CYTOMORPHOLOGICAL AND MORPHOMETRIC STUDIES OF SOMATOTROPHS IN THE PITUITARY PARS DISTALIS OF POSTNATAL FEMALE ALBINO RATS

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ABSTRACT

The present study was designed to demonstrate the cytomorphological and morphometric changes of somatotrophs in female rats during the postnatal period by using the light and transmission electron microscopy. Fifty female rats were used in this study which classified into age ten groups (5 animals / group) (neonate, 7, 15, 22, 30 & 40 days-old rats); adults (2, 4 & 6 months-old rats) and senile (2 years-old rats). All animals were sacrificed and their pituitary glands immediately dissected, fixed and processed for light and electron microscopy. At the first week of postnatal life, somatotrophs were scanty and showed signs of inactivity. They were revealed as sporadic, small-sized round or triangular-shaped cells with faint yellow stained cytoplasm after staining with PAS-Orange G. Ultrastructurally, it showed few secretory granules and ill-developed cytoplasmic organelles. With age, a significant increase in the number of somatotrophs associated with increasing in the secretory activity and organelles at the immature stage till reaches its maximum in adults. At 22-30 days-old rats, two types of somatotrophs were seen ultrastructurally; densely granulated and sparsely granulated type. No significant change in the number of somatotrophs was observed at the pubertal stage and most of somatotrophs appeared in the storage phase exhibiting by heavily granulated cytoplasm with ill-distinct organelles. While in senile rats, a significant decrease in the number of somatotrophs that exhibited signs of inactivity was observed. They showed some regressive changes including vacuolated cytoplasm, decreasing in granulation with degenerated organelles. In conclusion, there was a significant postnatal age-related change in the structure and number of somatotrophs in female rats.

Key words: Somatotrophs, ultrastructure, morphometry, postnatal, female rats.

INTRODUCTION

The anterior pituitary gland is considered a key regulator of body homeostasis during the development (Müller, 1999). It is a dynamic and complex endocrine gland that synthesizes and secretes trophic hormones from distinct endocrine cells (Lee, 2006). Somatotrophs secrete growth hormone considered as a primary regulator which plays an important role in body growth and metabolic hemostasis (Anderson *et al.,* 2004). Somatotrophs constitute about 40-50% of cell population in anterior pituitary gland located mostly at the lateral regions (Melmed, 2002). Structural and morphometrical changes in the pituitary gland may be caused by age and could influence the change in cell density and total cell number of somatotrophs (Shimokawa *et al.,*

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1996). The number of somatotrophs increased with age from suckling to adulthood in female mice (Sasaki, 1988) and reduced growth hormone level caused by reduced somatotroph cell population leading to dwarfism (Garcia-Tornadu *et al.,* 2010). The relative proportions of somatotrophs and lactotrophs showed a clear variation between sexes in the anterior pituitary gland of rats (Takahashi and Kawashima, 1982). The hypothalamus and sex glands are responsible for this difference during the postnatal life (Sasaki and Sano, 1986). Aging produces alterations in certain functions of the hypothalamo- pituitary axis resulting in sexually dimorphic changes in the somatotrophs (Jurado *et al.,* 1998). Structural and morphometric data concerning the age-related changes of somatotrophs, especially, in females are scarce. Therefore, the aim of this study was carried out to investigate the normal age-related changes on the cytological, morphometric and histochemical characteristics of somatotrophs in female albino rats (from birth to senile ages) with the aid of light and transmission electron microscopy.

MARTIALS AND METHODS

The present work was conducted on 50 female albino rats which obtained from Helwan Laboratory Breeding Animal Farm. They were classified according to postnatal ages (5 animals of each age) into: neonates (at birth ˈ0 dayˈ, 7 days-old rats); immature (15, 22, 30 days old-rats); pubertal (age of vaginal opening; 40 days-old rats); adults (2, 4 & 6 months old-rats) and senile (2 years old-rats). In adult females, vaginal smears were examined daily to achieve diestrous smears and only animals with normal and regular estrous cycles were used. All rats of different ages were killed by over dose of ether inhalation. Pituitary glands were quickly dissected and fixed in Bouinˈs fluid. The samples were subjected to classical histological procedures of dehydration, clearing and paraffin embedding & 4-6 µm thick sections were obtained and stained with hematoxylin & eosin stain. For demonstrating somatotrophs, pituitary sections were stained with PAS-Orange G technique (Bancroft and Gamble, 2008).

