

Immunocytochemical and Ultra Structural Identification of Different Cell Types of the Anterior Pituitary Cells of Egyptian Herbivorous Adult Female Non Pregnant Rabbit *Oryctolagus cuniculus*

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Abstract

The immunocytochemical and the ultrastructure identification of the pituitary gland of many species of herbivorous mammals were few in Egypt and may be in the world, so the present study is carried out on some herbivorous mammals such as *Oryctolagus cuniculus* in order to elucidate the similarities and the differences of the pituitary cells in rabbits and some mammals of the world and those of Egypt. The results indicate that, the gland is pyramidal in shape and its apex is long, directed posterior and its base interiorly directed. The acidophilic and the basophilic cells distributed heterogeneous in the body of the gland. The identification of the cells based on specific morphological characterizes, staining reaction and immunocytochemistry. (STH) Somatotropin: Numerous, the nucleus is central, irregular or lobed with chromatin granules. The secretory granules are very enormous and more denser. Luteotropic Hormone (LTH): The nucleus is eccentrically near the plasma membrane, the mitochondria are spherical or elongated. Rough ER is poor and the granules are light and mostly collected at the periphery of some cells. (ACTH) Adrenocorticotropic: These cells are found singly, round with eccentric nucleus. Secretory granules are small and spherical shaped, while the (TSH) Thyroid Stimulating Hormone: with very small secretory granules but the (FSH and LH) Follicle Stimulating Hormone and Luteinizing Hormone: are found singly, angular in shape with eccentric nucleus and the secretory granules are spherical or ovoid in shaped and exhibit variation in electron density than STH cells. We can differentiate between them by immune reactivity.

Keywords: Pituitary cells; Rabbit; Electron microscope; Immune reactions

Introduction

There were much information on the cytology of the pituitary gland of species belonging to order rodent, carnivore and insectivore but with herbivore may be low, so the present study concern with herbivore animal such as Rabbit *Oryctolagus cuniculus*, of family Lagomorpha. The morphological differentiation of the pituitary gland of mammals attended many authors [1] identified six types of secretory cells of both *Vesperugo savi* and *Vesperugo piccolo*. The ultrastructure and functional characteristics of the anterior pituitary cells in the Indian fruit *Rousettus leschenaultia* studied [2].

The Local regulation of gonadotroph function by pituitary gonadotropin-releasing hormone studied [3]. The ultrastructural changes in gonadotropic and prolactin cells of *Myotis myotis* under experimental conditions have been studied by Muniz et al. [3]. In Vespertilionid bat, *Scotophilus heathi* electron microscopic studies revealed six distinct cells types in the pars distalis on basis of specific morphological characters, staining reactions and immunocharacteristics [4,5].

The distribution of the gonadotropes, LH and FSH is immunohistochemically in the pituitary pars distalis of the adult male viscacha (*Lagostomus maximus maximus*) using specific antibodies against LH and FSH, with the streptavidinbiotin- peroxidase complex [6]. The distribution, size and percentage immunopositive area of these

cells were analyzed by image analysis in viscachas captured during the annual reproductive cycle. The LH cells were found widely distributed throughout the pars distalis during the reproductive period, and they were found in the ventro-medial region in the pars distalis during the gonadal regression and gonadal recovery periods.

In other Egyptian bats such as *Rhinopoma hardwekei* who observed that the gland is irregular in shape and two types of cells appeared by the semi thin sections [7]. The acidophilic and the basophilic cells distributed heterogeneous in the body of the gland. The histological characterizes of the pituitary gland of common bats from Egypt. They observed that there is a wall developed pars nervosa, pars intermedia and infundibular stalk but the residual lumen is poorly developed in some species than others [8].

