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**MORPHO-HISTOMETRIC EVALUATIONS OF PRE-PUBERTAL,
PUBERTAL AND POST PUBERTAL PROSTATE GLANDS OF
INDIGENOUS BULLS (*Bos indicus*) of BANGLADESH**

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ABSTRACT

The present study was aimed to evaluate the morpho-histometric parameters of prostate gland of indigenous bulls. Twenty eight bulls of three age groups were selected from the local market: the pre pubertal group (<1 year n=4), pubertal group ((1.5- 2.5 years, n=16) and post pubertal group or adult (>3 years, n=8). The prostate glands of bull consisted of corpus prostate and pars disseminata portion were distinguished. In all three groups of bull showed the corpus prostate was a band form. The both portions enclosed by the connective tissue capsule composed of mainly collagen and smooth muscle cells. Interlobular connective tissue decreased significantly ($p<0.01$) with the advancement of age. The secretory ends pieces were lined by simple cuboidal epithelium, simple low columnar epithelium and simple high columnar epithelium in prepubertal, pubertal and adult bulls respectively. The height of the lining epithelium increased with the advancement of age significantly ($p<0.01$). In ducts, height and number of the typical enfolding were increased with age. The ducts were lined by simple columnar or pseudostratified epithelium. The average diameters of the ducts and the height of the lining epithelium increased significantly ($p<0.01$) with the advancement of age. The findings revealed that the morpho-histometric changes were associated with the advancement of age.

Key Words: Prostate gland, Histometry, Puberty and Indigenous bull.

INTRODUCTION

Prostate gland is the most important accessory genital gland of male genital system which has major contribution in seminal plasma which plays important role in male reproduction. Its functional significance lies in neutralizing the seminal plasma and to initiate active movement of the ejaculated spermatozoa (Eurell &

Frappier, 2007; Hafeez 1987). Before eighteenth century (Hunter, 1792) first believed that the sound animal economy depends upon a perfect relationship between the testes and the accessory reproductive glands. Later, in the twentieth century used the prostatic acini to isolate, characterize and assay the androgens (Moore *et al.*, 1930). Histological and histochemical studies have been made on the prostate gland of cattle (Stallcup, 1969), buffalo (Sudhakar *et al.*, 1984, 1985), camel (Ali *et al.*, 1976), ram (Roy *et al.*, 1985), man (Copenhaver 1964; Bloom and

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Fawcett 1968) in the light microscope and in rat (Brandez and portella, 1960; Kanai and Tadao, 1961) by the electron microscope. The staining characteristics and the secretory granules have been studied in bovine male (Trotter 1959). Hypertrophy and cancer of the prostate are the two major problems facing both veterinary and human medicine. Abundant literatures are available regarding the anatomical features of the prostate gland of the most mammals, but the literature on the indigenous bulls of Bangladesh is lacking. Hence this work is taken to record the sequential changes in anatomical and histological features from the prepubertal stage to adult.

MATERIALS AND METHODS

The experiment was conducted in the laboratory of the Department of Anatomy and Histology, Sylhet Agricultural University, Bangladesh from July 2012 to June 2014. Twenty eight bulls of three age groups were selected from the local market before Muslim sacrifice on the year round: the prepubertal group (<1 year, n=4), pubertal group (1.5- 2.5 years, n=16) and adult or post pubertal group (>3 years, n=8) bearing 0, 2- 4 and 6 permanent incisor teeth, respectively. The age was determined based on tooth eruption patterns (Getty, 1975). The bulls were kept individually in the stall and fed with ad libitum balanced ration for 4 weeks. Immediately after slaughter, the gland was cut into the few pieces and part of each glandular sample were fixed in more than 20 times the volume of each in Bouin's fixatives for 24 hour. Dehydrated in series of submerging progressively more concentrated ethyl alcohol (70%, 80%, 90%, 95%, 100%). The tissues were kept for 3 hours in each grade of ethyl alcohol. When the tissues were dehydrated, cleared and infiltrated, they were placed in liquid paraffin for embedding, which were then allowed for hardening. This was achieved by cooling in the room temperature. The hardened tissue blocks containing the tissue samples were then sectioned at 6 μ m thickness using a rotary microtome (Microm GmbH, type HM 325, Germany) and the sections were floated in a water bath at 45°C flatten out on water. Then floated sections picked up carefully on clean slides, which had been smeared with Mayer's egg albumin and dried on the slide warmer. The good sections were chosen and from the different position of tissue about every 21st section so that the tissue examined were not in the same portion.

