



## Some histological studies on thymus gland of mature and senile rabbits

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### ABSTRACT

The present study was carried out to investigate some histological changes of thymus gland of mature and senile rabbits. The study was carried out on fifteen apparently healthy mature (six-month-old) and senile (twelve-month-old) rabbits which were humanly sacrificed, eviscerated and subsequently tissue specimens from the thymus were taken. It was found that the thymus of mature rabbits was lobulated organ consisted of stroma and parenchyma, the stroma consisted of connective tissue capsule and septa. The septal connective tissue rich in collagen fibers and contain low elastic fibers. Within the septal connective tissue found the muscular artery which characterized by duplicated internal elastic lamina. The artery branched to give small arteriole. Also, septa contained medium size vein. Parenchyma consisted of peripheral dark cortex and central light stained medulla. The cortex heavily populated by small lymphocytes which had dark nucleus and also contained epithelial reticular cells which had large vesicular nucleus. Medulla contained large lymphocytes, epithelial reticular cells and Hassall's corpuscles. The medullary epithelial reticular cells showed positive reaction for PAS stain. The thymus of senile rabbits showed signs of involution, but it not completely disappeared. There were some changes, occurred thinning and irregularity of thymic lobule, progressive cortical thymocyte depletion, thymic cyst formation and lipid infiltration within the capsule and septa. The present study revealed that thymus was present through all the life, after maturity occurred involution but complete absence of thymus not occurred.

**Keywords:** *Rabbit, Thymus, Histology, Involution*

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### 1. INTRODUCTION

The breeding of domestic rabbits is used for multipurpose meat and fur production and used as lab animals for laboratory diagnosis and research. Rabbit's meats show high values for human consumption, it has a higher percentage of protein than other meats. The rabbits meats are highly digestible so for this reason it is recommended for sick people (Zotte, 2002).

The thymus is a primary or central lymphoid organ and the site for development of T cell within the cortex, without the need for

antigenic stimulation, it is morphologically similar across species (Elmore, 2006). It is actually an epithelial organ in which its epithelial cells forming a framework containing T cells as well as smaller numbers of other lymphoid cells. A symbiotic interaction exists between the thymic microenvironment and developing T cells, and the specificity of T cell release into the systemic circulation is under thymic control. Some epithelial cells are functionally essential

for the maturation of T lymphocytes and thus are called nurse cells (Shimosato and Mukai, 1997). The thymic cortex in mature rabbits is heavily populated by developing T cells along with a smaller proportion of associated epithelial reticular cells. Larger, more mature T cells are found in the medulla where epithelial reticular cells and Hassall's corpuscles are present (Pearse, 2006). After maturity thymus undergo stages of involution as increased amount of adipose tissue in connective tissue capsule and septa, Hassall's corpuscles decreased both in number and volume (Karan and Dinc, 2004).

The present study aimed to study some histological changes of thymus gland of mature and senile rabbits.

## 2. MATERIALS AND METHODS

### 2.1. Animal population and samples:

Fifteen apparently healthy mature and senile rabbits were collected from farm of Faculty of Agriculture, Benha University and transported to laboratory of histology at Faculty of Veterinary Medicine, where they were humanly sacrificed, eviscerated and subsequently tissue specimens from the thymus were taken.

### 2.2. Preparation of specimens for light microscopy:

One cubic centimeter (1cm<sup>3</sup>) specimens were used in light microscope study. The specimens were washed in distilled water for removal of blood clots and other debris, then fixed in 10% buffered neutral formalin, dehydrated in ascending grades of ethyle alcohol, cleared in xylene and embedded in paraffin wax. 5µm thick sections were cut, placed onto glass slides, and stained with the following stains:

- 1- Harris's haematoxylin and eosin stain for general histology of the specimens.
- 2- Masson trichrome stain for demonstration of collagen fibers.

- 3- Periodic acid Schiff technique (PAS) for demonstration of neutral mucopolysaccharides.

- 4- Verhoeff's stain for demonstration of elastic fibers.

All these methods and techniques were quoted from Bancroft and Gamble (2002). Representative fields were photographed for morphology.

