solutions (Sabatini et al., 1962) and epon inclusion (Wanson and Drochman, 1968).

Labelling of cells was done according to peroxidase-antiperoxidase method -PAP- (Baker, 1977), described as follows:

After paraffin, removal sections were put into a lugol bath to eliminate the fixer sublimate. They were then blanched in 5% sodium hyposulphite, and after a long wash in running water, they were left in phosphate-buffered saline (PBS). After treatment with 5% hydrogen peroxide for 15 min (elimination of internal peroxidase activity) and a wash in PBS for 5 min, sections were covered with normal pig serum diluted in 0.05M Tris buffer, pH 7.6 for 20 min. Primary antibody against human FSH or human LH (IgG rabbit fraction prediluted in 0.05 Tris buffer pH 7.6, Dako), was used in a dilution 1:500 and incubated for 3 h at room temperature; then it

was washed in PBS for 20 min. Secondary antibody (pig IgG against rabbit Ig G) was diluted in 0.05M Tris buffer, pH 7.6 and incubated for 20 min. After, it was washed in PBS for 20 min. The PAP complex was diluted in 0.05 m Tris buffer, pH 7 (Dako) and incubated for 20 min, then it was washed in PBS for 20 min. Development was achieved with DAB (3, 3'-diamino-bencidine tetrachloride, Sigma) for 5 min.

The immunohistochemical controls reccomended by Childs and Ellison (1980) were also applied using adjacent sections, and evaluating the sensibility of the reaction with the following tests: a) Primary antiserum was substituted by normal pig serum or PBS. b) The first antiserum was absorbed with its homologous (FSH and LH hormones, Sigma) before incubation of the section. c) The first antiserum was applied to the sections in increasing dilutions. In test a) and b), there was no evident reaction after immunostaining of the sections that were not exposed to the first antiserum or in which this was previously absorbed. In test c) colouration diminished in proportion to antiserum concentration and at high dilutions it was not evident.

Different histological and histochemical stainings such as Hematoxylin-eosin (H-E), PAS, PAS-Orange G (PAS-OG), Herlant's tetrachrome, Blue alcian PAS-Orange G (BA-PAS-OG), and Cleveland-Wolfe were made in order to correlate the stain preferences of different cells with those developed immuno-histochemically.

For ultrastructural study ultrathin sections, about 40 nm thick, were cut with adjacent thick sections (1 µm thick) for light-microscopic immunostaining. The ultrathin sections were counterstained with 2% uranyl acetate and lead citrate and examined with an electron microscope. Semithin sections were obtained adjacent to the previous ones for observation under a light microscope. The cells were marked using the PAP method after being deplastified with sodium metoxide, using the same technique for sections in Paraplast, except for the following variations: times reduced to 10 min; incubation with the primary antiserum for 24° at 4° C; and after incubation in PAP complex all steps from the incubation of the first antiserum were repeated.

A morphometric study was carried out with a semi-automatic image analyzer scree (IBAS1) directly from the histological 3 to 5 µm thick sections from the adenohypophysis which had been embedded in paraplast and immunostained. The following parameters were measured: maximum and minimum diameter of the ellipse; maximum diameter and circle diameter of the cell. The 40 to 60 nm thick sample cuts were electronographed and magnified 15000 times for the morphometric study of the secretion granules. The diameter of the secretion granule was the statistical data obtained. Confidence intervals were used to compare the parameters studied in the different populations included in the analysis (anoestrus, gestation and milk production). The level of confidence was pre-fixed at 95% (x = 0.05).

Results

Light Microscopic Observations

In the three groups of animals, cells marked with both anti-FSH and anti-LH sera were slightly PAS-positive and were stained blue after Cleveland-Wolfe and after Alcian blue-PAS-orange G staining. They were sparse in the centre and periphery of the gland and more numerous in the anterior half. They appeared isolated and sometimes formed clusters. The morphology was variable although the oval shape predominated with small cytoplasmic vacuolisations. The mean diameter was 13.38 µm. The nucleus was central or eccentric with scarce chromatin and a large, central nucleolus. During gestation they showed a distribution similar to that of animals in anoestrus, a spherical morphology with a mean diameter of 13.10 µm predominating and showing a more vacuolised cytoplasm.