In others mammals studied the pituitary gland of the carnivore *Vulpes zerda*, the herbivore *Oryctologus cuniculus* and observed that the gland in *V. zerda* was pyramidal in shape with an apex dorsally directed and its base is cleft and ventrally directed while in *O. cuniculus*, the gland was pyramidal in shape but with a slight long apex posteriorly directed while its smooth base interiorly directed [9]. There are randomly distributed extracellular colloidal accumulation were observed in the pars distalis of Viscacha (*Lagostomus maximus maximus*) located in the peripheral zone of the gland and showed variability in shape and size [10]. In vespertilionid bat, *Scotophilus heathi* the electron microscopic studies revealed six distinct cells

Material and Methods

Collected animals

For the present study, the animals of *Oryctolagus cuniculus* were brought alive from Abo-Rawash Giza-Cairo-Egypt in the laboratory and the specimens were killed by decapitation through the years 2013 and 2014.

Immuno cytochemical examination

Immunocytochemical Examination used for FSH, LH and GH cells of the pituitary gland by Karanovsky [11]. Sections were treated by xylene for 20 minutes to remove the wax, hydration was carried out in descending series of ethyl alcohols, then incubate slide in Hydrogen Peroxide Block for 10-15 minutes (to reduce nonspecific background staining due to endogenous peroxidase), wash 2 times in buffer, incubate tissue in digestive enzyme, wash 4 times in buffer.

Apply Ultra V Block and incubate for 5 minutes at room temperature to block nonspecific background staining, apply primary antibody and incubate, apply biotinylated Goat Anti-Polyvalent and incubate for 10 minutes at room temperature, wash 4 times in buffer, apply Streptavidin Peroxidase and incubate for 10 minutes at room temperature, rinse 4 times in buffer, add 1 drop DAB Plus Chromogen to 2 ml of DAB Plus Substrate, mix by swirling and apply to tissue, incubate for 5-15 minutes, counter stain and cover slip using a permanent mounting media.

Transmission electron microscopy

For transmission electron microscope, small pieces of $1 \times 1 \times 1$ mm of pituitary gland of bats were obtained and were rapidly processed according to as follows [11]:

Fixation: Small pieces of fresh specimens were fixed in a mixture of Formaldehyde: Glutraldehyde (4:1) at pH 7.4 at room temperature for 4 hours, and then rinsed twice in 0.1M phosphate buffer (15 minutes for each).

Post-fixation treatment: Specimens were post fixed in 2.0% buffered Osmic acid for half an hour at 4°C. The tissue specimens were then washed twice in phosphate buffer for 30 min

Dehydration: The tissues were dehydrated in ascending grades of ethyl alcohol (50, 70, 80, 90 and 100%) for two changes each of 15 minutes (2×15 minutes) in each Grade. Specimens then cleared in propylene oxide for 2 changes 5 min each.

Infiltration: Dehydrated tissues were initiated in 1:1 solution of propylene oxide and upon mixture. Infiltration was continued with 1:3 (propylene oxide: upon mixture) overnight at room temperature.

Embedding: Embedding was carried out using freshly prepared araldite upon mixture in capsules pre-dried for 1-3 hours.

Polymerization: The capsules were polymerized at 60°C and then the polymerized capsules were cured at room temperature for at least a day before attempting to section.

Sectioning and staining of semi thin sections for light microscopy: Blocks were trimmed under the binocular microscope of ultra-cut Reichert jung ultra-microtome. Semi thin sections of 1µm thickness were obtained with the aid of glass knives made on Leica EM KMR

(Knife marker). Semi-thin sections were stained by toluidine blue and examined for general orientation with the light microscope.

Sectioning and staining of ultrathin sections for electron microscopy: Specimens were then ret rimmed to the selected region and ultra-thin section 60 nm thickness were cut and picked up on copper Grids. Sections mounted on grids were double stained using uranyl acetate and lead citrate. Seven percent uranyl acetate solution in methyl alcohol was prepared and centrifuged at 5000 rpm for 10 minutes. A drop of uranyl acetate solution was placed on a dental wax sheet placed in a Petri-dish. Grids were then placed on the uranyl acetate drop (the surface grids loaded with section being facing the stain).

