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Histogenesis of Human Adenohypophysis and Differentiation of Corticotrophs and Thyrotrophs

[1]Anandi Neel Bande, [2]Dr.Megha Arun Doshi,[3]Dr. Priya Santosh Patil, [4]Dr.Nitin Radhakishan Mudiraj

[1]Anandi Neel Bande, PhD student, Department of Anatomy, Krishna Institute of Medical Sciences (Deemed to be University), Karad, Maharashtra, India.

[2]Dr. Priya Santosh Patil, Associate Professor, Department of Anatomy, Bharati Vidyapeeth (Deemed to be University) Medical College & Hospital, Sangli, Maharashtra, India.

[3]Dr.Megha Arun Doshi of Department of Anatomy, Krishna Institute of Medical Sciences (Deemed to be University), Karad, Maharashtra, India.

[4]Dr.Nitin Radhakishan Mudiraj, Professor and Head, Department of Anatomy, Bharati Vidyapeeth (Deemed to be University) Medical College & Hospital, Sangli, Maharashtra, India.

Corresponding Author: Anandi Neel Bande, PhD student, Department of Anatomy, Krishna Institute of Medical Sciences (Deemed to be University), Karad, Maharashtra, India.

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Abstract

The pituitary gland is the master endocrine gland which has a complex embryological origin from Rathke's pouch and diencephalon. [1] They contribute to form two main parts of the gland namely adenohypophysis and neurohypophysis. The functional role of pituitary in the development of other glands is crucial. The appearance and differentiation of various cells in the gland have implications on the overall development and function of different organ systems.

The studies carried out on histogenesis of pituitary show varying results and confusion in identification of various cells. [2] The contributing factors may be lack of availability of suitable material and specific staining methods. Many authors have tried to elaborate different methods for the study of histogenesis of pituitary gland. The adenohypophysis especially has chromophobe and chromophil cells having different appearance, staining properties and functional significance. [3] There are studies wherein staining methods are mentioned however most of the studies are carried out on animals. [4]There is paucity of recent data on histogenesis of adenohypophysis at increasing gestational ages. This study aims at describing in detail the development of pituitary gland with special focus on adenohypophysis. The study mainly helps in identifying the various cells in adenohypophysis using H&E stain and a special stain. The study helps to identify the cells as well as the exact time of appearance of the cells throwing light on the stepwise differentiation of adenohypophysis until it assumes the adult architecture by 30 weeks of gestation. The knowledge of histogenesis of adenohypophysis will definitely help to correlate with the histogenesis and functional activity of other glands. The background of histogenesis will help in a better understanding of certain congenital anomalies and

pathological conditions with altered architecture of the gland.

Materials and Methods

The current research was a cross-sectional study carried out on40 spontaneously aborted dead human fetuses with gestational age ranging from 10 weeks to 38 weeks. The fetuses were collected over a period of four years, from Bharati Vidyapeeth (Deemed to be University) Medical College and Hospital, Sangli and other private hospitals with the kind permission of the concerned authorities, consent of parents and ethical approval. Dead, aborted and still born normal human fetuses were included. Twins and fetuses with gross congenital anomalies as well as mothers having metabolic or hormonal disorder were excluded. The gestational age was estimated by using Crown Rump Length (CRL) and foot length confirmed by referring Last Menstrual Period (LMP). These fetuses were divided into five groups according to their gestational age.

Groups	Gestational age	Crown Rump length (cm)	Foot length (cm)	Number of fetuses
I	10-15 Weeks	6 – 12.6	0.9-1.7	05
II	16- 20 Weeks	13.7-18.3	2.6-3.6	15
III	21-25 Weeks	19.6 -25	4.1-5.5	12
IV	26-30 Weeks	25.5 -28	5.8-6.5	05
VI	>30 Weeks	31 - 30	6.8-7.8	03

A. Fixation of fetuses: It was done by injecting 5% formalin locally at various sites with the help of 10 ml syringe and needle in abdominal, thoracic, cranial cavity and subcutaneously in the upper and lower limbs. After injecting formalin, fetuses were kept in 5% buffered formalin filled glass jars before dissection for 10 days.

B. Dissection and Fixation of Pituitary Gland:

Fetuses were dissected carefully by taking required incisions. First the scalp was detached from the skull cap. Then cranial vault was removed with the help of big scissors. The brain was removed with its enveloping meninges. The pituitary gland was scooped out from hypophyseal fossa after separating the diaphragmasellae.

The tissue slices of 3-4mm thickness were taken and fixed with Bouins fluid for 24 hours. The tissue was passed through graded alcohol [50%, 70%, 80%, 90% and absolute alcohol] for dehydration. Clearing was done by using two changes of xylene for 1 hour each. Embedding of tissue was done in paraffin wax for two changes [melting point 560C] of 1 hour each. The paraffin blocks were made by using L moulds. The sections were cut at 5 µm thickness by using rotary microtome. The ribbons of sections were thrown on the surface of warm water in tissue floating bath then sections were taken on slides coated with egg-albumin. Slides were kept for drying on a hot plate at 400- 500 C for 2 hours or more as per requirement.