For standard Hematoxyline and Eosin staining, the sectioned tissues were deparaffinized and maintained the procedure stated by (Gridly, 1960). The stained tissue sections of prostate gland of bull were studied under compound binocular microscope (Abota Corporation, USA). The evaluation of prostate gland included thickness of interlobular connective tissue, diameter of the lumen, non-lumen acini and epithelial height of the secretory end pieces, diameter of the duct and epithelial height of the duct by image J software. The data generated

from this experiment were processed for further analysis. Mean value, standard error (SE) and correlations were estimated using the software Statistical Analysis System (SAS, 1998).

RESULTS

Macroscopic Anatomy

The prostate glands of bull consisted of two portions, corpus prostate or external portion and internal or pars disseminata portion. The two portions were distinguished, compact external portion or corpus prostate was observed dorsal of the pelvic urethra at the level of the colliculus seminalis. The pars disseminata portion was occupied along the entire wall of the pelvic urethra. The corpus prostate was a white colored band of tissue observed below a fibrous cord which joined the two vesicular glands at the junction of urinary bladder with pelvic urethra. The corpus prostate was found as a band in all three groups of bulls. Internal portion of the gland occurred in the urethra from its junction with the urinary bladder to the level of the ischial arch. It was surrounded by the urethral muscle which was thin dorsally, increased in thickness laterally and became thin ventrally. In the cranial portion of the pelvic urethra, the glandular tissue was mostly present in the dorsal wall only. It was relatively sparse in the lateral and ventral walls of the pelvic urethra. In the middle portion of the pelvic urethra, the distribution of the gland (in cross section) was almost uniform encircled the whole pelvic urethra except in the few area of very small ventral part. In the caudal segments of the pelvic urethra, the distribution of glandular elements was lack or less to the half of the pelvic urethra (in cross section) in all three groups of bull. In prepubertal, pubertal and post pubertal animals, the corpus prostate was frequently observed in various samples as a distinct white color band below the cord joining the two vesicular glands. In post pubertal animals, it was observed approximately higher in raised area of the gland with dorsal depressing line (Figure 1), whereas in prepubertal bull dorsal surface was convex.

Microscopic Anatomy

The corpus portion and disseminate portion of the prostate gland observed similar in histological structures except the histometric parameters. The corpus prostate was well demarcated with the pars disseminate prostate by the fibro muscular connective tissue septa. There was a capsule enclosed by the connective tissue composed of collagen, elastic, and reticular fibers along with smooth muscle cells. The smooth muscle fibers were observed more in the capsule, trabecule and interstitial connective tissue septa than the fibrous components. The capsules gives off trabeculae and the trabeculae gives off interlobular septa and the glandular parenchyma divided into lobe and lobules. The thickness of capsule and trabeculae were varies with the location of the gland.

Usually, capsular thickness was higher than the trabecular and finally interlobular septa lesser than the trabecular. The average interlobular septal thickness was represented in Table 1. The observation was interlobular connective tissue thickness decreased (Figure 2) significantly ($p < 0.01$) along with the advancement of age. The capsule composed of collagen and reticular connective tissue fibers and abundant smooth muscles arranged inner circularly and outer longitudinally. The collagen fibers were extended along the wall of blood vessels. Several autonomic ganglia, encapsulated nerve endings were observed in the connective tissue.