Staining was done in the dark for about 20 mines. Grids were washed in three successive glass bottles containing distilled water. After the last bath, Grids were dried on a filter paper. Sections were then stained with freshly prepared lead citrate that centrifuged at 5000 rpm for 10 minutes and used for staining for 10 minutes as in uranyl acetate, then washed with 0.02N NaOH and finally with freshly distilled water. Grids dried on filter paper and examined with electron microscope (TEM Philips 400T at 80 Kv). Photos were made on Kodak EM sheet films developed then enlarged and printed and investigated.

Results

Morphologically the gland is pyramidal in shape and its apex is long, directed posterior and its base interiorly directed (Figure 1).

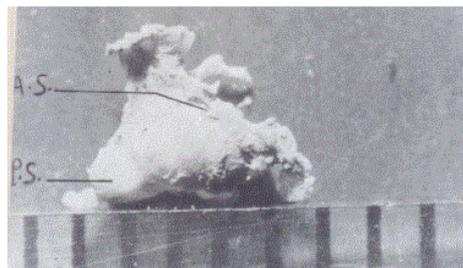


Figure 1: The pyramidal shape of the pituitary gland of presented rabbit (A.S) Anterior Surface, (P.S) Posterior surface after El Dosoki and selim (1996).

Transmission electron observations

We can depend on the shape and the size of the nucleus, the secretary granules and the morphology of the mitochondria.

Somatotroph (STH-cells)

These are the most enormous in the pars distalis of the present specimen. The nucleus is spherical and eccentric with chromatin granules at the periphery of it. The Mitochondria are poor in some cells. RER is poor. Secretary granules are ovoid or irregular in shape with high electron density scattered through the margin of the nucleus. Large number of small vesicles is present (Figure 2).

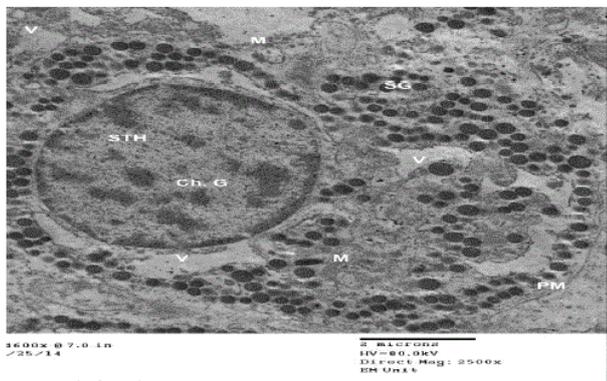


Figure 2: Transmission electron microscope of the pituitary gland of *Oryctolagus cuniculus* showing the Somatotrophic cell- STH, Ch.G- chromatin granules, M- Mitochondria, PM- Plasma Membrane, SG- Secretory Granules, V- Vacuoles.

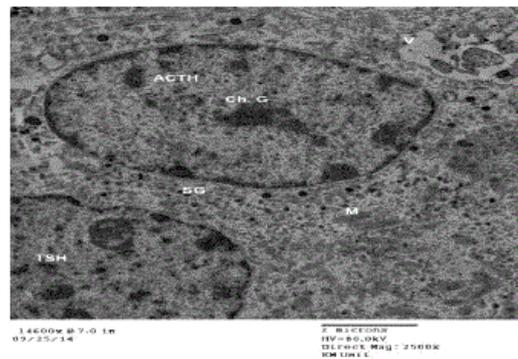


Figure 4: Transmission electron microscope of the pituitary gland of *Oryctolagus cuniculus* showing the Adrenocorticotrophic cell ACTH cell, Chromatin Granules (Ch. G), Mitochondria (M), Plasma Membrane (PM), Secretary Granules (SG), Nucleolus (Nu).

Lactotroph (PRL-cells)

The Nucleus is eccentrically with chromatin granules present attached to inner surface of nuclear membrane. The rough endoplasmic reticulum is in the form of short and long tubular profiles dotted with ribosome are seen scattered in the cytoplasm, some collection of mitochondria present Figure 3.

