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Histological and structural alterations in the bursa of fabricius of rajasree birds across different ages

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Abstract

The bursa of Fabricius in Rajasree birds from day old to 135 days of age. The bursal mean weight, length, width, circumference and mean number, length, width of plicae, number of follicles per plicae and follicular diameter were increased from day old to 75 days while decreased thereafter at 135 days of age. The invaginations into follicular region and vacuoles in epithelial region at 60 days of age. The involutory process picked up at 105, 120 and 135 days. Deformed follicles and bursal epithelium expressed alcian blue positive while the plica, inter Follicular Epithelium (IFE) and septa has moderately Periodic Acid-Schiff (PAS) positive. PAS positive in the distorted follicles, developing cysts and lymphoid follicles. The plical epithelium is moderately positive for Acid Phosphatase (ACP) and intensely positive for Alkaline Phosphatase (ALP) in young age. The total number of serum immunoglobulins is more at 30 days of age.

Keywords: Rajasree birds, bursa of fabricius, gross morphology, morphometry, histology and histometry, histochemistry, serum immunoglobulins

Introduction

The bursa of Fabricius is a sac-like lymphoepithelial organ present in gallinaceous birds such as chickens, turkeys, pheasants, geese, partridges, and quails. It is situated dorsal to the cloaca (Toivanen *et al.*, 1972) [23]. The history of the bursa of Fabricius was described by Hieronymus Fabricius and its pivotal role in humoral immunity was noticed in 1950. Glick in (1956) [6] revealed the central role of the bursa in antibody production. The ontogeny of B-cell differentiation (Alboghobeish and Mayahi, 2003) [3].

The bursa was round or pear shaped and measured about the maximum mean length, width and weight was 22 mm $\pm$ 2.54, 17 mm $\pm$ 1.79 and 800 mg $\pm$ 0.86 in at 75 days old. Involution of the organ started at 135 days, and a fatty nodule appeared at 165 days of age. The bursal mucosal plica in desi birds was lined by pseudostratified columnar epithelium in all age groups (Purushotham *et al.*, 1989) [14].

Histologically the wall of bursa comprised the tunica mucosa, muscularis and serosa. The follicular surface comprised of two kinds of epithelium: Follicular associated epithelium (FAE) and IFE (Sari *et al.*, 2015) [18].

The present study was designed on Rajasree birds which considered to be less susceptible to disease by virtue of having desi fowl inheritance. Under the presumption that Rajasree birds thrive well even under unhygienic backyard conditions leads to a conclusion that these birds may have a better defense mechanism against disease which depends on age, breed, sex and environment. Hence the present research on Rajasree birds is taken up to throw light on the hidden facts about the morphological, histological and histochemical changes that take place from day-old chick up-to 135 days of age.

Materials and Methods

Study location and sample collection

The present study on the "Influence of Age on the Anatomy of Bursa of Fabricius in Rajasree Birds" was conducted in the Department of Veterinary Anatomy, College of Veterinary

Science, Rajendranagar, Hyderabad. A total of six healthy Rajasree layer chickens from each age group (day-old, 15, 30, 45, 60, 75, 90, 105, 120, and 135 days) were procured from the Department of Poultry Science, College of Veterinary Science, Rajendranagar, and private poultry farms in and around Hyderabad. Bursa of Fabricius specimens were harvested through gentle dissection on the dorsal wall of the cloaca in the Department of Veterinary Anatomy, College of Veterinary Science, Rajendranagar, for further anatomical studies.

Morphological and morphometric analysis

Morphometric measurements such as length, width, and circumference were obtained using a scale and digital Vernier calipers. Data analysis involved the following parameters:

1. Mean length (mm) and weight (g) of the bursa across different age groups.
2. Mean width (mm) and circumference (mm) of the bursa across different age groups.

Histology and histometry

Tissue samples of the bursa were fixed in 10 percent Neutral Buffered Formalin (NBF) and Bouin's fluid immediately after collection. The specimens were processed using the routine paraffin embedding method (Singh and Sulochana, 1998) [20]. Sections of five μm thickness were prepared and subjected to the following staining techniques:

- Haematoxylin and Eosin for routine histological examination (Singh and Sulochana, 1998) [20].
- Masson's Trichrome for collagen and muscle fiber differentiation (Singh and Sulochana, 1998) [20].
- Van Gieson method for collagen fiber identification (Singh and Sulochana, 1998) [20].
- Wilder's method for reticular fiber demonstration (Luna, 1968) [11].
- Methyl Green Pyronin for DNA, RNA, and plasma cell demonstration (Chayen, 1969) [4].

