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Histomorphological And Histochemical Study Of Pituitary Gland In Adults Male New Zealand Rabbits

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ABSTRACT

The present study aimed to examine the morphology, histology, and histochemistry of the pituitary gland in adult male rabbits. Ten adult male rabbits were used, and samples from their pituitary glands were collected. The tissue specimens were processed and stained with Hematoxylin and Eosin (H&E), with an additional special stain (Orange G) applied. Morphological observations were made regarding the shape and location of the pituitary gland. Histologically, the pituitary gland was encased in a thin capsule derived from the dura mater, tightly surrounding the gland. The results revealed that the pituitary gland(hypophysis) was primarily composed of the adenohypophysis and neurohypophysis, with the adenohypophysis being the main component. The adenohypophysis included the pars distalis, pars intermedia, and pars tuberalis. The pars distalis was located laterally, while the pars nervosa was situated at the center of the gland. Additionally, the pars distalis contained three main cell types: acidophils, basophils, and chromophobes. The H&E staining results highlighted that acidophils were the most prominent cells. The cellular structure of the pars distalis consisted of irregularly arranged clusters and cords of chromophilic and chromophobic cells, mainly concentrated at the periphery of the pars distalis.

Keywords: *pituitary; morphology; Rabbit; Adenohypophysis.*

1. INTRODUCTION

The rabbit is a calm, non-aggressive animal, making it simple to manage. It is frequently cultivated and is economically advantageous relative to larger species. (Ismail and Atyia, 2013). Rabbits have brief reproductive cycles, encompassing gestation, nursing, and puberty. (Hussein et al., 2024) reported that the rabbits being classified as small animals. The local ethical council governs smaller animals, whereas larger animals necessitate further approval from the central ethical committee, a procedure that is more protracted and subject to more stringent laws. (Mapara et al., 2012).

The hypophysis is a central gland located within sella turcica of the sphenoid bone at the brain's base. It is considered the master endocrine gland because it regulates a variety of physiological functions such as growth, puberty, metabolism, energy balance, fluid regulation, and fertility. The pituitary receives signals from the central nervous system, relayed via the hypothalamus, and produces hormones from various cell types. These hormones target specific organs and regulate physiological functions, with some hormones also stimulating the target gland. A key characteristic of hormone axes (hypothalamus-pituitary-target organ) is a negative feedback system, except for estrogen, which operates in a feed-forward cycle for luteinizing hormone (LH) (Trautman, 1909b). The dura mater covers most of the gland, leaving an opening through which the hypophyseal stalk passes (Dyce et al., 1996).

The pituitary gland comprises three primary components: the anterior lobe, posterior lobe, and intermediate lobe. The front lobe (pars distalis) constitutes the largest and most crucial segment of the gland (Herring, 1908). The intermediate lobe and cleft connect the anterior and posterior lobes. The intermediate cleft divides the gland into two discrete lobes: both the anterior and posterior ones (Trautman, 1909a). Adenohypophyseal cell types (acidophils, basophils, and chromophobes) can be distinguished using a combination of acidic and basic stains, owing to the affinities of their secretory granules for these dyes (Tucker, 1999; Greaves, 2007; Ross et al., 1989). Acidophils are categorized into growth hormone (GH)-producing cells and prolactin (PRL)-producing cells (Kovacs and Horvath, 1981). Acidophils, responsible for the production of GH and PRL, exhibit orange or light green staining, whereas basophils (gonadotrophs and adrenocorticotropic cells) are stained with basic dyes and some cells that stain poorly are referred to as chromophobes. Based on their reaction to various stains, the cells in the horse's anterior lobe are categorized as either chromophilic or chromophobic (Flesch, 1884). Cells in the pars distalis can be categorized into acidophils, basophils and chromophobes, the latter referred to as "chief cells" due to their resemblance to cells in the peptic glands (Trautman, 1909a).