## 3. RESULTS

### 3.1. In mature rabbits:

The present study revealed that the thymus consisted of stroma and parenchyma, the stroma formed from thin connective tissue capsule (Fig.1) which gives rise to septa that partially subdivide the thymus into interconnecting lobules of variable size and orientation, these septa carried the blood vessels of the thymus (Fig.2), rich in collagen fibers (Fig.3.) and contained few elastic fibers (Fig.4).

The muscular artery observed in septal connective tissue. It consisted of Tunica intima, media and adventitia. Tunica intima consisted of endothelial cell layer and subendothelial connective tissue, followed by internal elastic lamina and several smooth muscle layers of tunica media and external elastic lamina. Externally surrounded by connective tissue layer of tunica adventitia (Fig.5).The internal elastic lamina duplicated (Fig.6).The muscular artery branched giving rise to small arteriole which consisted of endothelial layer of tunica intima, several layers of smooth muscle of tunica media and connective tissue layer of tunica adventitia (Fig.7).Medium size vein present in septal connective tissue. The wall of this vein consisted of endothelial cell layer of tunica intima, several layers of smooth muscle of tunica media and connective tissue layer of tunica adventitia (Fig.8).

The bulk of the supporting framework in the thymus was composed of the network of epithelial reticular cells that divided into three distinct subtypes: cortical which characterized by large light stained nucleus and light stained cytoplasm (Fig.9), medullary and Hassalls corpuscles epithelial reticular cells (Fig.10). The medullary epithelial reticular cells showed PAS positive staining reaction (Fig.11 and Fig.12). The parenchyma consisted of peripheral dark cortex and central light stained medulla which continuous between adjacent lobules and form small buds that reached deep into the cortex (Fig.13). Cortex was darkly stained and contained densely packed, small lymphocytes, which overshadow the sparse epithelial reticular cells (Fig.14). Medulla was paler staining, less densely cellular than the cortex. It contained large lymphocyte which had large nucleus with prominent nucleolus, epithelial reticular cells which characterized by light cytoplasm and large nucleus with prominent nucleolus but it lighter stained than that of large lymphocyte and Hassall's corpuscles (Fig.15). Hassall's corpuscle appeared as group of epithelial cells which undergo degeneration to its nucleus or may form cyst that may had amorphous protein in its center (Fig.16) or may be appeared as concentric arranged layers of epithelial reticular cells with keratinized center (Fig.17).

### **3.2. In senile rabbits:**

The thymus showed signs of involution. The results Showed decreased size and irregularity of the thymic lobule and infiltration by adipose tissue in the connective tissue capsule and septa (Fig. 18). Loss of corticomedullary demarcation (Fig.19). Epithelial cells became progressively more prominent which had stellate or oval shape and eosinophil also present in which it characterized by bilobed nucleus and high eosinophilic cytoplasm due to eosinophilic granules (Fig.20). Thymic cyst formation more prominent with age, the cyst

may be filled with homogeneous pale staining protein (Fig.21).

## **4. DISCUSSION**

The obtained data showed that the thymus of mature rabbits consisted of stroma and parenchyma, the stroma formed from thin connective tissue capsule which gives rise to septa that partially subdivide the thymus into interconnecting lobules of variable size and orientation this in agreement with Haley (2003) and Pearse (2006). The results showed that septa contained well developed collagen fibers this as mentioned by Pearse (2006). The obtained data showed few elastic fibers around thymic lobule and in wall of muscular artery, this confirm the results of Kandil (1972) and Contreiras et al. (2004) in which they recorded that the elastic fibers were found only in the perithymic region in the camel and vascular wall in equine respectively.

The results recorded three types of epithelial reticular cells; cortical, medullary and Hassall's corpuscles cells and this in agreement with Aly et al. (1988) but disagree with Suster and Rosai (1990), Kuper et al. (1995), DeWaal et al. (1997) and Greaves (2000) that mentioned that the epithelial cells were divided into distinct four subtypes, however Gartner and Hiatt (2006) mentioned that there were six types of epithelial reticular cells. The results showed positive PAS staining reactions in cytoplasm of some epithelial reticular cells indicating secretory activity of these cells this confirmed the results of Bodey et al. (1987) who recorded positive PAS staining reaction in cytoplasm of epithelial reticular cells and Aly et al. (1988) in which recorded three types of epithelial reticular cells, two of them showed secretory activity. The cortex was darkly stained contains densely packed, small lymphocytes and few epithelial cells and this confirmed the results of Ham and Cormack (1979); Junqueira and Carneiro (2005) and Pearse (2006). The obtained data showed that the medulla was paler staining,