For electron microscopy, some specimens were cut into very small pieces and fixed in 4% gluteraldhyde in phosphate buffer solution, washed in the same buffer (Hayat, 1986), post-fixed in 1% osmium tetroxide then embedded in epoxy medium. Semi-thin sections were obtained and stained with toluidine blue then examined by light microscope (Richardson *et al.,* 1960). Ultra-thin sections were obtained, mounted on copper grids, contrasted with 5% uranyl acetate and stained by lead citrate (Reynolds, 1963) and examined by a JEOL transmission electron microscope (JEM-1400 TEM 80 kv) in the electron microscopical unit of Assuit university.

Morphometric analysis:

The counting the somatotrophs in different ages of female rats was done manually by using Image – J v1.45s, free software version for image processing and analysis in Java, under an binuclear research microscope (Leica, Hannover, Germany) attached with digital camera (Canon, Tokyo, Japan). Ten non overlapping fields of each section of the pars distalis stained with PAS-Orange G were randomly selected at different levels (ventral, medial, and dorsal) and images were captured at 400x magnification.

Statistical analysis

Statistical analysis was performed on SPSS package Version 20. Data were presented as the Mean ± SE. Differences among the study groups were detected by one way analysis of variance (ANOVA) and post hoc comparisons were made by LSD test. *P values* < 0.05 were considered statistically significant.

RESULTS

Light and ultrastructural results:

During the first week of life (0-7 days-old rats), somatotrophs were rarely to be recognized and sporadically distributed between other cells. They were oval, rounded or triangular shaped cells with lightly acidophilic cytoplasm (Fig.1). Their nuclei were large, spherical, vesicular and eccentrically located. Some cells showed mitotic figures. With PAS-Orange G staining method, they had faint yellow cytoplasm (Fig.2). Ultrastructurally, they contained few round electron-dense secretory granules that peripherally distributed uniform, and large euchromatic nucleus. Most of cytoplasmic organelles were poorly-developed (Fig.3).

At 15 days-old rats, somatotrophs were slightly increased in number than that observed at the first week of life. While this cytoplasm appeared to be more condensed and more deeply stained (Fig.4). Ultrastructually, the secretory granules increased and appeared to be filling the majority of the cellular cytoplasm. Some of cytoplasmic organelles such as mitochondria and RER saccules appeared well-developed. The nucleus showed euchromatic chromatin and two prominent nucleoli (Fig.5).

At 22-30 days-old rats, marked increasing in the number of somatotrophs were recognized. They arranged mostly in groups or cords surrounding the blood sinusoids. Some of them appeared granulated with deeply stained cytoplasm while others were degranulated with faintly stained cytoplasm after PAS-Orange G staining (Fig.6). The electron microscope showed two types of cells; sparsely granulated and densely granulated type (Fig.7). The sparsely granulated cells showed an electron-lucent cytoplasm with numerous well-developed organelles as dilated cisternae of RER, mitochondria. While the granulated ones were characterized by electron-dense cytoplasm, numerous of secretory granules accumulating at one pole of the cell that associated with few organelles. Both cell types were shown to face the blood capillary.

At 40 days-old rats (pubertal age), somatotrophs appeared to be distributed all over the pars distalis that either singly or in clusters. Most of the examined cells appeared granulated and contained deeply stained cytoplasm (Fig.8). Ultrastructurally, the cytoplasm appeared packed with electron-dense secretory granules that intermingled with few organelles. The sparsely granulated cells were rarely to be seen in microscopical fields (Fig.9).