During the milk production stage there was an increase in GN cells. The distributional pattern was maintained, but small groups of cells intensely immuostained with an irregular morphology appeared. The mean diameter was 12.38 µm. The nuclei were more stained and vacuolisation could be observed in the cytoplasm which occupied large areas.

Ultrastructural observations

Immunomarked cells of semithin cuts with both anti-FSH and anti-LH sera showed the same ultrastructural morphological characteristics as the corresponding adjacent ultrathin cuts. This means that two cellular types can be found irrespective of the antiserum used, whose proportion varied in each phase of the cycle.

During anoestrus the predominating morphological type (Fig. 1) was characterized by the presence of a large-sized nuclei and a spherical morphology. They had a small amount of heterochromatin lying against the nuclear envelope and large nucleoli with abundant associated heterochromatin. In the cytoplasm a large number of secretory, spherical and electrondense granules appeared. The mean diameter of the granules was 255.7 nm, with 41% of the granules having a diameter between 244.6 and 400.3 nm. The rough endoplasmic reticulum was made up of narrow cisternae dispersed throughout the cytoplasm, although they were more numerous in the less granulated cells. The Golgi complex, which was well-developed, occupied a large juxtanuclear area, and images of granule formation were frequently observed. In the cytoplasm there were may large lysosome-like granules with a mean diameter of 1.7078 µm and with a heterogeneous content that were not immunostained in adjacent semithin sections, appearing as cytoplasmic vacuolisations (Fig. 1). The mitochondria were large and elongated with an electrondense matrix and were dispersed throughout the cytoplasm.