less densely cellular than the cortex, and contained more large lymphocytes, prominent epithelial cell, Hassalls corpuscles, as mentioned by Haley (2003) and Pearse (2006). The results showed three types of Hassalls corpuscles one appeared as group of epithelial cells which undergo degeneration to its nucleus, the second form appeared as cyst that may had amorphous protein in its center while the third type had onion like appearance in which the epithelial reticular cells concentric arranged with keratinized center this in agreement with Kandil (1972) who recorded three types of corpuscles and recorded that the most common type is onion like form.

In senile rabbits the thymus enters in stage of involution in which occurred progressive cortical lymphocyte depletion and shrinkage of thymic lobule this in agreement with Contreiras et al. (2004). The study showed thymic cyst formation were present this confirmed results of Khosla and Ovalle (1986) who mentioned that with age prominent cyst may be filled with homogeneous pale staining protein. There was lipid infiltration as mentioned by Karan and Dinc (2004) who mentioned that the lipid cells, one of the involution signs, were first to be seen. The eosinophils were present with in the periphery of the lobule this results also recorded by Kandil (1972) but within the interlobular connective tissue septa.

## 5. Conclusion

the present study indicated that the thymus of mature rabbits had lobulated appearance with well definite peripheral dark cortex and central light stained medulla but after maturity it undergo involution as occurred thinning and irregularity of thymic lobule, loss of corticomedullary demarcation and infiltration by adipose tissue. Complete atrophy or absence of thymus not occurred.