Histometric analysis included:

1. Mean number of plicae in different age groups.
2. Mean plicae length (μm) across age groups.
3. Mean plicae width (μm) across age groups.
4. Mean number of follicles per plica in different age groups.
5. Mean follicular diameter (μm) across age groups.

The micrometric analysis was performed by calibrating ocular and stage micrometers, and the data was subjected to statistical analysis as per Snedecor and Cochran (1994) [21].

Histochemistry

For histochemical studies, paraffin sections of the bursa were subjected to the following techniques:

1. PAS reaction for glycogen detection (Singh and Sulochana, 1998) [20].
2. PAS-Alcian Blue reaction for identifying acid and neutral mucopolysaccharides (Singh and Sulochana, 1998) [20].

Cryostat sections (10-15 μm thickness) obtained from fresh bursa tissues prefixed in chilled acetone were used for:

1. Gomori's acid phosphatase method for localizing acid phosphatase enzyme (Singh and Sulochana, 1998) [20].
2. Gomori's alkaline phosphatase cobalt method for localizing alkaline phosphatase enzyme (Singh and Sulochana, 1998) [20].

Serum immunoglobulin estimation

The total serum immunoglobulin levels were estimated using

the sodium sulfate precipitation method (Pfeiffer *et al.*, 1977) [15]. The results were recorded as mean total serum immunoglobulins (mg/ml) across different age groups.

Results and Discussion

The bursa of Fabricius was pear shaped and light pinkish in colour when collected fresh. It was sac like and connected to the proctodeum with a stalk. These findings are similar to the (Purushotham *et al.*, 1989) [14] in desi birds and in turkey (Nnadozie *et al.*, 2014) [12].

In the day old chick the body and bursal weights were $34 \text{ gm} \pm 0.56$ and $0.060 \text{ gm} \pm 0.05$, at 75 days it was $729.54 \text{ gm} \pm 2.73$ and $0.740 \text{ gm} \pm 0.14$ and at 135 days of age it was $1256.89 \text{ gm} \pm 64.32$ and $0.510 \text{ gm} \pm 0.12$ respectively. Bursal weight decreased from $0.740 \text{ gm} \pm 0.14$ at 75 days to $0.510 \text{ gm} \pm 0.12$ at 135 days of age (Table.1 and Fig 4). The present investigation revealed that there was no consistent relation between chick body weight and bursal weight as it was 0.18% in day old while at 75 days age where bursal physical features are highest recorded 0.1% and at 135 days 0.04%. The present findings are in acceptance with Glick, (1956) [6] who observed that the bursa weight increased at 8th week in both sexes of chicken. Our findings are also in complete acceptance with Glick, (1959) [7] who reported that the most rapid growth of the bursa at 22 and 36 days of age in white leghorn. According to Purushotham *et al.* (1989) [14] the maximum weight of bursa in desi birds was $800 \text{ mg} \pm 0.86$ in at 75 days. There after decreased up to $575 \text{ mg} \pm 3.04$ in at 135 days of age.

In day old chick the mean length, width and circumference were $5.12 \text{ mm} \pm 0.03$, $4.62 \text{ mm} \pm 0.01$ and $11.3 \text{ mm} \pm 0.03$, at 75 days it was increased upto $17.08 \text{ mm} \pm 0.92$, $13.38 \text{ mm} \pm 0.83$ and $35.9 \text{ mm} \pm 0.09$, at 135 days of age it was decreased upto $7.11 \text{ mm} \pm 0.15$, $5.90 \text{ mm} \pm 0.14$ and $21.5 \text{ mm} \pm 0.03$ respectively (Table.1 and Fig 4) comparatively more than those observed by Purushotham *et al.* (1989) [14] in desi birds was $22 \text{ mm} \pm 2.54$ and $17 \text{ mm} \pm 1.79$ respectively in at 75 days of age and also Onyeansi *et al.* (1993) [13] reported in helmeted guinea fowl at 18th day of age about 18 mm and 15 mm. According to Singh *et al.* (2006) [19] observed that the maximum diameter of bursa in guinea fowl was 1.35 cm at 35th days of age.