During gestation the most common type of GN cell

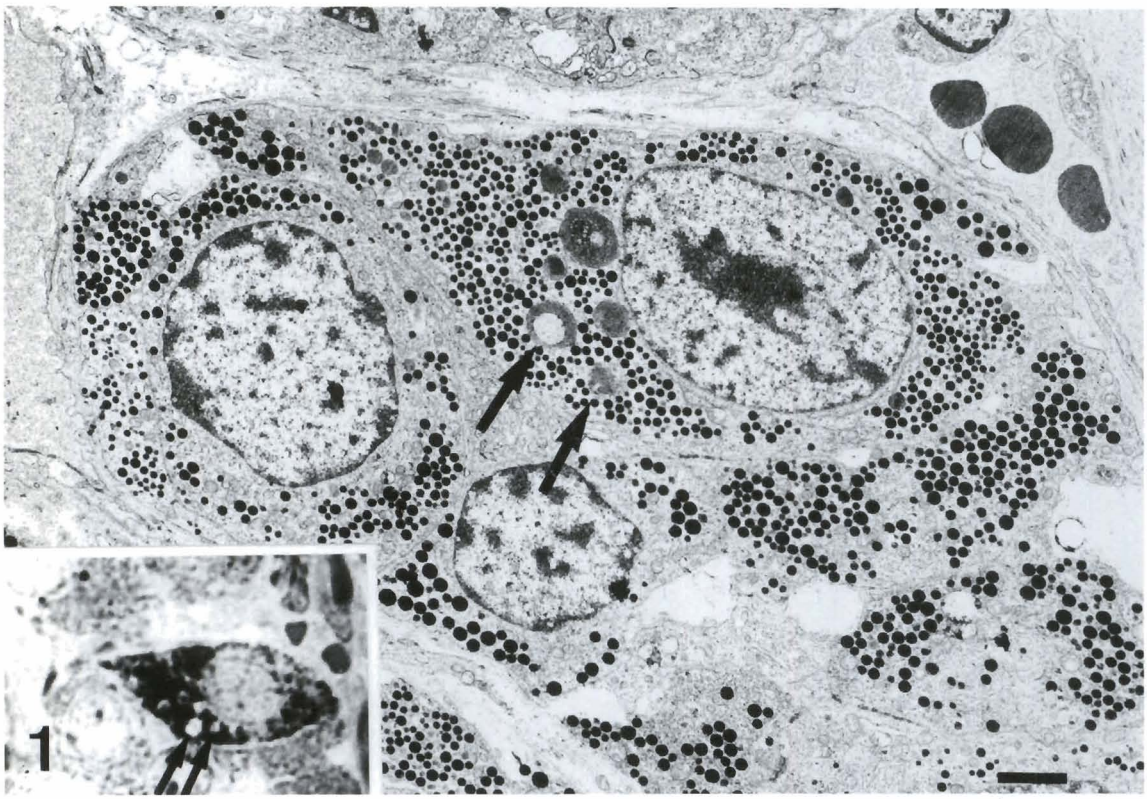

Fig. 1. GN cell of a goat in anoestrus immunostained with anti-FSH serum on the adjacent section, with abundant granules and lysosomes that are not immunostained in adjacent section (arrows). Bar = 2 μm. Inset x 1.107

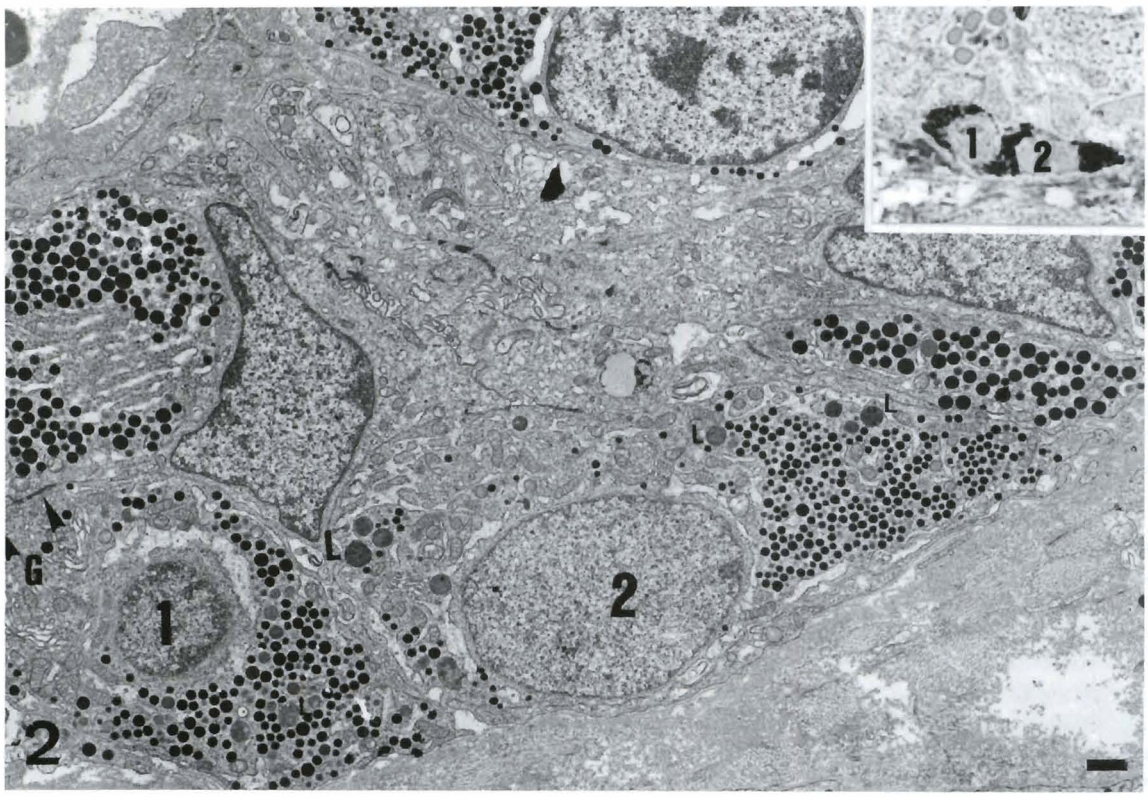

Fig. 2. GN cells (1, 2) of a goat in gestation immunostained with anti-FSH serum on the adjacent section, with dilated cisternae of the rough endoplasmic reticulum, well developed Golgi complex (G), small lysosomes (L) and next complex between cells (arrow head). Bar = 2 μm. Inset x 750