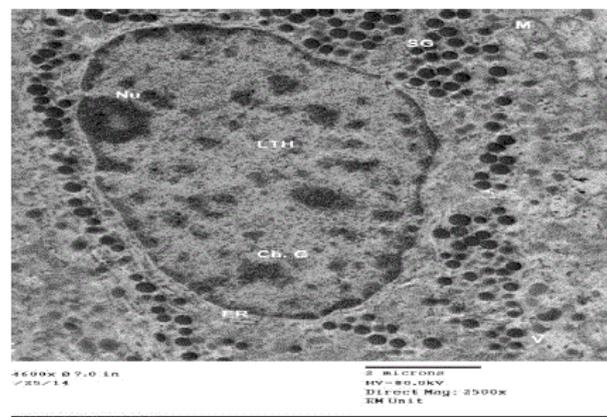


Figure 3: Transmission electron microscope of the pituitary gland of *Oryctolagus cuniculus* showing the Lactotrophic cell (LTH), Chromatin Granules (Ch. G), Mitochondria (M), Plasma Membrane (PM), Secretary Granules (SG), Nucleolus (Nu).

Thyrotroph (TSH cells)

These are elongated, polygonal or triangular in shape with large cytoplasm processes. The nucleus is irregular in outline and eccentrically; chromatin granules are seen distributed in nucleus. Mitochondria are very few. The secretary granules are very small, some vacuoles present (Figure 5).

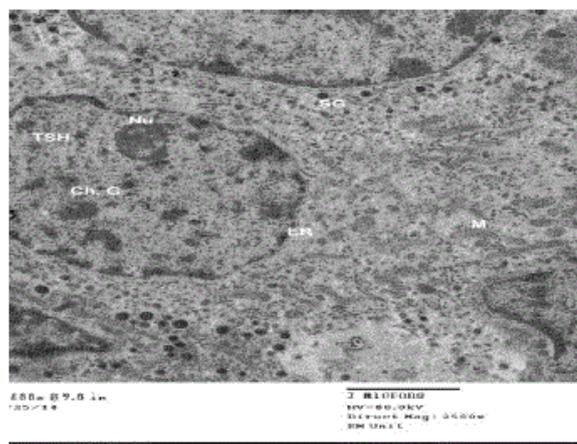


Figure 5: Transmission electron microscope of the pituitary gland of *Oryctolagus cuniculus* showing the Thyrotrophic cell (TSH), Chromatin Granules (Ch. G), Mitochondria (M), Plasma Membrane (PM), Secretary Granules (SG), Nucleolus (Nu).

Adrenocorticotroph (ACTH-cells)

These cells are elongated or angular with long cytoplasm processes with eccentric nucleus. Nucleus is large, oval or irregular with different shapes. Numbers of mitochondria are present. Golgi apparatus is not well developed. Rough endoplasmic reticulum is present. The secretary granules are numerous, small in size, uniform, round and very dense (Figure 4).

Gonadotrophs

In the FSH cells the nucleus is irregular in outline and shows indentations. Heterochromatin in the form of chromatin granules are seen scattered throughout the nucleoplasm. Mitochondria are very few. Secretary granules are electron dense, spherical, variable size and are distributed the cytoplasm RER present (Figure 6).

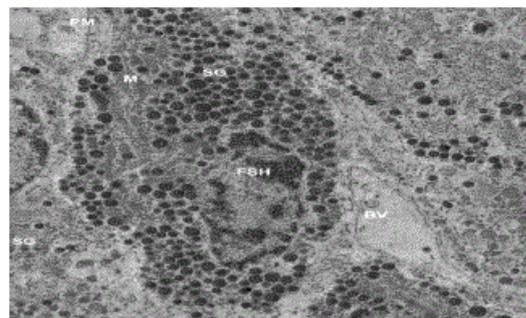


Figure 6: Electron microscope of the pituitary gland of *Oryctolagus cuniculus* showing the Follicular Stimulating Cell (FSH) Chromatin Granules (Ch. G), Mitochondria (M), Plasma Membrane (PM), Secretary Granules (SG), Nucleolus (Nu).