C. Staining Procedure

1. Haematoxylin and Eosin

Paraffin was removed with xylene followed by graded alcohol [100%, 90%, 80%, 70% and 50%]. Then the sections were stained with haematoxylin solution. Excess stain was removed by dipping into acid alcohol for a few seconds followed by bluing. Counter staining wasdone witheosin.Sections were washed with alcohol, cleared with xylene and mounted with DPX.

2. **PFA-AB-PAS-OG stain**:

The sections were dewaxed in xylene and dehydrate through graded alcohols to distilled water.After the paraffin wax was removed, the sections were immersed in performic acid for 5 minutes. They were then rinsed in tap- water for 10 minutes, absolute alcohol followed by

again in tap-water and then gently heated to 50-600c temperature until just dry.The sections were once more rinsed in absolute alcohol and finally washed for 1 minute in tap-water.These steps were essential to prevent irregular deposition of alcian blue on loose edges, creases and around bubbles of gas trapped under the section.

Sections were dipped in alcian blue (2.5 PH) for 1 hour. Then they were washed in tap-water for 5 minutes, oxidized with periodic acid for 5 minutes and rinsed in three changes of distilled water for 2 minutes each. They were treated with Schiff reagent for 15 minutes and rinsed in running tap water for 5-10 minutes. Then the nuclei were stained with haematoxylin, differentiation and blueing was carried out.

The sections were dipped in 2%orange G andthen in 5% phosphotungstic acid, whichwas used as counterstain for 1-5 minutes. Then they were differentiated in tap-water. The sections were dehydrated, cleared and mounted with DPX . [5]

The sections were observed under a CX21i Biological microscope and the images were captured with Olympus CCD camera (U-TV0.5XC-3). Processing of images was done with Magnus Pro 3.7 software and analyzed.

Observations And Results

Group I: (10 – 15weeks)

Histogenesis of adenohypophysis was studied in H&&E stained slides at different gestational ages. The adenohypophysis of fetuses in Group I with gestational age 10 weeks to 15 weeks showeddifferentiation of anterior, posterior and intermediate lobes. Rathke's cleft was seen between anterior lobe and intermediate lobe. The gap between neurohypophysis and pars intermedia was noted. Three parts of adenohypophysis; larger part pars distalis, pars tuberalis and pars intermedia were identified. The capsule was thin well defined and made up of connective tissue.

Pars distalis – The parenchyma was arranged in the form of acini or nests lined by polygonal cells and were supported by reticular connective tissue. Sinusoidal capillaries were found throughout the adenohypophysis lined by endothelium. [Microphotograph 1] Two types of cells were found viz: chromophobes and chromophils.

The chromophobes were lightly stained due to lack of secretory granules and scanty cytoplasm. The nuclei were oval, vesicular and eccentric in position and contained prominent nucleoli.It was noted that the cells in pars distalis showed differential staining for haematoxylin and eosin. The chromophils were classified according to their size, shape, position of the nucleus and the staining affinity of their granules into two major types: acidophils and basophils. [Microphotograph 1]

Acidophils -Acidophil cells were small, strongly eosinophilic found at peripheral region of the adenohypophysis. The basophilic cells were oval or elongated medium sized cells with spherical nuclei seen at the central area and also seen near the Rathke's cleft.

Basophils – Basophil cells formed the majority of cells and were arranged in the form of acini and cords. In this group the number basophils predominated while the number of acidophils and chromophobes were less.

Pars Tuberalis - The parenchyma of pars distalis extended upward as pars tuberalis around the infundibulum and showed high vascularity. As compared to pars distalis, the cells of pars tuberalis were arranged in the form of cords along the blood vessels. Undifferentiated cells were found arranged in the form of small follicles and filled with an amorphous substance.

Pars Intermedia -The glandular cells of pars intermedia were arranged in the form of follicles lined mostly by basophils. The follicles were seen between posterior margin of Rathke's cleft and pars nervosa. Chromophobes and acidophils were not found. Many small cysts without

colloid were observed in intermediate lobe. Few blood capillaries were noted as compared to pars distalis and pars tuberalis. The posterior margin of Rathke's cleft was lined by stratified epithelium. [Microphotograph 1c]

PFA-AB-PAS-OG stain

The basophils of adenohypophysis of fetuses in Group I with gestational age 10 to 15 weeks were stained with special stain PFA-AB-PAS-OG to differentiate corticotrophs and thyrotrophs.