2. MATERIAL AND METHODES

1. Morphological technique

The hypophysis was painstakingly excised from the ventral aspect of the cranium utilizing specialized equipment. Procedure began with the removal of the mandible, followed by the breaking of the hyoid bone. Next, the basisphenoid bone was taken out, and the hypophysis was photographed to document its location and surrounding structures. The gland was subsequently excised from its location in the sella turcica utilizing a knife and hemostatic forceps. After excision, the gland was resected, weighed, and irrigated with 9% normal saline. Finally, photographs captured using a digital camera(AL-Okaily et al., 2013)

2. Histological technique

Pituitary gland Samples were kept in 10% neutral buffered formalin for two to three days for examination under a light microscope. The tissue underwent dehydration via sequential alcohol dilutions, was cleared in xylene, and subsequently embedded in paraffin wax. (AL-Mahdawi et al., 2015).The gland was sectioned and stained with Hematoxylin and Eosin. Sections of the pituitary stained with PAS showed a uniform distribution of mucopolysaccharides across all cells in the anterior and posterior regions. Orange G special stain It was utilized for the differential stains for different cell types, including acidophils and basophils (Adams and Swettenham, 1958; ElSakhawy et al., 2012).

3. ETHICAL STATE

All studies were performed in accordance with Iraqi law and the University's protocols for the welfare of experimental animals. The protocols followed in this study received approval from the Committee of the Faculty of Veterinary Medicine, Baghdad University, Iraq (PG:2306) on 3/12/2024.

4. RESULTS AND DISCUSSTION

1. Morphological Results

The present study found that the pituitary gland in adult male rabbits is a single, oval-shaped organ with a creamy color. It is located at the base of the brain in the sella turcica of the sphenoid bone (Fig. 1a). These findings align with those of (Al-Zubaidy, 2012; Luay, 2016) in guinea pigs and (Rosamura, 1983) in rats. However, (Nagamalleswari Y. et al., 2015; Al-Dbag, 1998), disagreed with these results, stating that the pituitary gland of rats at the weaning stage is small, white to yellowish, and lentiform in shape, weighing approximately (0.05900± 0.005g). It consists mainly of anterior and posterior parts, which are not visible to the naked eye. The gland is anchored at the base of the brain by a slender infundibular stalk. (Fig. 1b).

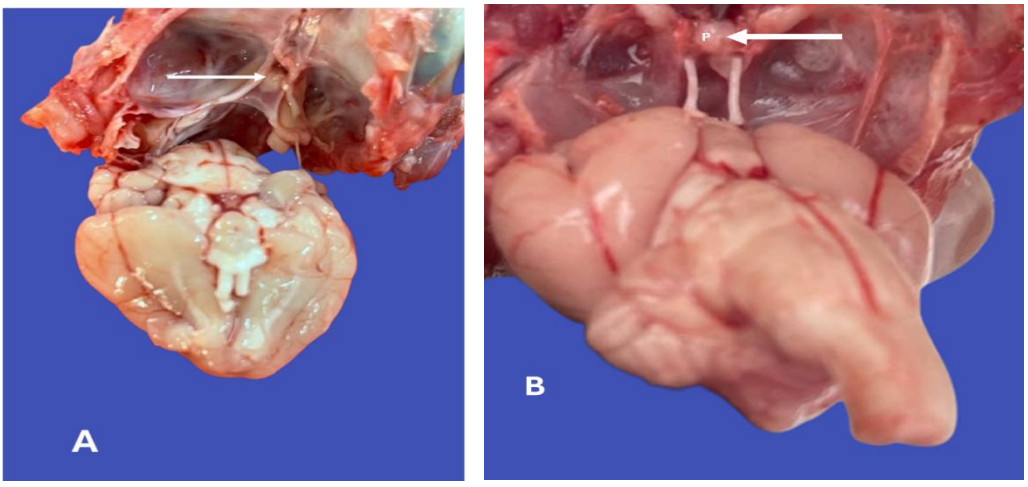

Fig. 1: The pituitary gland in its normal position inside the basisphenoid fossa from the ventral surface, A "white arrow refer to pituitary gland surrounded by diaphragma Sella of dura mater. B" white arrow referred to pituitary gland with the intact of infundibular stalk.