With advancement of age (2-4 months-old rats), the somatotrophs were more numerous than that observed in previous ages. Most cells were large with pale yellowish cytoplasm containing large, rounded

vesicular nuclei (Fig.10). With electron microscope, they appeared sparsely granulated with electron-lucent cytoplasmic matrix. Few secretory granules were accumulated near the plasma membrane. Numerous mitochondria and well developed dilated cisternae of RER were seen. The nuclei were rounded and had euchromatic chromatin which surrounded by a well-defined nuclear membrane (Fig.11). No cytological changes in the somatotrophs were noticed at 6 months-old rats.

With advancing of age (2 years-old rats), the examined pars distalis of senile rats showed decreased number and size of somatotrophs compared to the adults. They lost the typical structure and showed vacuolated cytoplasm and ill-distinct cell boundaries. Some cells were seen to be destroyed (Fig.12). Ultrastructurally, the cytoplasm had few, small sized secretory granules and degenerated cytoplasmic organelles which included highly dilated, destructed RER, swollen mitochondria and irregular nucleus (Fig.13).

Morphometric results:
Relations of the number of somatotroph cells detected in the postnatal ages of female rats are summarized in (Table 1 & Fig.14). The number of somatotrophs showed a significant increase with progressing of age from 7, 15, 22, 30 days-old rats compared with neonate rats. No significant changes were recorded in the number of somatotrophs between (30 & 40 days & 2 months-old rats), after that time the number increased significantly to reach its maximum at adults (4 months & 6 months-old rats) compared with rats at birth. Marked significant decrease in the number of somatotrophs was seen in senile rats (2 years old-rats) compared to the other ages except at 15-days old rats with no significant.