Corticotrophs: The corticotrophs were large, round to oval cells with eccentrically placed nucleus and contained a prominent nucleolus. The corticotrophs were identified by its characteristic staining with PAS and orange G as umber colored cell after staining with PFA-AB-PAS-OG. Umber colored corticotrophs were seen mainly in the central area, along the posteromedian zone of pars distalis, alongside the developing trabeculae and their extensions. They were slightly larger than other type of cells. At some places the whole cords were lined only by corticotrophs.

Posterior limb of Rathke's cleft was predominantly lined by corticotrophs.

Thyrotrophs: The thyrotrophs were elongated, oval cells with large cytoplasmic processes. The nucleus was eccentrically placed with prominent nucleolus and showed a coarse chromatin material. The thyrotrophs were identified by its characteristic staining with alcian blue and weak staining of its granules by the PAS reaction resulting in blue-grey colored cell after staining with PFA-AB-PAS-OG**.** They were situated more deeply in the cords and were not in direct contact with sinusoids although their processes were often seen to reach the sinusoids.

Thyrotrophs were not observed at earlier gestational period ie10th to 13th weeks. They were first seen at 14th week mainly in anteromedian zone of pars distalis. They were also seen in the 15th week of gestation.

Group II: (16 – 20 weeks)

The adenohypophysis of fetuses in Group II with gestational age 16 weeks to 20 weeks were stained with H &E stain and the microscopic features were observed. The capsule was well developed in this age group. The three lobes of pituitary gland were well demarcated.Rathke'scleft was seen between anterior lobe and intermediate lobe. The gap between neurohypophysis and pars intermedia was noted up to the 18th week of gestation. [Microphotograph 2] 19 weeks onward the gap between pars intermedia and pars nervosa disappeared.

Pars distalis- The parenchyma of adenohypophysis showed glandular cells in pars distalis arranged in the form of thick irregular cords anastomosing with each other. The cells were supported by a network of delicate reticular fibers. Between the cords a large number of sinusoidal spaces lined by endothelial cells were observed. Long follicular cells with star shaped cytoplasmic processes were seen, forming network and supporting stroma of the glandular cells. The trabeculae entered into the substance of the adenohypophysis from periphery and converged towards the pars intermedia. It was noted that the cells in pars distalis showed differential staining for haematoxylin and eosin. Eosin stained cells (acidophils) predominated in the postero-lateral region and haematoxylin stained cells (basophils) in the central region around the mesoderm as well as along with trabeculae. Lightly stained chromophobes were seen either in groups or single. Chromophobes were small and the nucleus was usually oval enveloped by a thin rim of cytoplasm.

Pars Tuberalis -Well developed portal vessels were found in pars tuberalis. The epithelial cells were arranged as longitudinal cords and follicle like structures which were supported by meshwork of reticular fibers.

Pars Intermedia - The lining epithelium of posterior margin of Rathke's cleft was not seen and the tubular structures or follicular arrangements of epithelial cells grew from pars intermedia towards the pars nervosa so that the size of intermediate lobe was increased. The irregular follicles were lined mainly by polygonal basophilsand few acidophilsbut chromophobes were absent.

PFA-AB-PAS-OG stain:

The basophils in adenohypophysis of fetuses in Group II with gestational age 16 to 20 weeks were stained with special stain PFA-AB-PAS-OG to differentiate corticotrophs and thyrotrophs. [Microphotograph 3]

Corticotrophs: Well developed, umber colored corticotrophs were observed in increased numbers in this group. The majority of corticotrophs were found along the follicles in pars intermedia.

Thyrotrophs: The small, elongated blue-grey colored thyrotrophs were observed in the anteromedian zone and a few similar cells were seen in pars tuberalis.

Group III: (21 – 25weeks)

The adenohypophysis of fetuses in Group III with gestational age 21 to 25 weeks were stained with H & E stain and the microscopic features were observed.

The capsule was thick and the stroma was well developed in this group.

Pars distalis -At this gestational age, the cells of pars distalis were fully differentiated and were arranged in the form of cords supported by reticular fibers. Lymphocytes and fibroblasts were found in the stroma. Well developed portal vessels were found throughout the adenohypophysis. The acidophils were present at the marginal region whereas basophils were in the central region. The size and numbers of acidophils and chromophobes was increased. [Microphotograph 4]

Pars tuberalis - Similar to previous age group.

Pars Intermedia -The size of pars intermedia increased up to the 23rd week of gestational age, but later on it decreased. The colloid filled follicles were seen. The follicles were irregular in shape lined by basophils and few acidophils. The basophils were also arranged in groups in between the follicles. Only a few chromophobes were found in this group.

PFA-AB-PAS-OG stain:

The basophils in adenohypophysis of fetuses in Group III with gestational age 21 to 25 weeks were stained with special stain PFA-AB-PAS-OG to differentiate corticotrophs and thyrotrophs. [Microphotograph 5]

Corticotrophs: The size and number of corticotrophs increased than previous gestational age group. Large sized angular, umber colored corticotrophs lined the follicles in pars intermedia.