The histoarchitecture of the bursa of Fabricius was comprised of tunica mucosa, muscularis and serosa (Fig.1). The tunica mucosa thrown into folds called "Plicae" towards lumen in all age groups. In day old bursa the mean number of plicae, plical mean length and width were 12.63 ± 0.21 , $573.53 \mu\text{m} \pm 4.93$ and $359.29 \mu\text{m} \pm 6.36$, at 75 days it was increased upto 20.51 ± 0.61 , $3783.94 \mu\text{m} \pm 32.15$ and $1892.30 \mu\text{m} \pm 34.57$, at 135 days of age it was decreased upto 13.16 ± 0.42 , $2185.72 \mu\text{m} \pm 37.25$ and $833.45 \mu\text{m} \pm 7.14$ respectively (Table.1 and Fig 5). The plical mucosa in all age groups was lined by pseudostratified columnar epithelium similar to the findings of Purushotham *et al.* (1989) [14] in desi bird and in broiler chicken (Akter *et al.*, 2006) [2]. According to Jawad (2011) [9], they reported that the mucosal folds of bursa was lined with simple columnar cells in quails.

The lamina propria of plical mucosa was occupied by several lymphoid follicles. These follicles were surrounded and supported by connective tissue trabeculae made of collagen bundles (Fig.1) and few reticular fibres increased in at advancing age groups. Striking feature of 60 days of age group was invaginations in mucosa extending into follicular region and appearance of few vacuoles in epithelial region (Fig.2). The involutory process (Fig.3) hastened and picked

up momentum was noticed in at 105, 120 and 135 days and involving all follicles resulting in deep invaginations, indentation and formation of several cysts in epithelium and follicular zones which increased in number, thus indicating progressive and extensive degenerative changes. The current findings either are in complete or partial acceptance with Purushotham *et al.* (1989) ^[14] noticed that the involution at 135 days, fibrous and adipose tissue at 165 days in desi birds, Sari *et al.*, (2015) ^[18] in Long-Legged Buzzard stated that the lamina propria of bursa contained numerous lymphoid follicles.

The follicles in day old bursa were undifferentiated and showed extensive pyroninophilia. In day old bursa the mean number of follicles per plica and follicular diameter were 171.21 ± 5.72 and $120.11 \mu\text{m} \pm 6.0$, at 75 days increased upto 639.91 ± 5.55 and $362.66 \mu\text{m} \pm 6.05$, at 135 days of age decreased upto $145.83 \mu\text{m} \pm 4.84$ and 134.55 ± 3.57 respectively (Table.1 and Fig 5). Intense pyroninophilia in the cortex indicating the RNA activity in the lymphocytes of this region while low pyroninophilia in the medulla similar findings to the Purushotham *et al.* (1989) ^[14] stated that the submucosa of bursa was composed of mainly collagen fibres which increased with age in desi birds. These findings are in total acceptance with the observations of Fennell and Pearse, (1978) ^[5] who reported that the methyl green pyronin was used as a reagent brilliant red reactive centres (usually attributed to RNA) were evident as spherical or irregular entities, adjacent or contiguous to nuclei, in both cortex and medulla of follicles in chicken.

Tunica-muscularis was composed of inner circular and outer longitudinal smooth muscle fibres in all age groups. This observation is in total confirmation with Purushotham *et al.*, (1989) ^[14] in desi birds, Tamilselvan *et al.*, (2017) ^[22] in guinea fowl where all the researchers reported that the tunica muscularis was arranged into inner longitudinal and outer circular layers of smooth muscle fibres.

The tunica serosa was made up of thin and loose connective tissue which comprised of collagen fibres (Fig.1) and a few reticular fibres with mesothelial lining along with blood vessels and nerves. These findings are in total acceptance with the observations of Hashimoto and Sugimura, (1976) ^[8] reported that the outermost part of the bursa consisted of a circular strand of tunica serosa in White Pekin ducks while Purushotham *et al.* (1989) ^[14] noticed that the tunica serosa was very thin in desi birds.