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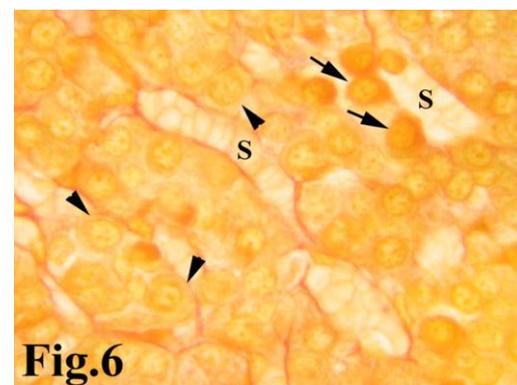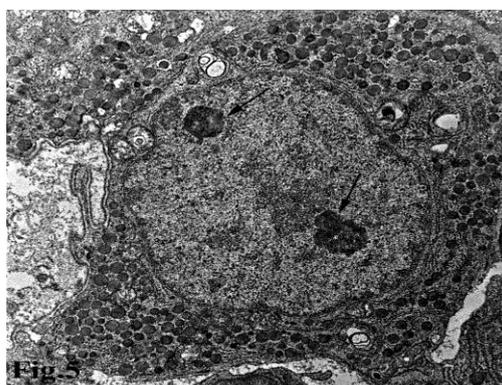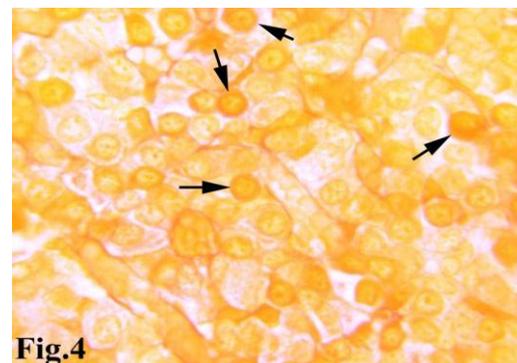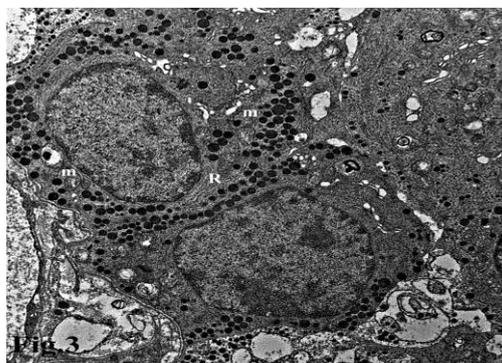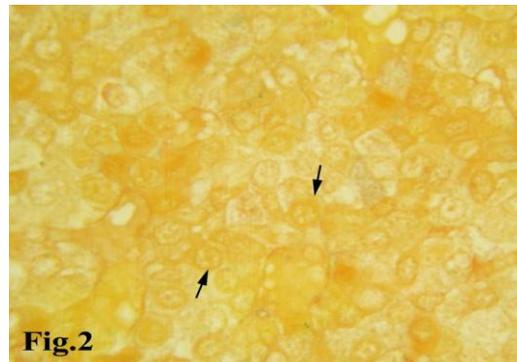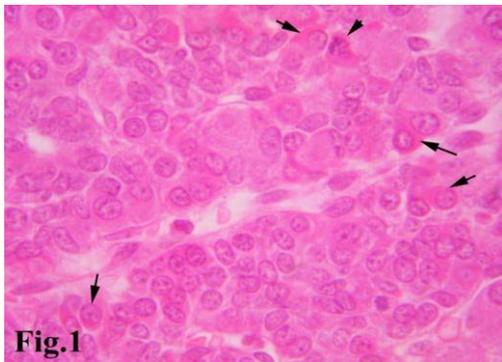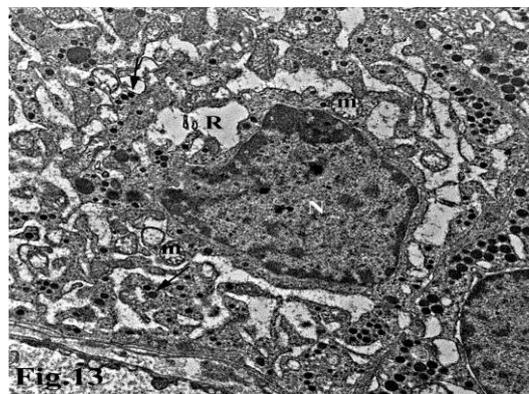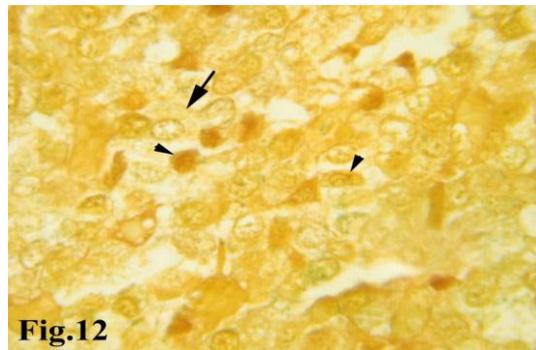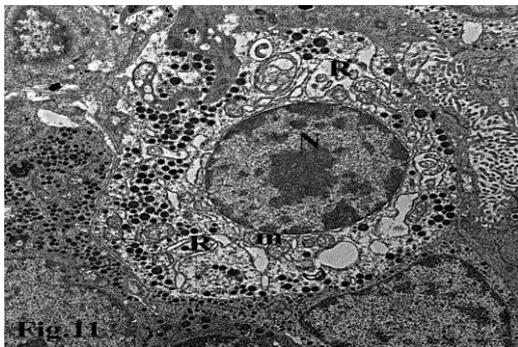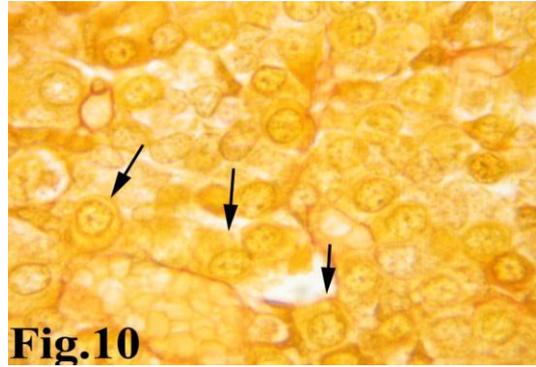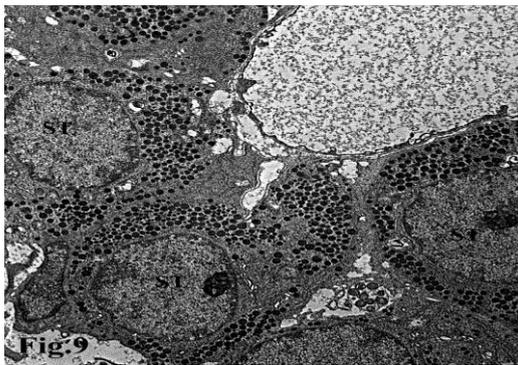
LIST OF FIGURES