Thyrotrophs: The thyrotrophs increased in size and became large sized, polygonal, blue-grey coloured cells in this age group.

Group IV: (26 – 30Weeks)

The adenohypophysis of fetuses in Group IV with gestational age 26 weeks to 30 weeks were stained with H & E stain and microscopic features were observed.

The capsule was thick, well developed and it surrounded the whole gland. The vascularity increased due to a well developed hypothalamo-hypophyseal portal system.

Pars distalis - The basophils were seen in the form of small groups at the periphery of pars distalis. The number and size of acidophils increased throughout the pars distalis. The number of chromophobes also increased. [Microphotograph 6].

Pars tuberalis – The undifferentiated epithelial cells were arranged as longitudinal cords and follicle like structures and well developed portal vessels were seen.

Pars Intermedia - The size of pars intermedia decreased in this group. The 26th week fetus showed, the pars

intermedia encircling the pars nervosa like a collar without discontinuity of pars nervosa. [Microphotograph 7] The irregular, large follicles were lined mainly by basophils.

PFA-AB-PAS-OG stain:

The basophils in adenohypophysis of fetuses in Group IV with gestational age 26 to 30 weeks were stained with special stain PFA-AB-PAS-OG to differentiate corticotrophs and thyrotrophs. [Microphotograph 8]

Corticotrophs: As the number of acidophil cells (yellow colored cells) increased the number of corticotrophs decreased in this age group.

Thyrotrophs: The number of thyrotrophs decreased but the size of cells increased.

Group V: (> 30weeks)

The adenohypophyses of fetuses in Group V with gestational age more than 30 weeks were stained with H &E and the microscopic features were observed.

In this group, the microscopic picture of adenohypophysis was similar to that of adult pituitary gland.

Pars distalis - The cells of pars distalis of pituitary gland were clearly distinguishable with H & E stain. Eosin stained cells i.e. acidophil cells predominated in lateral regions of pars distalis and were also observed at the peripheral region probably they were somatotrophs and lactotrophs. Hematoxylin stained cells i.e. basophils predominated in middle region of pars distalis and probably they were corticotrophs, thyrotrophs and gonadotrophs. The numbers and size of acidophils increased as gestational age advanced as compared to the basophil cells indicating probably prolactin hyperplasia. [Microphotograph 9].

Pars tuberalis - The undifferentiated epithelial cells were arranged as longitudinal cords and follicle like structures and well developed portal vessels were seen.

Pars intermedia- The rudimentary intermediate lobe showed cysts filled with colloid and lined by corticotrophs and melanotrophs.

PFA-AB-PAS-OG stain:

The basophils in adenohypophysis of fetuses in Group V with gestational age more than 30 weeks were stained with special stain PFA-AB-PAS-OG to differentiate corticotrophs and thyrotrophs.The microscopic feature of adenohypophysis was more or less similar to adult type. Thyrotrophs constituted about 5% and corticotrophs 15% to 20% of the parenchymal cells. [Microphotograph 10]

Discussion

The microscopic structure of adenohypophysis at different gestational age was observed by using H & E and points noted were: a) capsular development and vascularity b) stroma c) Appearance of acidophils, basophils and chromophobes. The basophils were further differentiated by using combination of PFA-AB-PAS-OG stain.

Development of the pituitary gland broadly occurs in three stages:

1. Initiation of pituitary organogenesis and formation of Rathke's pouch
2. Evagination of Rathke's pouch and cell proliferation
3. Lineage determination and cellular differentiation [6]

Morphogenesis of the anterior lobe begins with the appearance of "buds" in the rostral wall of Rathke's pouch which extend antero-laterally and engulf the vascularised mesenchymal tissue. [4]

Rathke's pouch is first evident in human foetuses at around 3.5 weeks of gestation. [7]

Capsule:

Chi JG and Lee MH (1980) observed that by 7 to 8 weeks of gestation the collection of mesenchymal cells became cellular and started to form a capsular structure. At 12thweek of gestation prominent capsule common for both adenohypophysis and neurohypophysis was

observed. From 13 weeks the capsule became thicker and finally fused with periosteum of the sella turcica.[8] Rao BS et al (2017) reported that well defined connective tissue capsule was observed at 14 weeks.[3] In the present study, a thin capsule was seen at 10 to 11 weeks of gestational age. From 12 weeks onward a well defined capsule was noted and as the gestational age advanced the thickness of the capsule increased.