Immunocytochemistry

Prolactin positive cells as single or double – triple groups were detected in both zones of the pars distalis. Cells had an ovoid shape (Figure 7) while big nucleus was found to have an eccentric cytoplasm. PRL immune reactive cells were detected with large nucleus within the cytoplasm and moderate reactive with immune antibody reaction. In LH and FSH cells collected singly or in groups. The cells were circular or oval in shape with circular nucleus and granular cytoplasm; they react strongly with immune antibody reaction than STH.

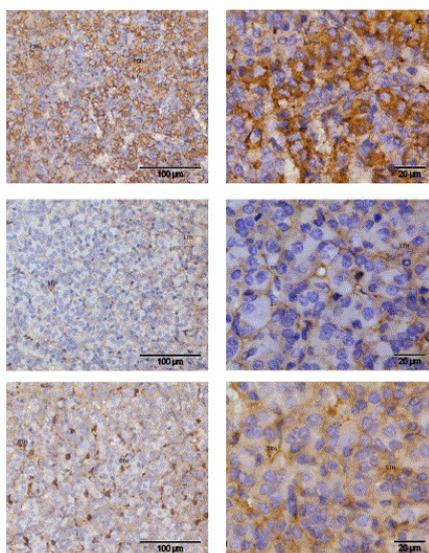


Figure 7: Immunohistochemistry of LH, STH and FSH cells.

The number of FSH *Oryctolagus cuniculus* 76 0.20–0.30, 0.26 ± 0.03 , LH *Oryctolagus cuniculus* 54 0.18–0.32, 0.26 ± 0.05 STH *Oryctolagus cuniculus* 41 0.16–0.33, 0.22 ± 0.04 (Table 1) and Histogram (Figure 8).

<i>Oryctolagus Cuniculus</i>	FSH	LH	STH
Range	0.20 – 0.30	0.18 – 0.32	0.16 – 0.30
Mean \pm SD	0.257 ± 0.035	0.259 ± 0.046	0.216 ± 0.042
F. test	17.149		
p. value	0.001*		
FSH & LTH	FSH & STH	LTH & STH	
0.873	0.001*	0.001*	

Table 1: The mean number of cells of FSH, LH and STH.

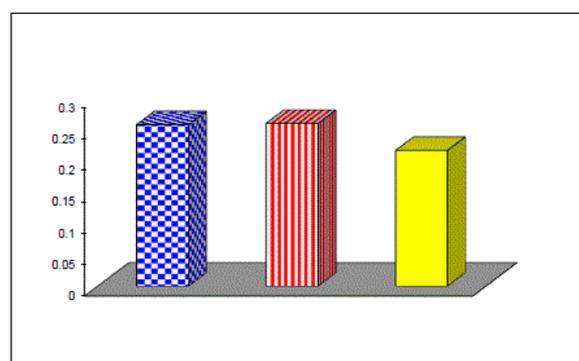


Figure 8: Number of cells of FSH, LH, and STH. Blue column is FSH- red column is LH and yellow column is STH.

Discussion

The morphology of the pituitary was pyramidal in shape but in carnivore mammals such as *Vulpes zerda* was irregular [9], may be due to feeding habit but in bats such as *Rinopoma hardwicki* was irregular in shape [7] different from *Taphozous nudiventris* which was semicircular in shape [12] and was that of emballonurid bat, *T. melanopogon* [1], Rhinopomatid bat, *Rinopoma hardwicki hardwicki* [13] and *Megaderma lyra lyra* were semicircular in shape [14].

Electron microscopic studies reveal the state of activity of the cells and their morphological features in the non-pregnant adult females allow formulating the criteria for their functional analysis. In conjunction with the ultra-structure of the mammalian pituitary gland [2,4,5,15], the ultra-structural observations demonstrate the presence of six cells types in the pars distalis of the present animal. The increase in enlargement of nucleoli and nature of secretary granules of the cells have taken as the indicators of differentiation between the different cells.