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Plate 1

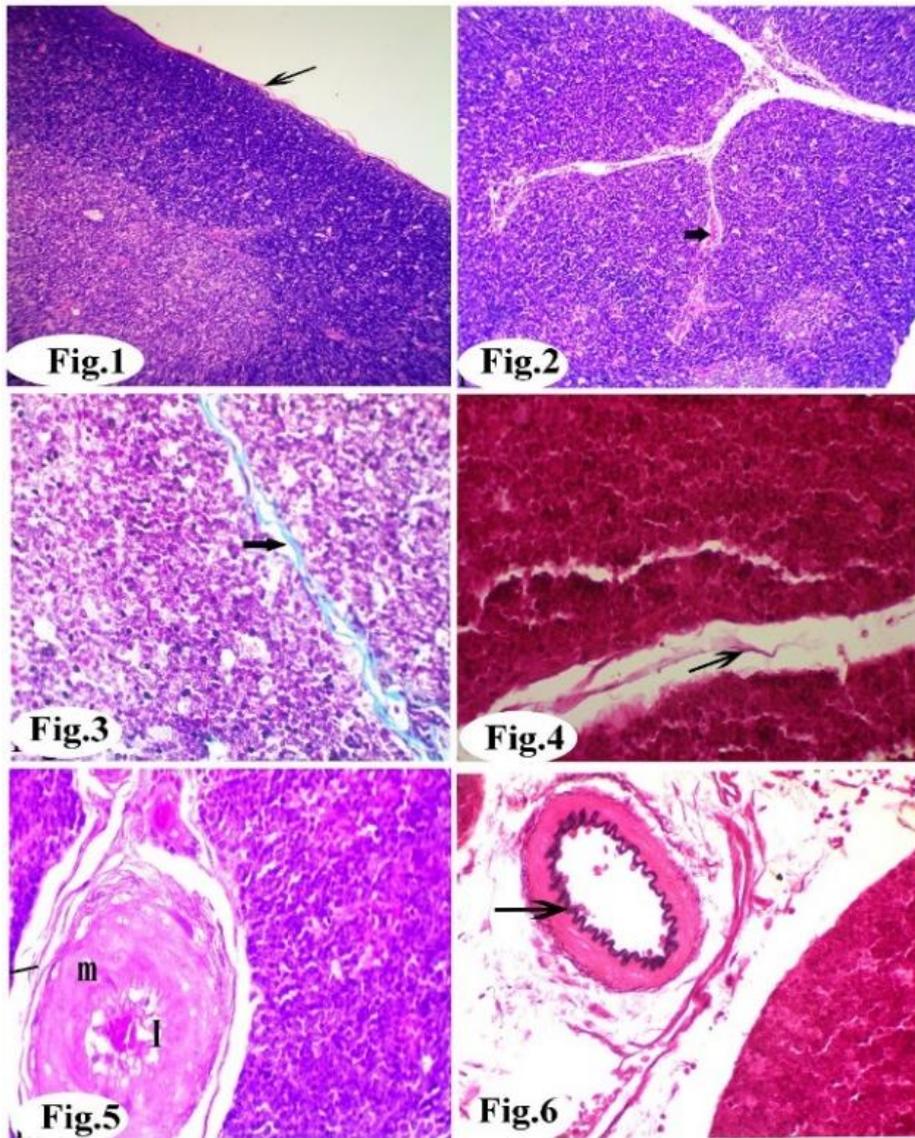


Fig.1: Photomicrograph of 6-month-old rabbits showing thymus surrounded by thin connective tissue capsule (arrow). H&E X 4.

Fig.2: Photomicrograph of 6-month-old rabbits showing septa that partially subdivide the thymus into interconnecting lobules of variable size and orientation, these septa carry the blood vessels of the thymus (arrow). H&E X 10.

Fig.3: Photomicrograph of 6-month-old rabbits showing stromal connective tissue rich in collagen fibers (arrow). Masson trichrome stain X 40.

Fig.4: Photomicrograph of 6-month-old rabbits showing few elastic fibers in septal connective tissue (arrow). Verhoeffs stain X 40.

Fig.5: Photomicrograph of 6-month-old rabbits showing muscular artery consists of tunica intima (I), tunica media (m) and tunica adventitia (line). H&E X 40.

Fig.6: Photomicrograph of 6-month-old rabbits showing duplicated internal elastic lamina of muscular artery (arrow). Verhoeffs stain X 40.