Parenchyma of Adenohypophysis

Junqueira IC et al (2005) reported that the supporting stoma formed by reticular fibers and star shaped follicular cells having cytoplasmic process that formed a meshwork for supporting the cords or nests of glandular tissue and sinusoids. Similar cells were also noted in our study. [9]

Pearse AGE (1952b) observed the development of trabeculae in 8th week of gestational age. Two large vascular connective tissue strands were seen entering in the parenchyma of the hypophysis and a triradiate structure was formed. The vascular trabeculae were marked out as two radiating stars by the darkly staining mucoid cells which were seen in close contact with them. Same findings were seen in our study from 10week onwards.[10] Ikeda H et al (1988) observed that at 6 weeks of gestation, Rathke's pouch buds off from the oral ectoderm and subsequently undergoes massive expansion resulting in an 377-fold increase in total free surface area between 5 -21 weeks.[11] Solov'en GS et al (2008) observed the newly formed loose fibrous connective tissue with developing capillary bed around the epithelial rudiment at 6-7 weeks of gestation. [12] Rao BS et al (2017) reported well developed portal vessels observed 24 weeks onward. [3] Ikeda H et al (1988) noted that the portal vasculature was established at 20 weeks of gestation.[11] Atwell WJ (1926) reported that from early stages the pars tuberalis was more vascular than pars intermedia. [4] In the present study fully developed portal vessels were observed in 21-25 week fetuses. Pars tuberalis showed large number of blood capillaries. The pars intermedia showed fewer capillaries as compared to pars distalis. Our findings were comparable with Rao BS et al (2017), Atwell WJ (1926),Ikeda H et al (1988).[3,4 ,11]

Ikeda H et al (1988) observed that the size of pars intermedia increased until 21 weeks. In the present study, the size of pars intermedia was increased upto 25th weeks and then decreased. [11] Falin (1961) had reported differentiation of cells in the anterior lobe very early, during 7-8 weeks of gestation. The basophil appears first of all at the 8th week in adenohypophysis whereas the acidophils first observed at the 9-10 weeks of gestational age.[13] Rosen F and Ezrin C (1966) reported that basophils first appeared at 9 weeks of gestation.[14] Conklin (1968) noted that the basophils first appeared at 7 weeks and acidophils at 11 weeks.[15] In the present study, both acidophils and basophils were observed 10th week onwards. Our findings correlated with the findings of Falin (1961), Rosen F and Ezrin C (1966) and Conklin (1968). [13, 14, 15]

Pearse AGE (1952b) observed that in the horizontal section of pituitary gland, acidophils predominantly present in two postero-lateral wings while basophils and chromophobes in the median wedge.[10] Rao BS (2017) reported that eosin stained cells were located at the lateral region and haematoxylin stained cells at the central region. [3] Asa SL et al (1986) observed that acidophils were found primarily at the periphery of the lateral region and the basophils were distributed throughout the median wedge and posterior part of lateral wings. Basophils formed the majority of cells of pars intermedia. [16] Krishnan S and Fenn A (2017) reported that, in pars intermedia the majority of cells were PAS positive basophils. [17]

In the present study, in pars distalis the acidophils were mainly observed at postero-lateral region and along the periphery. The basophils predominated around the central mesenchymal tissue, along the trabeculae and were also seen in the posterior aspect of lateral region near Rathke's cleft. In pars intermedia the follicles were irregular in shape lined by basophils and few acidophils. Our findings were comparable with Pearse AGE (1952b) ,Asa Sl et al (1986) and Rao BS et al (2017) [10, 16, 3]

Rao BS et al (2017) observed hypophyseal cleft in between the anterior and intermediate lobe and also a gap between the intermediate lobe and pars nervosa at 14th week. At 19th week the gap between the intermediate lobe and pars nervosa were disappeared.[3] Similar findings were noted in the present study. Falin LI (1961) reported that between the anterior and posterior lobe there was a wide cavity called Rathke's pouch. The epithelium of posterior wall formed numerous outgrowths in the form of colloid filled follicles or epithelial nests forming the intermediate lobe of the hypophysis. [13]

PFA-AB-PAS-OG staining for basophils:

The use of combination of stains for differentiating basophils was the interesting aspect of this study. The basophils – corticotrophs and thyrotrophs play a crucial role in development of adrenal and thyroid glands. It was seen that a simple routine staining procedure could clearly differentiate corticotrophs from thyrotrophs under routine microscopy. However there are not many studies in the recent decades showing such observations.