Fig.1: A photomicrograph of pars distalis of female rat at birth showing few, sporadic, small-sized, oval or triangular-shaped somatotrophs with large rounded, vesicular nucleus (arrows) and mitotic figures (an arrowhead). H&E, X1000

Fig.2: A photomicrograph of pars distalis of female neonates at 7 days- old stained with PAS-Orange G showing somatotrophs with faint yellow cytoplasm (arrows). X1000.

Fig.3: An electron micrograph of somatotroph cells of neonates at 7 days-old female rats showing electron dense cytoplasm with few uniform rounded, electron dense secretory granules, few delicate RER canaliculi (R), rounded mitochondria (m) and euchromatic nuclei (N). X8000.

Fig.4: A photomicrograph of pars distalis of 15 days-old female rats stained with PAS-Orange G showing somatotrophs with deeply stained cytoplasm (arrows).X1000.

Fig.5: An electron micrograph of a somatotroph cell at 15 days-old female rats showing the cytoplasm packed with numerous secretory granules, ill-distinct organelles. Notice euchromatic nucleus with prominent two nucleoli (arrows). X12000.

Fig.6: A photomicrograph of pars distalis of 22 days-old female rats showing numerous and large-sized somatotrophs grouped in cords surrounding blood capillary (S), some of them were deeply stained (arrows) while others were faintly stained (arrowheads). PAS-Orange G stain, X1000.

Fig.7: An electron micrograph of somatotrophs at 30 days-old female rats showing sparsely granulated cells with electron lucent cytoplasm, well developed dilated cisternae of RER (R), numerous mitochondria (m); a heavy granulated cell with electron dense cytoplasm and ill-distinct organelles. Notice, both cell types facing the blood capillary.X8000.

Fig.8: A photomicrograph of pars distalis at 40 days-old female rats stained with PAS-Orange G showing most of somatotrophs were deeply stained.X1000

Fig.9: An electron micrograph of somatotrophs at 40 days-old female rats showing the cytoplasm packed with numerous electron dense secretory granules obscuring most of organelles.X6000

Fig.10: A photomicrograph of pars distalis of adult female rats (4 months-old) stained with PAS-Orange G showing hypertrophied somatotrophs with faintly stained cytoplasm (arrows).X 1000.

Fig.11: An electron micrograph of sparsely granulated somatotroph cell of adult female rat (4 months-old) showing electron lucent cytoplasm with numerous well-developed dilated cisternae of RER (R), mitochondria (m) and euchromatic nucleus (N).X11000

Fig.12: A photomicrograph of pars distalis of senile female rats (2 years-old) stained with PAS-Orange G showing degenerated, small-sized somatotrophs (arrowheads) while others showed vacuolated cytoplasm with ill-distinct cell boundaries (an arrow). X1000.

Fig.13: An electron micrograph of a somatotroph cell of senile female rat showing few, small-sized secretory granules (arrows), swollen mitochondria (m), highly dilated and destructed RER (R). X1000.

Table 1: Showing results of the number of somatotrophs in different postnatal ages of female rats (5animals / age).