The rabbit's pituitary gland consists of two separate regions: the anterior lobe and the posterior lobe, both of which are functionally active. An intermediate lobe is situated between them. The anterior lobe secretes several hormones regulated by hypothalamic hormones, whereas the neurohypophysis is subdivided into three regions: the median eminence, infundibular stalk, and a slender layer of glandular tissue in the pars tuberalis (Malallah and Hussin, 2010). Furthermore, we noted that the anterior lobe is somewhat larger than the posterior lobes and is encased by a delicate, closely adhered capsule, a component of the dura mater known as the sella diaphragm. (Fig. 2a & b).

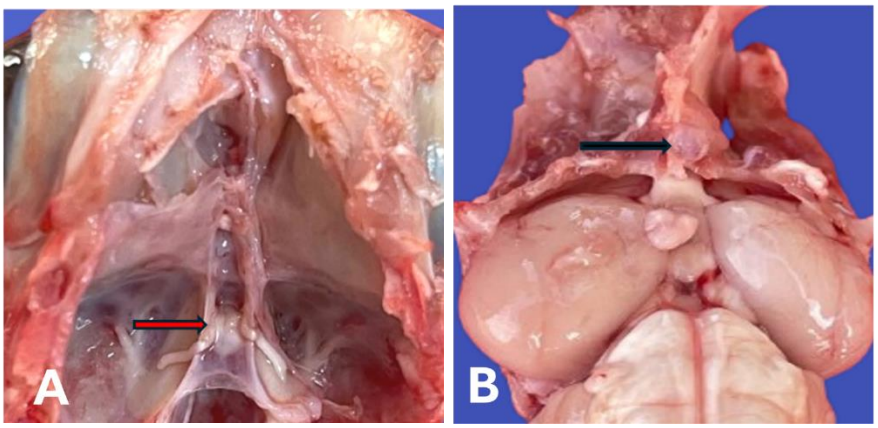

Fig. 2: Adult male rabbit pituitary gland in its normal location inside the basisphenoid fossa from the ventral surface, A "red arrow refers to pituitary gland surrounded by diaphragma Sella of dura mater .B" Black arrow referred to pituitary gland after removal from the fossa with intact infundibular stalk .

2. Histological Results

The findings showed that the hypophysis of male rabbits consists of two primary sections: the anterior pituitary, also known as the adenohypophysis, and the posterior pituitary, referred to as the neurohypophysis. The adenohypophysis is made up of three parts: pars distalis, pars tuberalis, and pars intermedia(Fig.3). This study aligns with previous research by authors such as (Manojlović-Stojanoski et al., 2007; Alnoor et al., 2024), who reported that these three components of the pituitary gland are fully differentiated in rats. The capsule of the male rabbit pituitary gland is composed of a very thin layer of fibrous connective tissue, predominantly collagen fibers (Fig. 4). This contrasts with the findings of (Gómez-Domínguez et al., 2023), who noted that the rat hypophysis capsule varies in thickness around the gland.

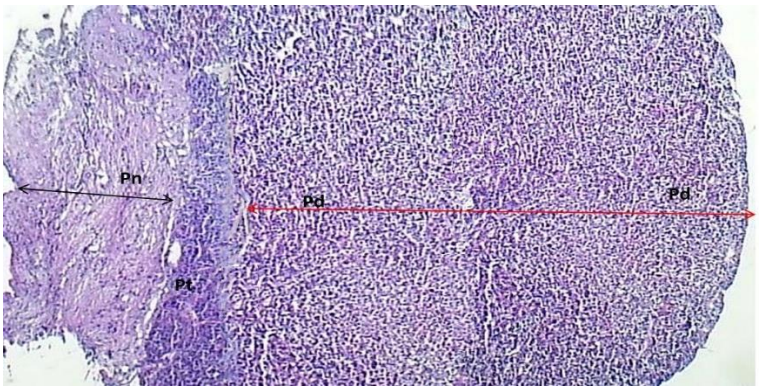

Figure 3 : section of pituitary gland shows pars distalis (Pd), pars nervosa (Pn) & pars tubralis (Pt) .H&E.40x