## Plate 2

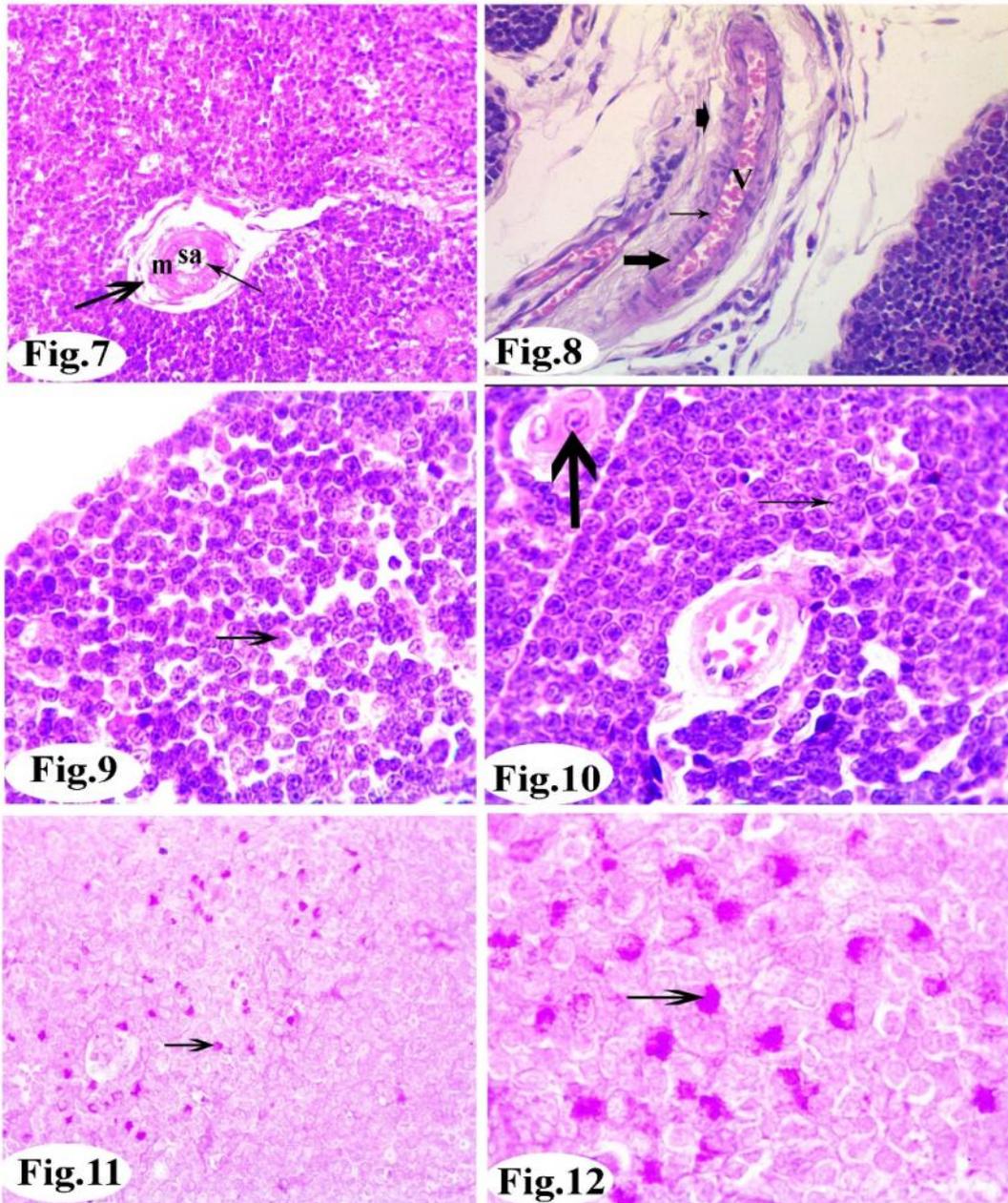


Fig.7: Photomicrograph of 6-month-old rabbits showing small arteriole (sa) consists of tunica intima (thin arrow), tunica media (m) and tunica adventitia (thick arrow). H&E X 40.

Fig.8: Photomicrograph of 6-month-old rabbits showing medium size vein (V) consists of tunica intima (thin arrow), tunica media (thick arrow) and tunica adventitia (half arrow). H&E X 40.

Fig.9: Photomicrograph of 6-month-old rabbits showing cortical epithelial reticular cells (arrow) which are large size cells contain vesicular light stained nucleus.H&E X 100.

Fig.10: Photomicrograph of 6-month-old rabbits showing medullary epithelial reticular cells that have large vesicular light stained nucleus (thin arrow) and Hassalls corpuscle epithelial reticular cells which have pyknotic nucleus (thick arrow).H&E X 100.

Fig.11: Photomicrograph of 6-month-old rabbits showing Positive PAS staining reaction in medullary epithelial reticular cells (arrow). PAS stain X 40.

Fig.12: Higher magnification of Figure 11 showing Positive PAS staining reaction in medullary epithelial reticular cells (arrow). PAS stain X 100.