The epithelial surface was Alcian blue (Fig.3) positive while the plical, IFE and septa was moderately PAS positive in all age groups. During the advanced stages of involution at 135 days, the involuting bursa (Fig.3) with deformed follicles expressed Alcian blue positive material in the cysts of follicles. The present findings are in consonance with the Ackerman and Knouff, (1959) ^[1] reported that the luminal surface epithelium of bursal mucosa was PAS positive while remaining part of the epithelium was Alcian blue positive. And partial confirmatory findings of Purushotham *et al.* (1989) ^[14] who reported that the epithelium was Alcian blue positive.

The moderate ACP activity was noticed in the plical and follicular epithelium indicating the presence of glycoprotein. IFE and FAE was strongly positive during involution indicating degenerative changes. The present findings are in acceptance with Fennell and Pearse, (1978) ^[5] in light Sussex chicken at day old to 9.5 weeks of age and in desi birds

Purushotham *et al.* (1989) ^[14] reported about the ACP reactions were moderately intense or greatly reduced with age.

The plical epithelium was intensely positive for ALP in young ages, less activity in advancing age groups. The inter follicular connective tissue, tunica muscularis, tunica serosa was negative for ALP. The present recordings are confirmation with Ruuskanen *et al.* (1977) ^[17] who reported that the ALP activity was weak between 4-6 weeks in chicken. According to Kempashi *et al.* (2017) ^[10] stated that the follicles showed moderate positive reaction to ALP and this reaction reduced from day old to 2 weeks of age in broiler chicken.

The mean total serum immunoglobulins increased upto 23.30 mg/ml ± 0.17 at 30 days of age and thereafter fluctuations was noticed (Table.1 and Fig 6). The current findings of presence of serum immunoglobulins at different growth stages of the bursa was confirmed by Yamamoto *et al.* (1977) ^[24] reported that the bursa synthesized IgM, IgG, and IgA in 1 day-old chicks. According to Ratcliffe (2006) ^[16] the normal serum concentration of chicken immunoglobulins was (IgM-1.3 mg/ml), (IgG-5.0 mg/ml), (IgA-0.3 mg/ml).

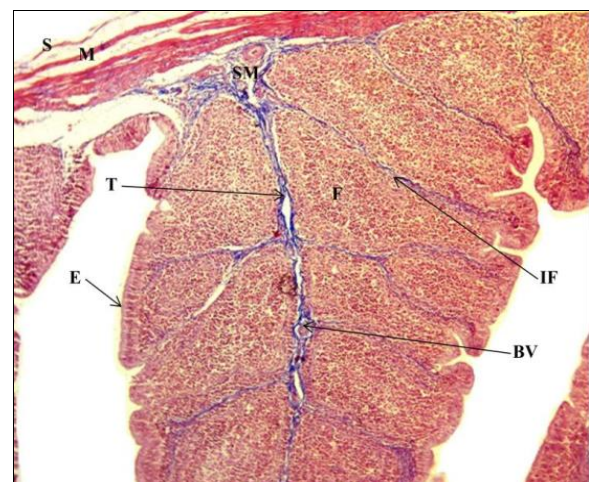


Fig 1: Photomicrograph of bursa of Fabricius in 45 days old Rajasree bird showing: Collagen fibres in trabeculae and inter follicular zones. Follicles (F), Inter follicular zones (IF), Trabeculae (T), Epithelium (E), Blood vessels (BV), Submucosa (SM), Muscularis (M), Serosa (S). (Masson's trichrome-10X10)

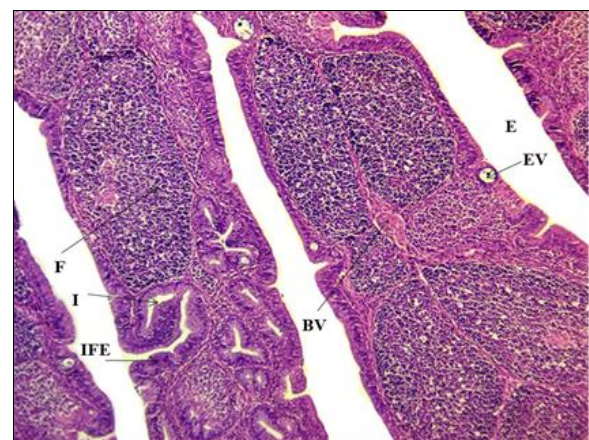


Fig 2: Photomicrograph of bursa of Fabricius in 60 days old Rajasree bird showing: Invaginations in to the follicular region and few vacuoles in epithelial region. Follicles (F), Invaginations (I), Epithelium (E), Epithelial Vacuoles (EV). (H&E-10X10)