Adams CWM and Swettenham KV (1958) used a combined performic acid-alcian blue- PAS- orange G staining method for the identification of two types of basophil granules. S type of granules was stained by alcian blue and R type of granules predominantly stained by PAS. Acidophil granules were stained by orange G. [5] Conkiln JL 1968) reported that the acidophil appeared as yellow coloured cell after using combined performic acid-alcian blue- PAS- orange G staining method. Basophils were further differentiated as thyrotrophs which appeared as blue-grey colored cells at 7 weeks and corticotrophs as umber coloured cell at 12 weeks.[15] Rosen F and Ezrin C (1966) noted that the primitive thyrotrophs became apparent by 13 weeks. [14] ConklinJL (1968) observed that corticotrophs appeared first at 7th week whereas thyrotrophs appeared at 12th week of gestation.[15] Baker BL and Jaffe RB (1975) reported that by immunocytochemical method corticotrophs were appeared along the ventrolateral border of the hypophyseal pouch by 7 weeks and thyrotrophs by13 weeks.[18] Asa Sl et al (1986) reported that PAS positive corticotrophs first appeared at 8weeks of gestation whereas thyrotrophs appeared at 12 weeks.[16] Dubois PM and Begeot M (1978) reported that the thyrotrophs were detectable from 15 weeks of gestation.[19] In the present study, corticotrophs were observed at 10th week whereas thyrotrophs first appeared at 14th week of gestation.

Conclusion

During the histogenesis of adenohypophysis, the chromophils can be first differentiated by 10th week into acidophils and basophils with H & E stains. A well-defined portal vasculature is not seen until 21 to 25th week. A combination of stains (PFA-AB-PAS-OG) can help to easily differentiate and describe the morphology of basophils into corticotrophs and thyrotrophs. This study highlights the histogenesis of adenohypophysis with cellular differentiation which can help to correlate the histogenesis and functional activity of other endocrine glands. It will also be of immense help to clinically correlate with pathological lesions such as hyperplasia or adenoma of the gland.

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Figures

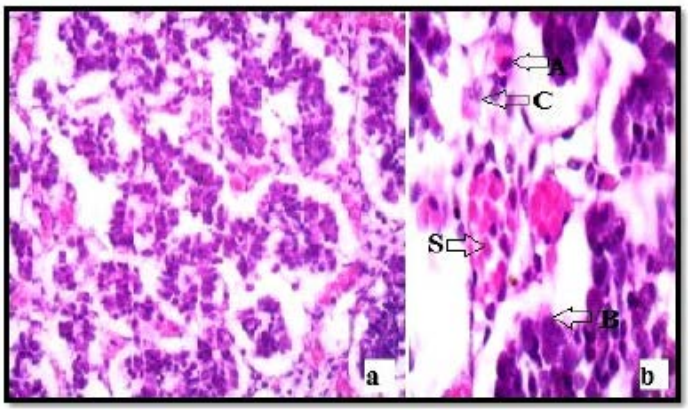

Figure 1 : Microphotograph 1: H&E stain, Showing pars distalis, at 12th week under 40X [a] and 100x [b] Arrows show A- Acidophils, B-Basophils, C-Chromophobes and S- Sinusoids.

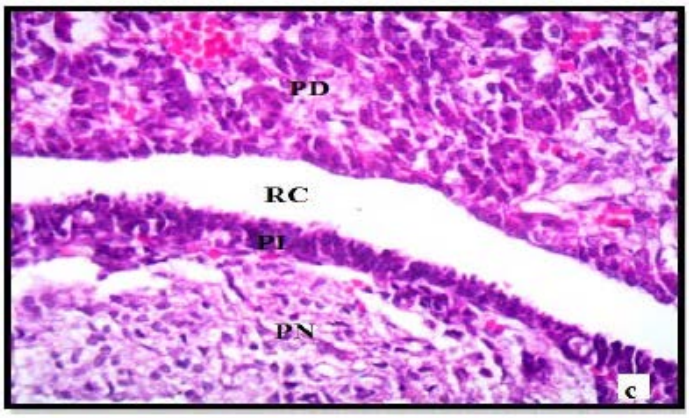

Figure 2 : Microphotograph 1c: H&E stain, Showing pars intermedia, at 13th week under 40X. PD- Pars Distalis, RC-Rathke's Cleft, PI- Pars Intermedia and PN- Pars Nervosa

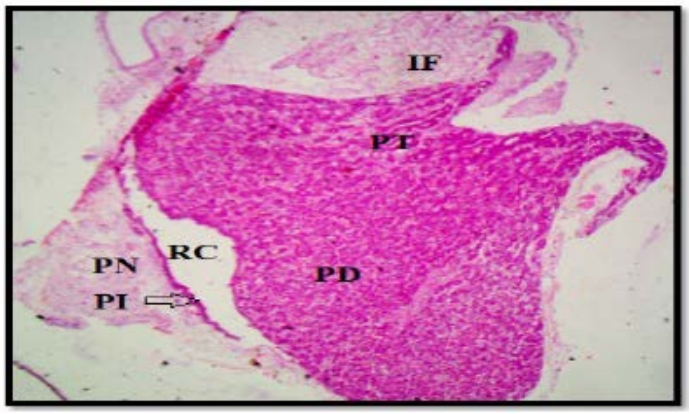

Figure 3 : Microphotograph No.2: H&E stain, Showing parts of pituitary gland, at 16 week under 10X. Parts of adenohypophysis: PD- Pars Distalis, PT-Pars Tuberalis, RC-Rathke's Cleft and PI- Pars Intermedia. Parts of neurohypophysis:PN- Pars Nervosa and IF- Infundibulum