**Plate 3**

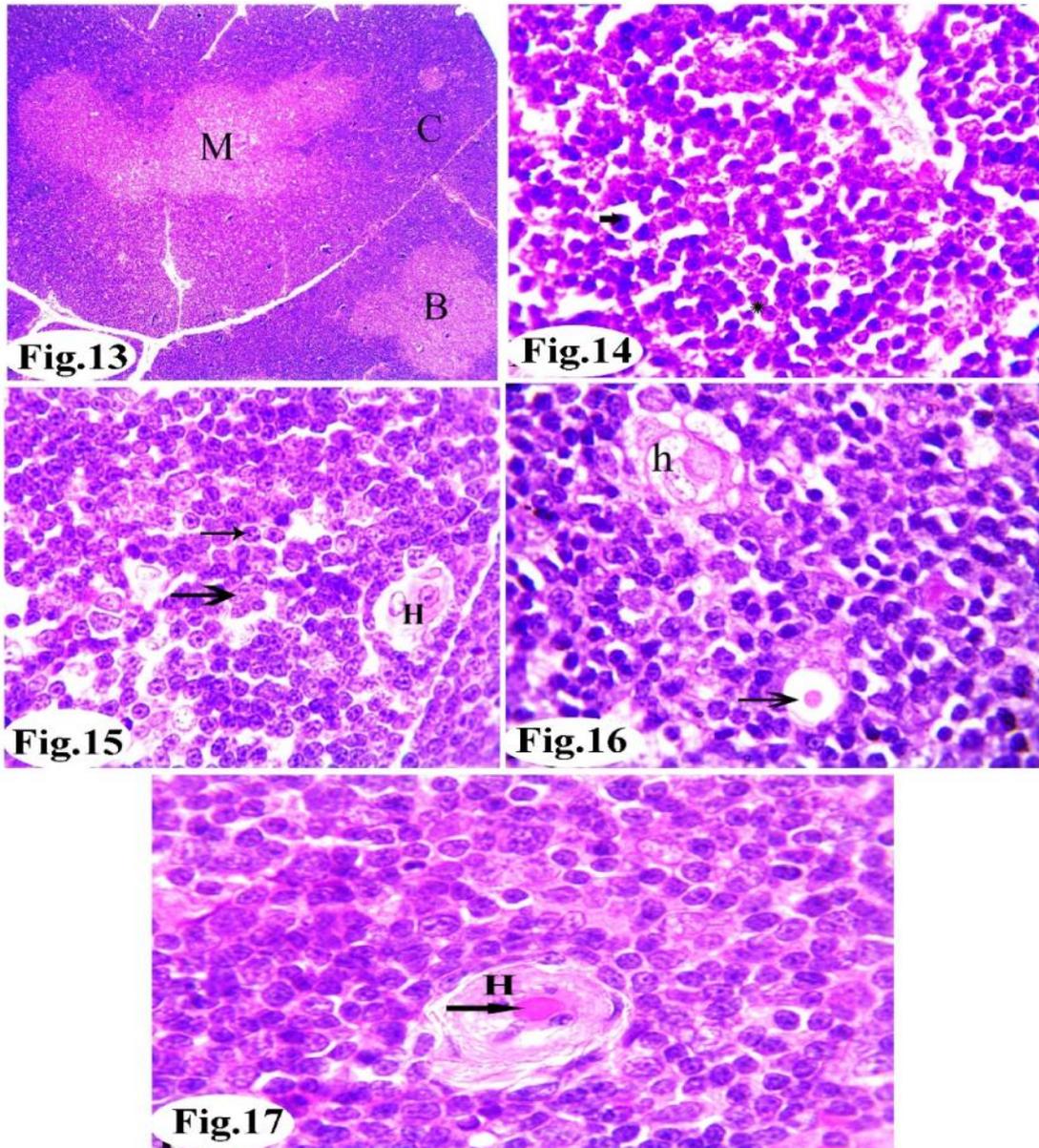


Fig.13: Photomicrograph of 6-month-old rabbits showing parenchyma consists of peripheral dark cortex (C) and central light stained medulla (M) which continuous between adjacent lobules and can form small buds that reach deep into the cortex (B). H&E X 4.

Fig.14: Photomicrograph of 6-month-old rabbits showing Cortex is darkly stained contains densely packed, small lymphocytes which have dark nucleus (arrow) and sparse epithelial reticular cells which have vesicular nucleus with prominent nucleolus (star). H&E X 100.

Fig.15: Photomicrograph of 6 month old rabbits showing medulla contains large lymphocyte which have large vesicular nucleus with prominent one or two nucleolus (thin arrow), prominent epithelial reticular cell which characterized by light stained nucleus and light cytoplasm (thick arrow) and Hassall's corpuscles (H). H&E X 100.

Fig.16: Photomicrograph of 6-month-old rabbits showing Hassall's corpuscle appeared as cluster of epithelial cells which undergo degeneration to its nucleus (h) or may form cyst that may has amorphous protein in its center (arrow). H&E X 100.

Fig.17: Photomicrograph of 6-month-old rabbits showing Hassall's corpuscle (H) has onion like appearance as epithelial reticular cells concentric arranged with keratinized center (arrow). H&E X 100.