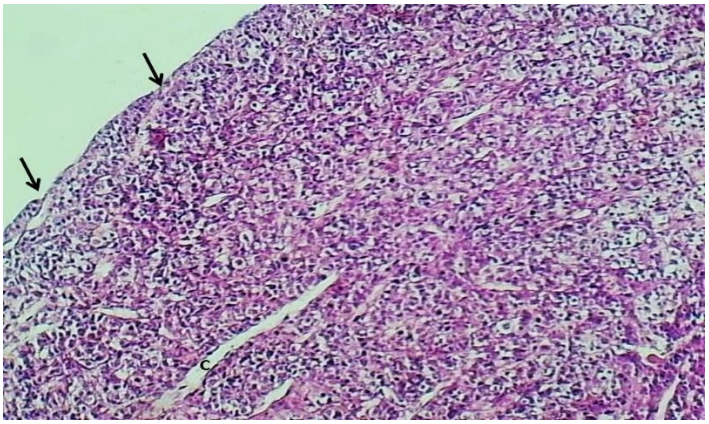

Figure4: section of pars distalis shows thin capsule (Arrows), sinusoidal capillary (C) & mass of cells clusters (Pt) .H&E.100x

A. Pars distalis of adenohypophysis

The cellular mass of the pars distalis consists of irregular clusters and cords of chromophilic and chromophobic cells, supported by a delicate stroma containing big capillaries (Fig. 4). The secretory granules within pars distalis exhibit an affinity for acidic (acidophilic) and basic (basophilic) dyes, as well as chromophobe cells. This finding aligns with the research of (Nagamalleswari et al., 2015), who reported that the pituicytes of adult male Sprague Dawley rats differentiated into chromophobes and chromophils. The cell details within the pars distalis include groups of light cells, consisting of large pale polygonal cells and small pale polygonal cells (chromophobes), while the chromophilic cells are primarily acidophilic, medium-sized cells with intracellular acidophilic secretory granules, and basophilic cells that are medium-sized with lightly basophilic intracytoplasmic granules (Fig. 4, 6, 7). The predominance of acidophils in rats was also observed in previous studies (Ibrahim et al., 2002), which strongly agrees with our findings.

Using Orang-G stain, the adenohypophysis-pars distalis section revealed two groups of cells with a positive reaction and two groups of cells with a negative reaction(Fig.5). Additionally, with PAS stain, the section of the adenohypophysis-pars distalis showed two groups of cells with strong neutral glycoprotein secretory granules and two groups of cells with a negative reaction (Fig. 6).

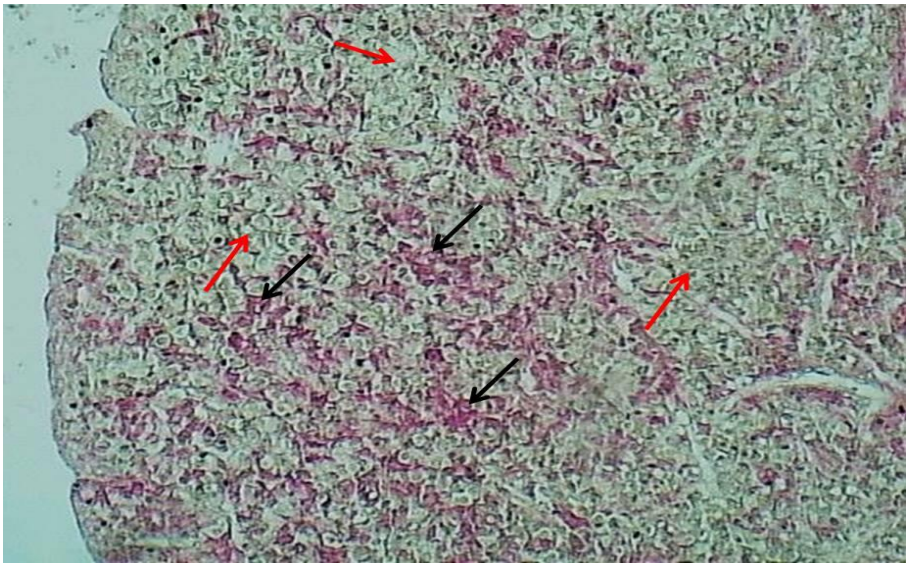

Figure 5: section of pars distalis shows positive reaction within cells (Black arrows) & negative reaction cells (Blue arrows). Orang-G stain.100x