Age	No. of animals / age	No. of somatotrophs/ x400 field
At birth	5	$14.52 \pm .806^{b,c,d,e,f,g,h,i,j}$
7 days	5	$18.96 \pm .756^{a,c,d,e,f,g,h,i,j}$
15 days	5	$25.80 \pm .879^{a,b,d,e,f,g,h,i}$
22 days	5	$32.06 \pm .918^{a,b,c,e,f,g,h,i,j}$
30 days	5	$40.50 \pm 1.648^{a,b,c,d,h,i,j}$
40 days	5	$40.42 \pm 1.719^{a,b,c,d,h,i,j}$
2 months	5	$41.24 \pm 1.406^{a,b,c,d,h,i,j}$
4 months	5	$72.64 \pm 2.249^{a,b,c,d,e,f,g,j}$
6 months	5	$70.98 \pm 2.718^{a,b,c,d,e,f,g,j}$
2 years	5	$25.76 \pm 1.265^{a,b,d,e,f,g,h,i}$

The data are given as means ± standard error (SE), with dissimilar superscript letters (significantly differ at $p < 0.05$): (a) letter is significantly differing from birth value; (b) letter is significantly differing from 7 days old ; (c) letter is significantly differing from 15 days old value;(d) letter is significantly differing from 22 days old value;(e) letter is significantly differing from 30 days old value;(f) letter is significantly differing from 40 days old value; (g) letter is significantly differing from 2months old;(h) letter is significantly differing from 4 months old value;(i) letter is significantly differing from 6 months old value; (j) letter is significantly differing from 2years old value.
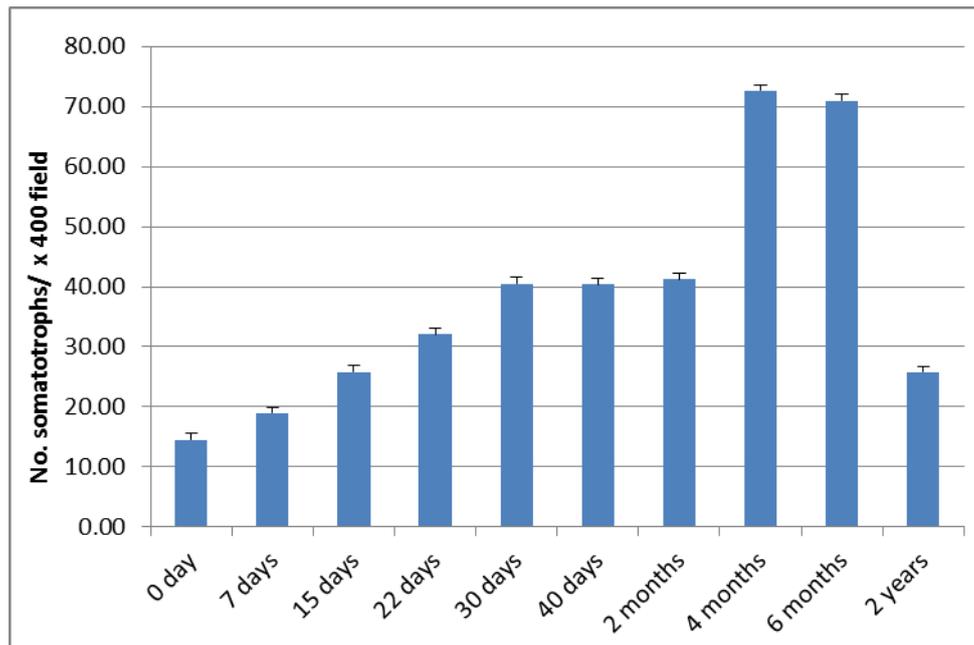
Fig.14: showing the relation between the number of somatotrophs at the different postnatal ages of female rats. The task performance was assist using number of somatotrophs / X 400 field. Bars on columns represent SE of the mean.

DISCUSSION

Somatotrophs were one of the acidophilic cells present in the pars distalis of different domestic animals which secrete growth hormone (Eurell and Frappier 2006). They react positive with histochemical stain PAS-Orange G and give yellowish orange stained cytoplasmic granules. The density of color depends on the amount of granules located (Baker, 1970 and Pearse, 1980). Also, they could be identified ultrastructurally by the characteristic large (300-500 nm in diameter), rounded and uniform electron dense secretory granules as recorded by (Nakane, 1970).