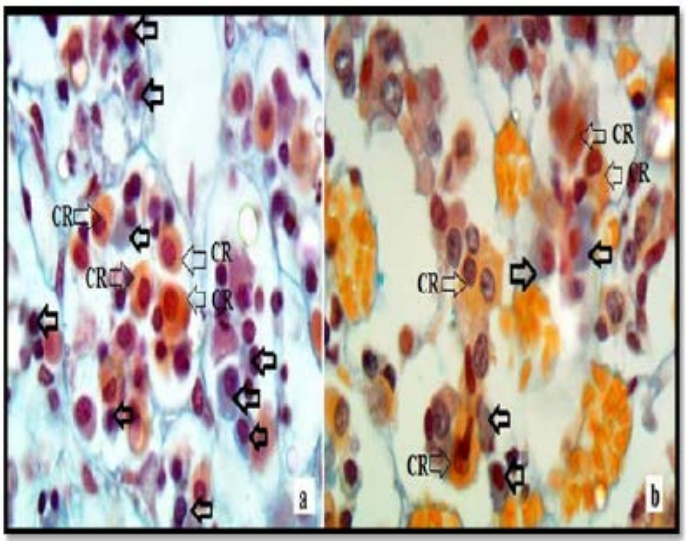

Figure 4 : Microphotograph No.3: PFA-AB-PAS-OG stain, Showing pars distalis, at 16 week [a] and 18 week [b] respectively under100X. Arrows show CR - Corticotrophs (Umber colour) Dark arrows show Thyrotrophs (Blue-grey colour)

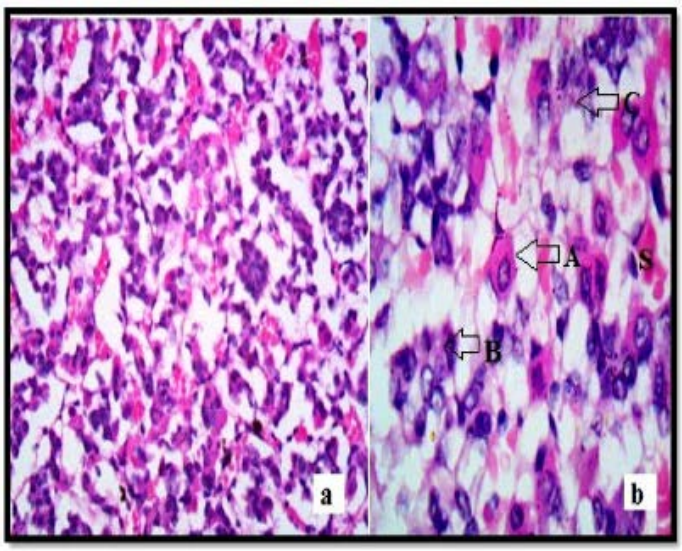

Figure 5 : Microphotograph 4: H&E stain, Showing pars distalis, at 23 week under 40X [a] and 100x [b] Arrows show A- Acidophils, B-Basophils, C-Chromophobes and S- Sinusoid

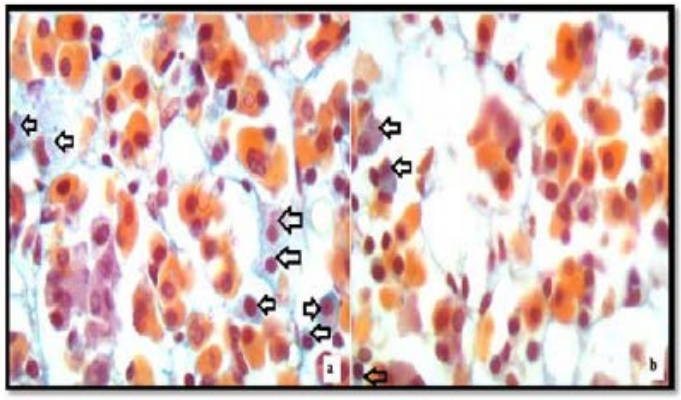

Figure 6 : Microphotograph 5: PFA-AB-PAS-OG stain, Showing pars distalis, at 22 week [a] and 25week [b] under100X respectively. Corticotrophs seen as umber coloured cells, Arrows show Thyrotrophs in blue- grey colour.