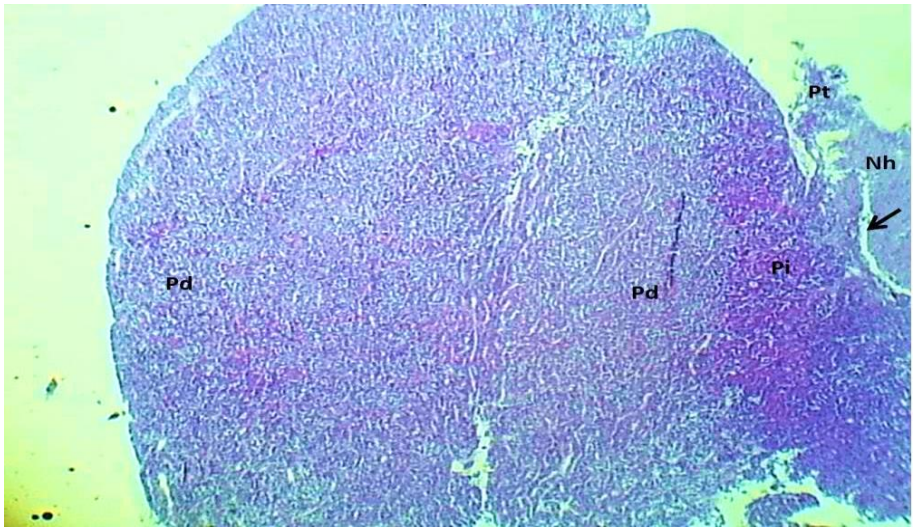

Figure 6: section of pituitary gland shows positive reaction within cells of pars distalis (Pd), intense positive reaction within cells of intermedia cells (Pi) & negative for cells of pars tuberalis (Pt) & neurohypophysis & (Nh). PAS stain.100x

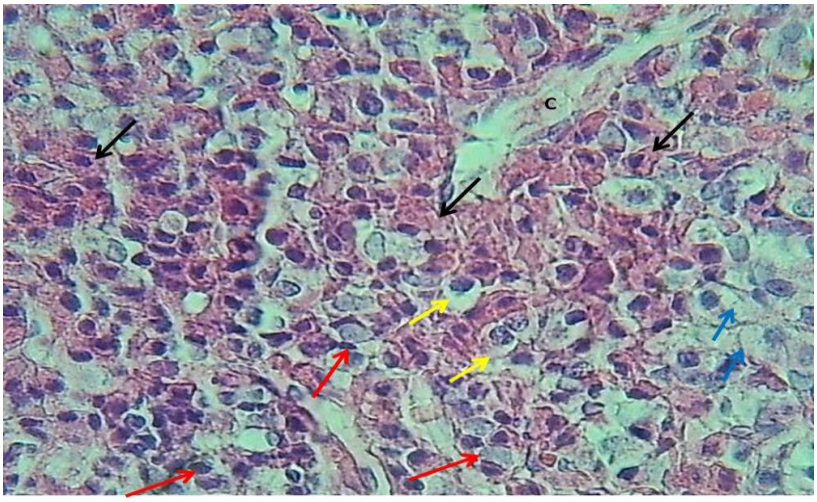

Figure 7: section of pars distalis shows acidophilic cells (Black arrows), basophilic cells (Red arrows), small size choromophobic cells (Yellow arrows) & large size choromophobic cells (Blue arrows).H&E.400x

B. Pars intermedia of adenohypophysis

Rabbits possess a well-developed pars intermedia, identifiable by a distinct cellular mass located between the neurohypophysis and the pars distalis of the adenohypophysis (Fig. 8). Our findings align completely with (Murakami et al., 1968), who noted that the human pars intermedia is also well-developed. However, our results contrast with those of (Turner and Bagnara, 1976) who reported that some mammalian species—such as humans, whales, dolphins, and elephants—lacking most or all their body hair, also lack a pars intermedia in the pituitary. Conversely our observations, human pars intermedia is rudimentary in adults, comprising a thin region between the adenohypophysis and neurohypophysis, often containing microscopic remnants of Rathke's cleft (Scott et al., 2024).