The present study showed the morphological and morphometric age-related changes in somatotrophs of postnatal female rats. They appeared in the first week of postnatal life as sporadic cells with prominent mitosis. Sasaki (1988) suggested that the increase in numbers of most of anterior pituitary cells specially somatotrophs was due to the cell division by mitosis. Also, the observed somatotrophs in our work showed signs of inactivity which regarded by faint staining cytoplasm with PAS-Orange G and presence of sparse secretory granules associated with poorly developed cytoplasmic organelles ultrastructurally. This finding agreed with that reported by Yamashita (1969) who stated that somatotrophs were poorly developed in newborn and during the first 10 days postnatally but showed steady increase in number by mitosis. While, Wilson and Christensen (1980) found that prominent

somatotrophs with well-developed organelles at birth. Consistent with our result, Sun et al. (2002) stated that serum growth hormone concentration decline abruptly immediately after birth.

At the 2nd week of postnatal life (15 days-old rats), we observed moderate increase in numbers of somatotrophs with marked granulation which manifested by densely stained cytoplasm after PAS-Orange G used. Also by electron microscopy, the cytoplasm packed with secretory granules which obscuring most of cytoplasmic organelles. According to our observation, we suggested that somatotrophs became active which appeared in synthetic and storage phases. Similarly, Wilson (1986) reported that the 14 days postnatally considered as crucial age to detect if the mice will be normal or dwarf as it showed negative reaction to anti-GH.

It has been found that progressive increase in number of somatotrophs and grouped in cords surrounding blood capillaries at 22-30 days postnatally as recorded by Sasaki (1988). While Gulyás et al. (1993) stated that somatotrophs reached plateau between p 13& p 20 followed by decreasing on day p35. We investigated that during this age, somatotrophs became active and secretory which manifested ultrastructurally by presence of electron lucent cytoplasm, sparsely granulated associated with numerous of well-developed organelles as dilated cisternae of RER and mitochondria. Similar observations were recorded by Wilson and Christensen (1980) and Sasaki (1988). Our results indicate that both synthesis and release of growth

128

hormone are enhanced as the animal approaches puberty. The level of growth hormone gradually rises postnatal and became significant between ages 26-30 days (Eden *et al.,* 1978). Changes in circulating growth hormone concentration in young pigs are caused by increase the total number of somatotrophs (Lee *et al.,* 2004).

At pubertal age (40-days old rats), we showed no significant changes in number of somatotrophs compared with ages of 30 days and 2 months and most of them appeared in storage phase. Most of them were repacked with secretory granules. Our findings were coincided with that recorded by Sasaki (1988) who stated that temporary slowing of somatotroph development around the puberty. Some researches recorded that the ovary has a role in somatotrophs differentiation. During the postnatal life, the ovary stimulates the differentiation of lactotrophs and inhibits that of somatotrophs. Also, ovariectomy before puberty induced increasing of somatotrophs to about 50% of the cell population while after puberty had less marked effect (Sasaki and Sano, 1980). The growth hormone secretion is progressively declined beginning in the adult life a phenomenon termed somatopause (Beatriz *et al.,* 2001, Hoffman *et al.,* and Lieberman & Hoffman 1997).

After puberty (2 months-old rats), our results showed marked increase in number of somatotrophs reaching maximum in the adults (4-6 months) compared to immature ages as recorded by Sasaki (1988). Accordingly, the plasma growth hormone reached the maximum level after puberty (Ojeda and Jameson, 1977) at time at which estrogen was also elevated (Parker and Mahesh, 1976). Some researchers suggested that estradiol may have a role in the regulation of GH secretion by modulating GRF release and acting directly on the somatotrophs (Shirasu *et al.,* 1999). It may be assumed that the ultrastructural features of somatotrophs at this age correlate with their functional activation which exhibited by presence of numerous and well - developed organelles.