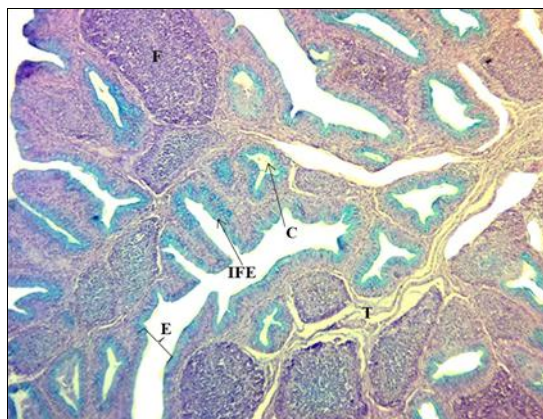


Fig 3: Photomicrograph of bursa of Fabricius in 135 days old Rajasree bird showing: Involuntary bursa with deformed follicles with Alcian blue positive in the cyst in majority of follicles (F), Epithelium (E), Inter follicular epithelium (IFE), Cyst (C), Trabeculae (T). (PAS-AB-10X10)

Table 1: Showing mean weight (gm), length (mm), width (mm), circumference (mm), number of plicae, plical length (μm), plical width (μm), number of follicles per plica, follicular diameter (μm), total serum immunoglobulins (mg/ml) of bursa of Fabricius in day old, 75 and 135 days of age

Age (Days)	MBW (gm) $\pm$ SE	MWBF (gm) $\pm$ SE	MLBF (mm) $\pm$ SE	MWBF (mm) $\pm$ SE	MCBF (mm) $\pm$ SE	MNP $\pm$ SE	PML (μm) $\pm$ SE	PMW (μm) $\pm$ SE	MNFP $\pm$ SE	MFD (μm) $\pm$ SE	MTSI (mg/ml) $\pm$ SE
Day old	34.0 $\pm$ 0.56	0.060 $\pm$ 0.05	5.12 $\pm$ 0.03	4.62 $\pm$ 0.01	11.3 $\pm$ 0.03	12.63 $\pm$ 0.21	573.53 $\pm$ 4.93	359.2 $\pm$ 6.36	171.21 $\pm$ 5.72	120.11 $\pm$ 6.00	5.15 $\pm$ 0.02
75	729.54 $\pm$ 2.73	0.740 $\pm$ 0.14	17.08 $\pm$ 0.92	13.38 $\pm$ 0.83	35.9 $\pm$ 0.09	20.51 $\pm$ 0.61	3783.9 $\pm$ 32.15	1892 $\pm$ 34.57	639.91 $\pm$ 5.55	362.66 $\pm$ 6.05	8.29 $\pm$ 0.18
135	1256.8 $\pm$ 64.32	0.510 $\pm$ 0.12	7.11 $\pm$ 0.15	5.90 $\pm$ 0.14	21.5 $\pm$ 0.03	13.16 $\pm$ 0.42	2185.7 $\pm$ 37.25	833.4 $\pm$ 7.14	145.83	134.55 $\pm$ 3.57	5.78 $\pm$ 0.11

MBW: Mean body weight, MWBF: Mean weight of bursa of Fabricius, MLBF: Mean length of bursa of Fabricius, MWBF: Mean width of bursa of Fabricius, MCBF: Mean circumference of bursa of Fabricius, MNP: Mean number of plicae, PML: Plical mean length, PMW: Plical mean width, MNFP: Mean number of follicles per plica, MFD: Mean follicular diameter, MTSI: Mean total serum immunoglobulins