## Plate 4

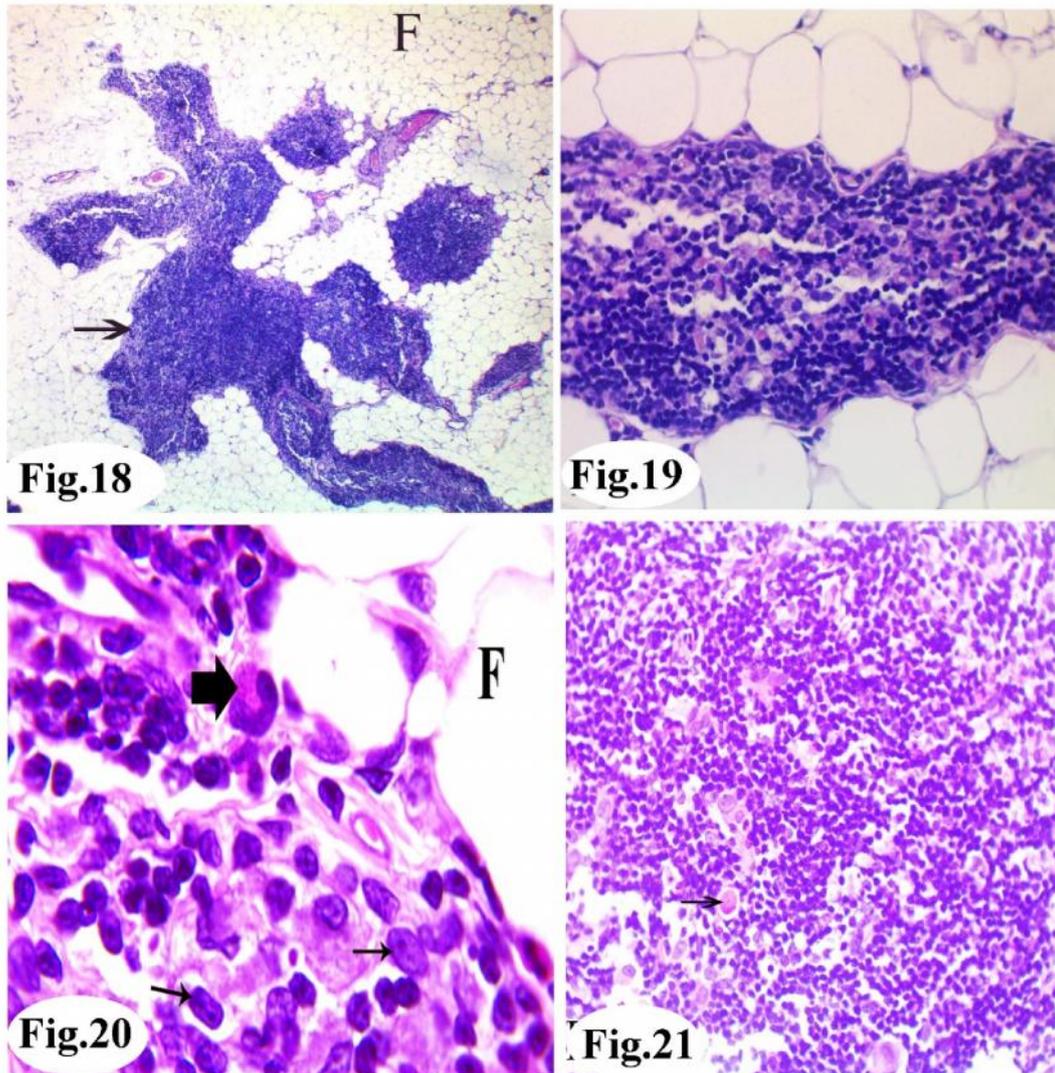


Fig.18: Photomicrograph of 12-month-old rabbits showing decreased size and irregularity of the thymic lobule (arrow) and infiltration by adipose tissue in the connective tissue capsule and septa (F). H&E X 4.

Fig.19: Photomicrograph of 12-month-old rabbits showing loss of corticomedullary demarcation. H&E X 40.

Fig.20: Photomicrograph of 12-month-old rabbits showing white fat around thymic lobule (F), epithelial cells become progressively more prominent which has stellate shape or oval shape (thin arrows), also eosinophil present which showed bilobed nucleus and high eosinophilic cytoplasm due to eosinophilic granules (thick arrow). H&E X 100.

Fig.21: Photomicrograph of 12-month-old rabbits showing thymic cyst formation more prominent with age, the cyst may be filled with homogeneous pale staining protein (arrow). H&E X 40.