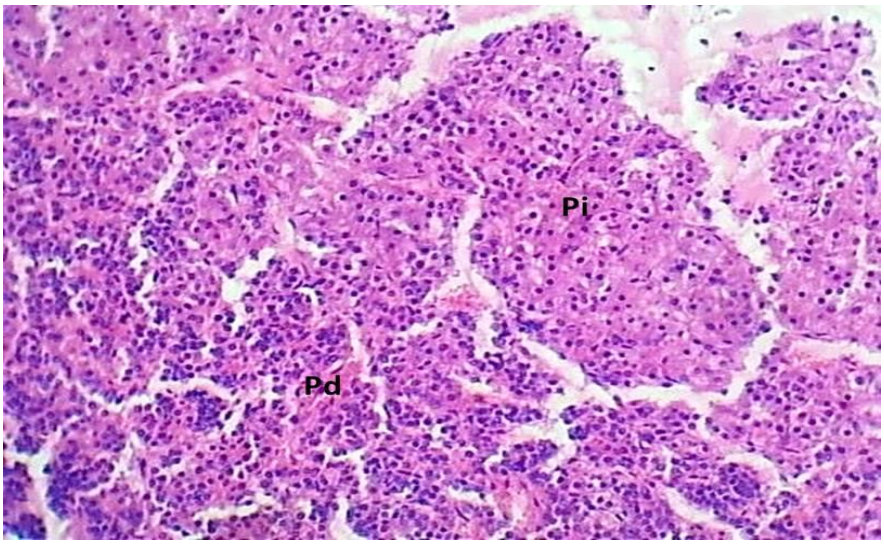

Figure 8: section of adenohypophysis shows cellar mass of pars distalis (pd) & pars intermedia (Pi). H&E stain.100x

Additionally, our study showed that the pars intermedia in male rabbits is of similar size to that in other laboratory animal species. Conversely, (Oishi et al. ,1992) reported that the mouse pars intermedia is larger compared to other species. The study identified three types of cells in the pars intermedia: (1) Predominantly magenta cells (dark cells) with dark intracytoplasmic granules, (2) a few large rounded chromophobe cells devoid of cytoplasmic granules, and (3) numerous flattened squamous cells (Fig. 9).

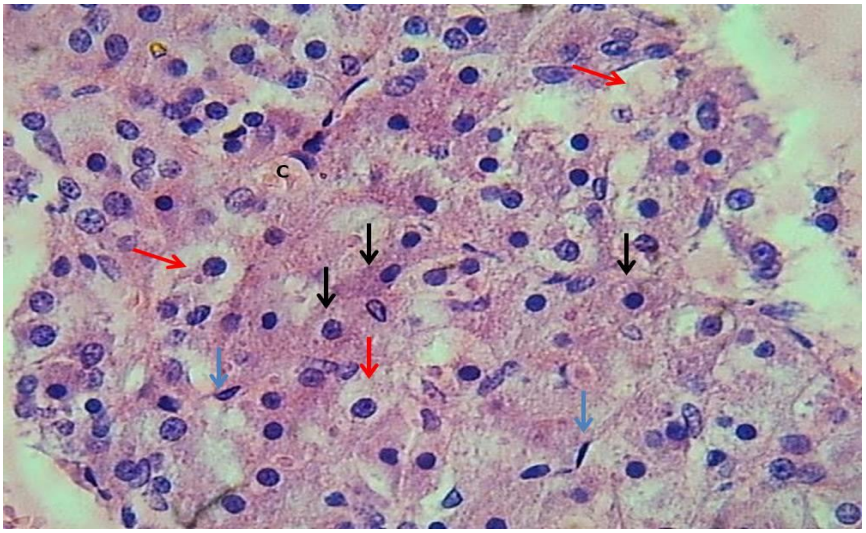

Figure 9: section of pars intermedia shows magenta cells (Black arrow) & light cells (red arrow) & flattened squamous cells (Blue arrow). H&E stain.400x

C. Paris tuberalis of adenohypophysis

The cell cords in the pars tuberalis were composed of four distinct cell types: (1) large, light, polygonal cells, (2) magenta or syncytial cells with ovoid nuclei and dark cytoplasm, (3) double squamous cells located along the margin of the pars tuberalis, interspersed among cell cords, consisting of double layers of small, flattened cells containing purple secretory granules (Fig. 10). The classifying of cells in our study is consistent with (Hussein, 2015) in rats.