In aged rats, our morphometrical results revealed a significant decline in the number of somatotrophs compared with other postnatal ages and reached to the level of 15 days-old rats. Such number decreasing was associated with appearance of regressive changes in somatotrophs with losing the typical structure as regarded by decreasing in granulation, vacuolation in cytoplasm with ill- distinct cell - boundaries as well as highly dilatation and destruction of mitochondria and RER. Therefore, such findings lead us to suggest somatotrophs were affected by age advancement. Our results were in agreement with that recorded by Abdl-Maksoud and Moustafa (2003), Takanashi *et al.* (2001), and Shinaki *et al.* (1995). They reported that

somatotrophs are the most sensitive cells with aging process associated with a reduction in their number and subsequent decline in growth hormone secretion. Jurado *et al.* (1998) recorded that this reduction was clearly more marked in senescent female than males and their ultrastructure changes suggestive of an immature state. The reduction of growth hormone with aging occurred either as a result of impairment in pituitary responsiveness to GHRH (Ceda *et al.,* 1986) or reduction in the pulsatile secretion of growth hormone in old rats (Sonntag *et al.,* 1983). Moreover, the pituitaries of older ages seem to be more sensitive to the inhibitory effects of somatostatin (GIH) (Spik and Sonntage, 1989). All the previous causes were attributed to intrinsic lesion in somatotrophic cells in aging animals (Müller *et al.,* 1993).

In conclusion, there was a significant postnatal age-related change in the structure and number of somatotrophs in female rats.

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130

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دراسات سيتولوجية وقياسية على الخلايا الحاثة للنمو فى الجزء القاصى للغدة النخامية أثناء مرحلة ما بعد الولادة في إناث الجرذان البيضاء

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اجريت هذه الدراسة لتوضيح التغيرات التركيبية والكمية على الخلايا الحاثة للنمو فى الغدة النخامية (الجزء القاصى) لاناث الجرذان البيضاء فى مرحلة ما بعد الولادة و ذلك باستخدام المجهر الضوئى والالكترونى النافذ. استخدم فى هذا البحث ٥٠ جرذا (خمسة فى كل عمر) وصنفت وفقا لاعمارهم الى : حديثى الولادة (عند الولادة ،٧ ايام) ،غير ناضجة (١٥، ٢٢، ٣٠ يوما) ، مرحلة البلوغ (٤٠ يوما) ، والبالغة (شهرين ، ٤ شهور و ٦ شهور) ، المسنة (سنتين). صبغت شرائح الغدة النخامية بصبغة الأورانج ج والشيف الحمضى البير ايودى ، وتم عد الخلايا الحاثة للنمو باستخدام جهاز التحليل الضوئى وقورنت النتائج احصائيا. وقد كشفت النتائج عن وجود ازدياد تدريجى فى عدد ونشاط الخلايا الحاثة للنمو بتقدم العمر، ففى الجرذان حديثة الولادة كانت الخلايا الحاثة للنمو قليلة العدد وبتقدم العمر، لوحظ ازدياد عدد الخلايا وكذلك نشاطها الافرازى حتى وصلت الى الحد الاقصى فى الجرذان البالغة. بالمجهر الالكترونى ، عند عمر (٢٢-٣٠ يوما بعد الولادة) ظهرت نوعين من الخلايا الحاثة للنمو احداهما تحتوى على حبيبات افرازية كثيفة والاخرى قليلة الحبيبات الافرازية. وفى مرحلة البلوغ (٤٠ يوما بعد الولادة) لم يلاحظ تغيير فى اعداد الخلايا ولكن ظهرت الخلايا فى مرحلة التخزين. أما فى الحيوانات المسنة ، فقد لوحظ انخفاضا كبيرا فى عدد الخلايا مع ظهور علامات الخمول. قد أوضح هذا البحث وجود تغيرات عمرية معنوية فى أعداد وتركيب الخلايا الحاثة للنمو فى مرحلة ما بعد الولادة. وفى الخلاصة، شوهدت تغيرات معنوية فى كمية وتركيب الخلايا الحاثة للنمو بالغدة النخامية لاناث الجرذان البيضاء أثناء مرحلة ما بعد الولادة بتقدم العمر حتى بلوغ سن الشيخوخة.