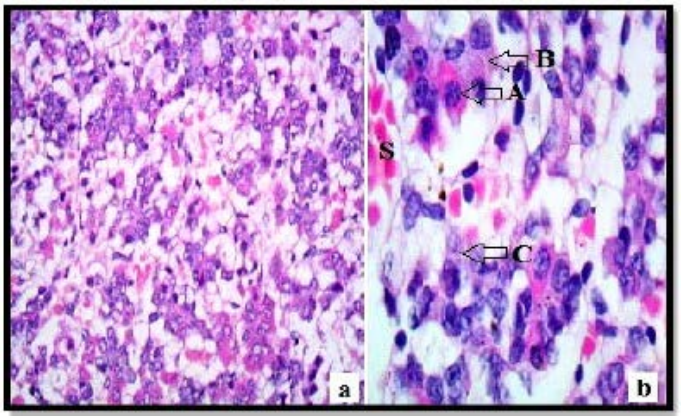

Figure 7 : Microphotograph 6: H&E stain, Showing pars distalis, at 28th weeks under 40X [a] and 100x [b] Arrows show A- Acidophils, B-Basophils, C-Chromophobes and S- Sinusoids.

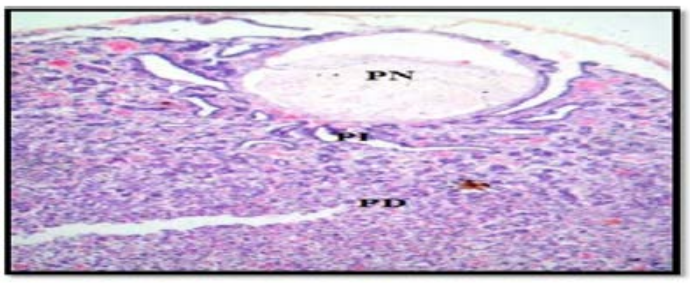

Figure 8 : Microphotograph 7: H&E stain, Showing pars intermedia encircling the pars nervosa, at 26 week under 10X . PD- Pars Distalis, PI- Pars Intermedia, F- Follicles and PN- Pars Nervosa.

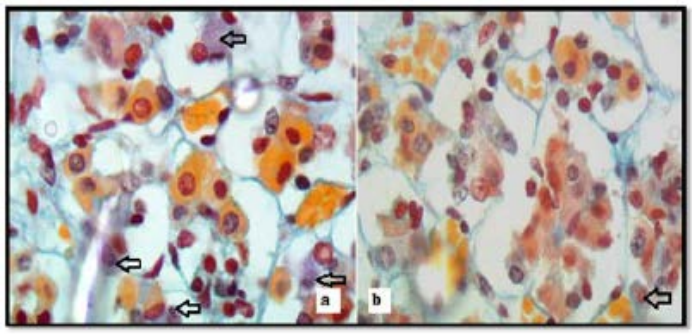

Figure 9: Microphotograph 8: PFA-AB-PAS-OG stain, Showing pars distalis, at 28 week [a] and 30weeks [b] under 100X respectively. Corticotrophs seen as umber coloured cells, Arrows show Thyrotrophs in blue-grey colour.

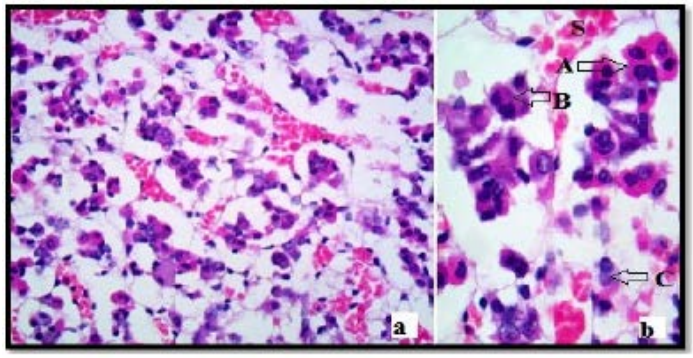

Figure 10 : Microphotograph No.9: H&E stain, Showing pars distalis, at 36 week under 40X [a] and 100x [b] Arrows show A- Acidophils, B-Basophils, C-Chromophobes and S- Sinusoids.

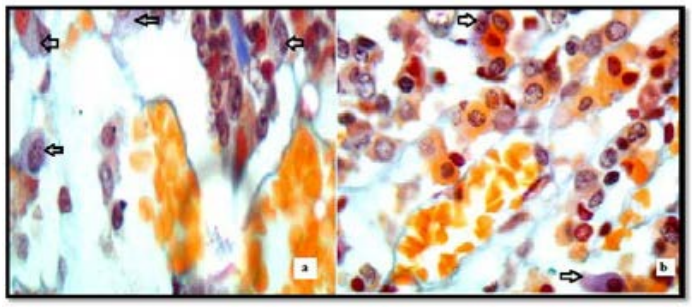

Figure 11 : Microphotograph 10: PFA-AB-PAS-OG stain, Showing pars distalis, at 32 weeks [a] and 36[b] under 100X respectively. Corticotrophs seen as umber coloured cells, Arrows show Thyrotrophs in blue-grey colour.