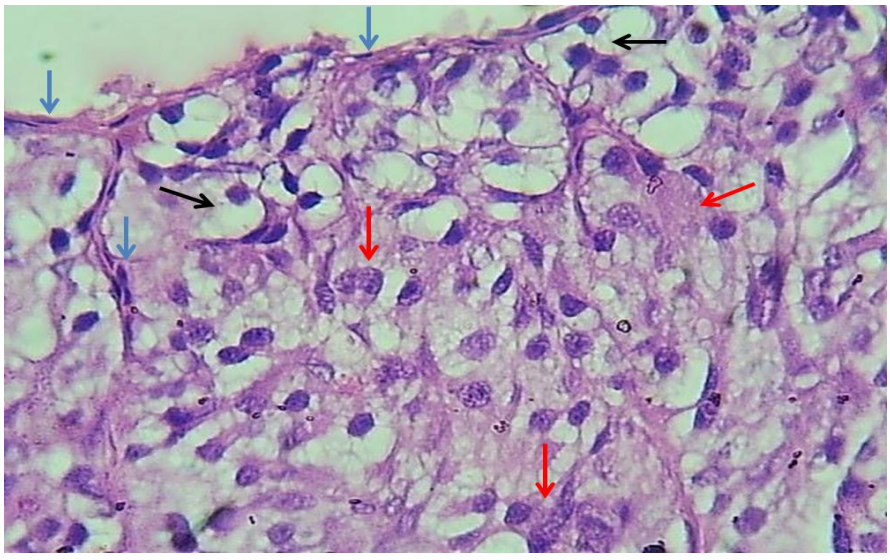

Figure 10: section of pars tuberalis shows double squamous cells (Blue arrows), dark syncytium cells (Red arrows) & large pale cells (black arrow) H&E stain.400x

Neurohypophysis

The pars nervosa consists of nervous tissue that is supported by connective tissue, which contains large groups of protoplasmic pituicytes, fibrous pituicytes, unmyelinated axons, and blood capillaries (Fig. 9). Previous findings indicated that the neurohypophysis in both humans and mice is hypocellular, primarily occupied by the unmyelinated axons of neurosecretory magnocellular neurons originating from hypothalamic supraoptic and paraventricular nuclei (Kelberman et al., 2009).

5. CONCLUSION

The present study provides a comprehensive morphological and histological analysis of the hypophysis in adult male rabbits. The gland was observed to be a single, oval-shaped structure located at brain base in the sella turcica of the sphenoid bone.

It consisted of distinct anterior and posterior lobes, with middle lobe between them. The adenohypophysis was composed of three parts: pars distalis, pars intermedia, and pars tuberalis, each containing different types of secretory cells. The neurohypophysis was divided into the median eminence, infundibular stalk, and pars nervosa, primarily consisting of nervous tissue and pituicytes.

6. RECOMONDATIONS

The current study recommended comparative studies across various mammalian species to gain a deeper understanding of the evolutionary and functional importance of the pituitary gland's structure. Future studies should focus on exploring the functional aspects of hormone secretion and regulation in the rabbit pituitary gland to clarify its physiological roles in growth, reproduction, and metabolism. Also, Examining the genetic and molecular mechanisms that govern pituitary development, and differentiation would offer greater insight into endocrine regulation.

7. ACKNOWLEDGEMENTS

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8. NOVELTY STATEMENT

This study provides a comprehensive morphological and histological characterization of the pituitary gland in adult male rabbits, filling a critical gap in comparative endocrinology. Unlike previous studies that focused on rodents such as rats and guinea pigs, our research identifies distinct structural features, including the well-developed pars intermedia and the cellular composition of the adenohypophysis. Additionally, we report novel insights into the differentiation of chromophilic and chromophobic cells, as well as the unique composition of pars tuberalis, aligning with and expanding upon previous findings in other species. These findings contribute valuable anatomical and functional data that can enhance the understanding of pituitary gland biology in lagomorphs.

9. AUTHORS' CONTRIBUTION

These authors contributed equally.

conflict of interest

The authors have declared no conflict of interest.

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