Somatotroph (STH cell)

We compare the Ultra structural characteristics of (STH) cells of the present animal with bats such as Indian fruit bat, *R. leschenaulti* studied by Bhiwgade et al. [2] which were round to oval in shape with centrally placed nucleus. The secretary granules are numerous, and mitochondria are round and scattered in the cytoplasm. The nucleus of the (STH) cells in the present rabbit is eccentric and irregular in shape

different from the case of *R. leschenaulti*. The secretory granules are numerous and large, no well-developed Golgi apparatus, but in *S. heathi*, round mitochondria and Golgi apparatus present in the (STH) cells [4].

The present finding study of (STH) cells similar to the case of *Hipposideros lankadiva* which are oval with eccentrically placed nucleus [16]. The secretory granules are numerous, mostly round to oval with uniform electron density.

Lactotrophic cells (LTH cells)

The ultra-structure features of LTH cells in the present animal reveal that numbers of mitochondria, rough endoplasmic reticulum are well developed and the electron dense granules are very large scattered in the cytoplasm similar to the case of Bhiwgade et al. [2]. In *S. heathi*, Singh et al. [4] reported numerous mitochondria, dilated endoplasmic reticulum and extensive Golgi complex, large number of secretory granules in the (LTH) cells, Ishibashi et al. [16] identified the (LTH) cells in the pars distalis of *Pipistrellus abramus* conforming to the present study. The morphometrical activity and PRL level were mostly low during follicular development while the PRL level increases after implantation but in the present animal we observe that the number of FSH and LH cells was higher than STH cells, may be due the breeding cycle period [17].

Adrenocorticotroph (ACTH cells)

In the present study the (ACTH) cells are elongated or angular found either singly or in groups in pars distalis. Mitochondria are moderate. Rough endoplasmic reticulum present and the cytoplasm appear vacuolated. The spherical or ovoid secretory granules of variable electron density are present just below the plasma membrane, which similar to the case of the (ACTH) cell of *T. longimanusa* and also in *S. heathi* [4,5], *R. leschenaulti* [2] and *H. inlakadiva* [16].

Thyrotroph (TSH cells)

The (TSH) cells show ultra-structural features except well-developed rough surfaced endoplasmic reticulum. It is in the form of array of elongated, tubular or lamellar cisternae frequently localized at one pole or periphery of the cell. The profiles of cisternae are parallel to one another or curved. Golgi is inconspicuous. The small secretory granules are very small with electron density scattered throughout the cytoplasm or show peripheral distribution similar to *S. heolhi* [4,5].

Gonadotrophs

The gonadotrophs in the present animal have irregular nucleus, numbers of mitochondria, electron density secretory granules. According to Bhiwgade et al., the variations observed in the electron density of the secretory granules are sufficient to differentiate two types of gonadotrophs [2]. The FSH secreting cells described in the present animal correspond to the, FSH cells of bat [15]. In the present study (FSH) cells are large ovoid and the rough surfaced endoplasmic reticulum is well developed. Secretory granules are spherical and show variable electron density

It is stated that prolactin positive cells were localized in the central and in the dorso-caudal zones of pars distalis as small groups when primary antibodies prepared against sheep prolactin was used similar to the present animal [18] while prolactin positive cells showed a

scattered distribution in pars distalis of mice pituitary [19]. In our study we observe that the number and the density of both FSH and LTH are higher than STH cells. In my opinion this increase depending on the effect of the genital development and increasing sunlight whereas the decrease in the reaction density is realized by the negative feedback effect, active FSH and LH hormones.

Conclusion

From the present study we can conclude that there are obvious differences in the pars distalis cells as regard immunocytochemistry and ultra-structure of the rabbit and bats or some other carnivorous and herbivorous mammals may be related to some extent to the phylogeny, difference of habit and feeding intake.

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