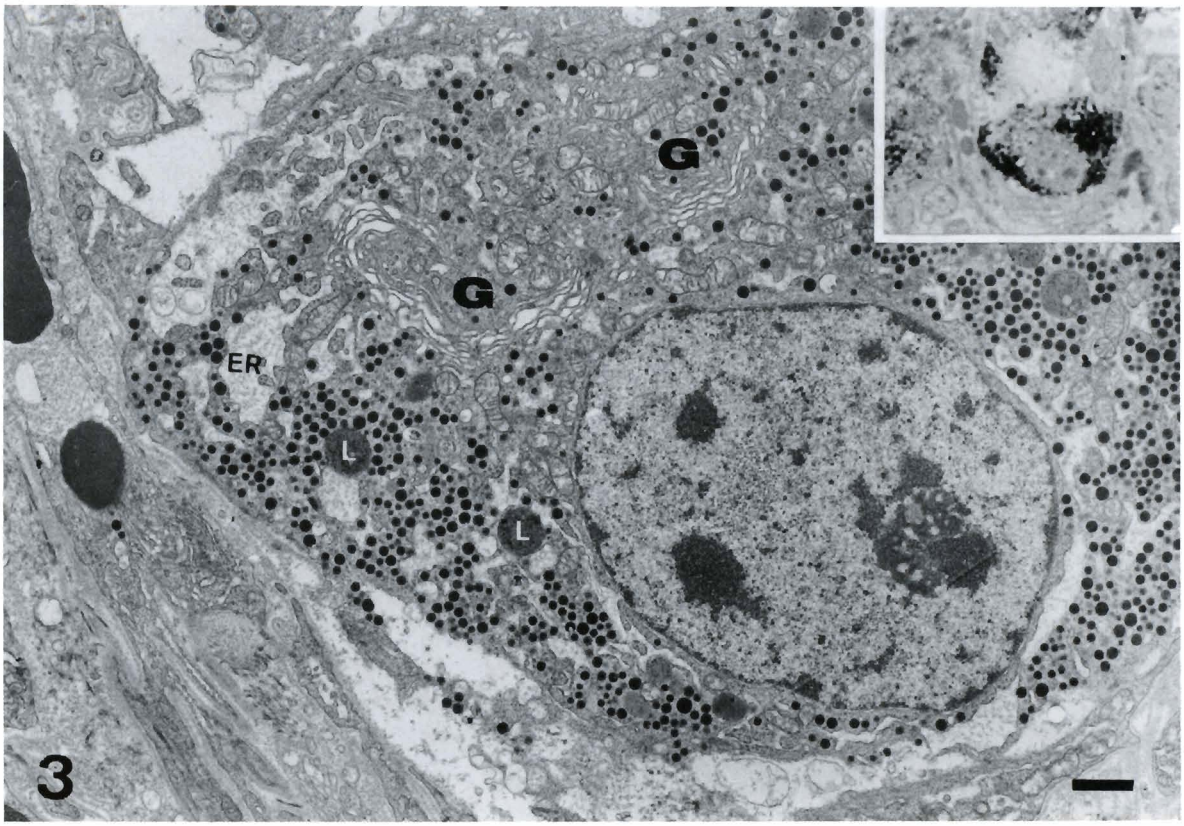

Fig. 3. GN cell of a goat in milk production immunostained with anti-FSH serum on the adjacent section, with dilated rough endoplasmic reticulum (ER) and well developed Golgi complex (G) and abundant lysosomes (L). Bar = 1 μm. Inset x 643

revealed immunocytochemically was seldomly seen in anoestrus; in this stage the predominating type was characterised by sparse secretory granules with a mean diameter of 230 nm, mostly small (76% between 140.9 - 244.6 nm and 23% between 244.6 - 400.3 nm). Images of exocytosis were frequently observed. The endoplasmic reticulum was well-developed with widely dilated cisternae interconnected among themselves, and with the nuclear envelope containing a finely granular electrodense material (Fig. 2). The scarce lysosomes were small in size, with a mean diameter of 504 nm.