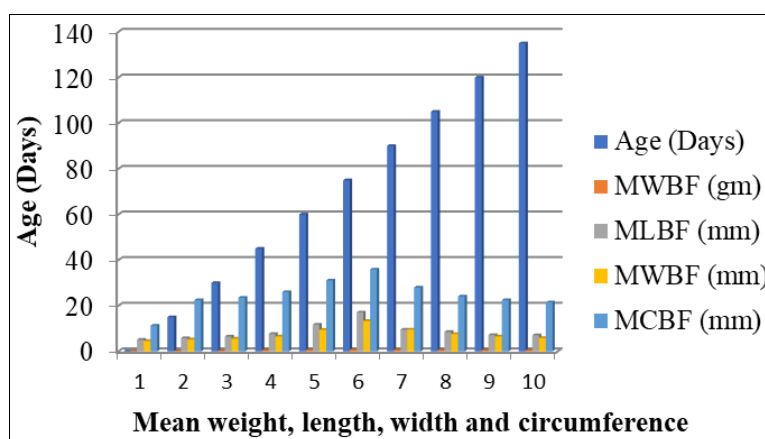


Fig 4: Showing mean weight (gm), length (mm), width (mm) and circumference (mm) of bursa of Fabricius in different age groups

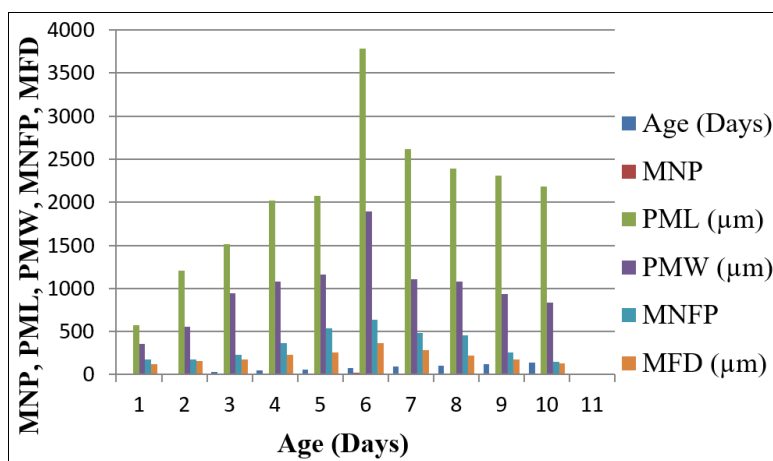


Fig 5: Showing mean number of plicae, plical mean length (μm), plical mean width (μm), mean number of follicles per plica and mean follicular diameter (μm) of bursa of Fabricius in different age groups

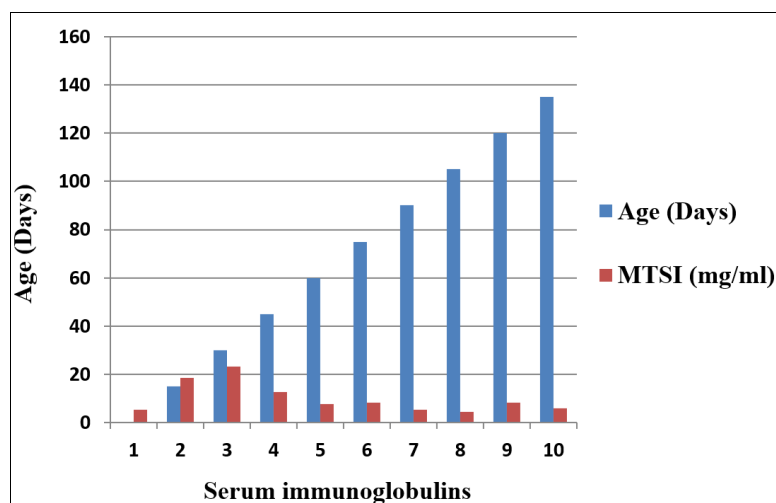


Fig 6: Showing mean total serum immunoglobulins (mg/ml) of bursa of fabricius in different age groups

Conclusion

Bursa of Fabricius was pear shaped and light pinkish in color. It was sac like and connected to the proctodeum with a stalk. The body and bursal mean weights, length, width, circumference and also the mean number of plicae, plical length and width were increased at 75 days and thereafter decreased at 135 days of age. The plical mucosa was lined by pseudostratified columnar epithelium. The lamina propria of plical mucosa occupied by several lymphoid follicles with surrounded and supported by connective tissue trabeculae made of collagen bundles and few reticular fibres and also noticed intense pyroninophilia. The mean number of follicles per plica and follicular diameter were increased at 75 days of age and thereafter decreased at 135 days of age due to the degeneration and involuntary process was noticed. PAS positive material noticed in distorted follicles with cyst. Strongly Alcian blue positive during involution. Moderate ACP activity, less ALP activity in advancement of age. The serum immunoglobulins highest at 30 days of age.

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