Secretory glandular end pieces

The secretory end pieces were lumined and non lumined distributed within the wall of the pelvic urethra and in the corpus portion of the prostate. In pre-pubertal bull, the mean diameter of lumined secretory end pieces was $32.37 \pm 1.46 \mu\text{m}$ whereas, those of non-lumined secretory end pieces was $27.87 \pm 2.28 \mu\text{m}$ (Table I). In pubertal and post pubertal bull, the average diameters of lumined acini were $43.00 \pm 2.27 \mu\text{m}$ and $56.75 \pm 2.79 \mu\text{m}$ respectively. Whereas, the average diameters of non lumined acini were $38.00 \pm 2.28 \mu\text{m}$ and $43.75 \pm 2.71 \mu\text{m}$ respectively. In prepubertal bull, the both types of secretory end pieces were lined by simple cuboidal epithelium with the rounded nucleus. In the pubertal bull, the both types of secretory end pieces were lined by simple low columnar epithelium with the large rounded nucleus. In the post pubertal bull, the both types of secretory end pieces were lined by simple high columnar epithelium with the oval or flattened basally placed nucleus (Figure 3). Due to the increased secretory granules, the nucleus gradually moved towards the basement membrane and become flattened. The lining epithelial cells of both pubertal and post pubertal glandular acini contained secretory granules, but the secretory granules were more in post pubertal

group. Secretory granules in the cells as well as secretion in the lumen increased along with the advancement of age. The height of the lining epithelium increased (Table 1) with the advancement of age significantly ($p < 0.01$). With the advancement of age, the number of lumined secretory end pieces increased over the non lumined secretory end pieces. The glandular alveoli were mainly mucous type, only a few serous alveoli placed in the peripheral part of the pars disseminate prostate.

Ducts

The ducts of the corpus prostate opened into numerous ducts in the pelvic urethra. The lobules of the pars disseminata contained ducts which opened into the pelvic urethra. Interlobular ducts or collecting sinuses had irregular lumen lined by pseudostratified columnar epithelium or simple columnar epithelium in prepubertal bulls. The average diameters of duct and epithelial height were represented in Table 1. The ducts were lined by multilayered stratified cuboidal at the developing enfolding in the lining epithelium. The ducts of the pubertal and post pubertal bulls contained folded mucosa were lined by simple columnar, pseudo stratified columnar or sometimes stratified cuboidal epithelium. The typical enfolding observed from prepubertal to adult bulls and height and number of the enfolding increased with age (Figure 4). The average diameters of the ducts and the height of the epithelium increased significantly ($p < 0.01$) with the advancement of age. The ducts of the disseminata prostate and the ducts of the corpus prostate had similar histological feature with histometric exception. The ducts were opened into the lumen of the urethra having transitional epithelium nearer the opening of all three groups of bulls. The lumen of the duct of prepubertal bulls showed clear, lumen of pubertal and post pubertal bulls showed secretion indicating full functional state.



Fig 2. Showing interlobular connective tissue septa (IC) decreases in diameters and size of the glandular unit increases along with the advancement of age of the bull. [Prepubertal (2a), pubertal (2b) and adult (2c). HE 10X]