During milk production cells with morphological characteristics similar to those of the first cell type described in anoestrus were observed, and another irregular-shaped cell type which tended to appear grouped in clusters of 3 - 4 cells with irregular nuclei and abundant heterochromatin. The cytoplasm contained numerous secretory granules similar to those described in the other cell types with a mean diameter of 222 nm; as in pregnancy there was a predominance of small-sized granules (72% between 140.9 and 244.6 nm in diameter). The endoplasmic reticulum was well-developed and was composed of long, dilated cisternae with an adielectronic content and there was also a large number of large-sized lysosomes and images which suggested the occurrence of crinophagy (Fig. 3).

Discussion

Both anti-FSH and anti-LH sera marked the same cellular type in samples included either in paraffin or epon. This supports the hypothesis that in the goat the two hormones are produced by a single cell, as found in other species (Batten and Hopkins, 1978; Dacheux, 1978; Gross, 1984; Gross et al., 1984). However the technique used does not allow us to determine the intracellular distribution of both hormones.

GN cells of goat were PAS- and Alcian blue-positive and showed patterns of distribution, shape, number and staining similar to those described previously (Singh and Dhingra, 1979; Gómez et al., 1987). We have not found the differences in PAS reaction reported by these authors but we think that this discrepancy is not significant because it is based on minor differences in the intensity of the staining and its subjective appreciation.

In anoestrus most GN cells exhibited a morphology similar to the denominated «type I GN» in kids (Gómez et al., 1987), which represents the classic FSH cells of the rat (Kurosumi and Oota, 1968); the cytoplasm was notably replete with secretory granules but the mean diameter of secretion granules was smaller than that reported in kids (Gómez et al., 1987), and similar to those of the gonadotropes described in adult males

(Shirasawa et al., 1985); we believe therefore that the larger granule size in kids is due to a low degranulation activity of impuber animal, which leads to a greater hormone accumulation in the granules, as is observed in other adenohypophyseal cellular types (Dannies and Tam, 1982). Granule size was similar to that of other species such as rat (Kurosumi, 1968; Kurosumi and Oota, 1968; Seinsch and Romler, 1978; Tougard et al., 1980); in this species other granules of a low electronic density have also been described, with diameters between 400 - 450 nm which are antigonadotropin-positive (Tixier-Vidal et al., 1975; Moriarty, 1976; Tougard et al., 1980). In goats the granules with a larger size and a lower electrodensity possibly correspond to lysosomes, as in the semithin sections they are not immunostained but appear as cytoplasmatic vacuolisations.

During gestation GN cells showed an increase in secretory activity and hormone synthesis. On the one hand there was a decrease in the number and size of the secretory granules, and on the other there was a greater development of the endoplasmic reticulum; this was observed in the LH cells of pregnant rats (Hassani and Ros, 1976). Furthermore, the images of possible crinophagy that were observed in animals in anoestrus did not appear, and lysosomes were fewer in number and smaller in size. These morphological characeristics coincide with the «type II GN» cells described in kids (Gómez et al., 1987), which also correspond to the typical LH cells described in rat (Kurosumi and Oota, 1968).

During milk production there was a decrease in GN cell size, also observed in rat (Merchant, 1974; Hassani and Ros, 1976) and bat (Richardson, 1991); cells appeared more immunostained forming small groups, a fact interpreted in the dog as a sign of non-activity (Carlon, 1967). It might be that after parturition the degranulation stops and the granules accumulated in the cytoplasm, this making the cells appear more immunostained. The increase in granules and lysosomes during postpartum suggests that these cells originate from the second type of GN cells described above, which after pregnancy would gradually tend to appear as in anoestrus.

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