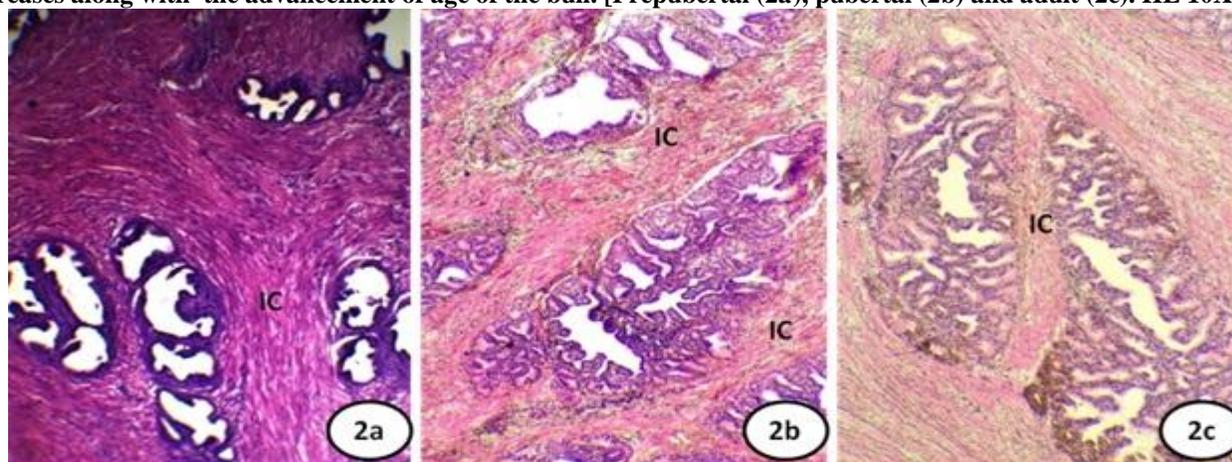


Fig 3. The secretory acini are lined by the simple low cuboidal epithelium with rounded nucleus simple columnar epithelium with large rounded nucleus, simple high columnar epithelium with oval or flattened basally placed nucleus in the lobules of prepubertal (3a), pubertal (3b) and adult (3c) prostate gland bull, HE 40X

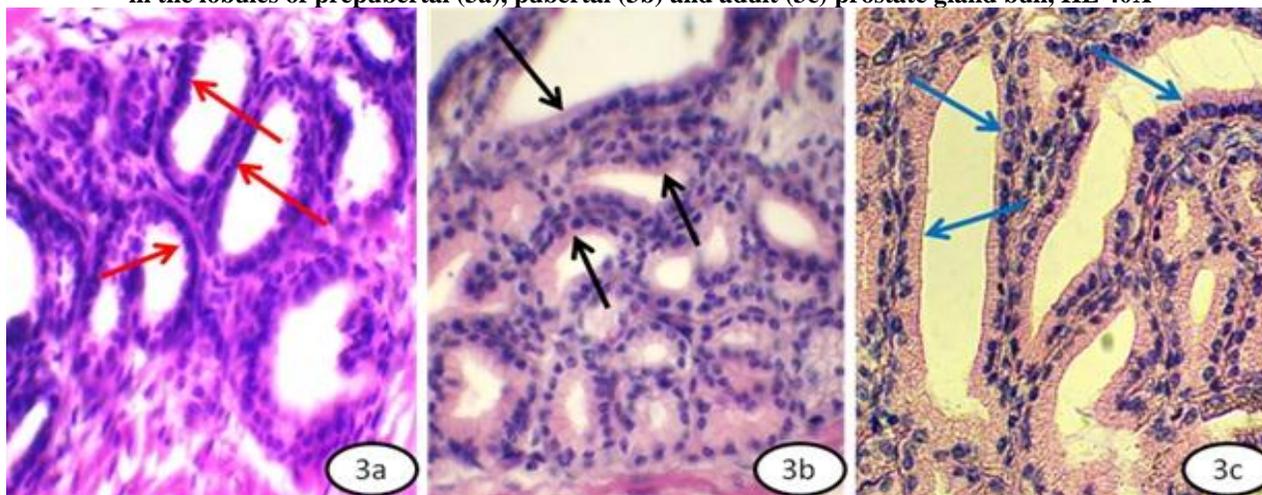


Fig 4. The duct of the glandular unit of the prostate gland with epithelial enfolding into the lumen (arrow head) increases in number with the advancement of age lined by columnar epithelium with different histometric appearance in bull. [Prepubertal (4a), pubertal (4b) and adult (4c). HE 40X]

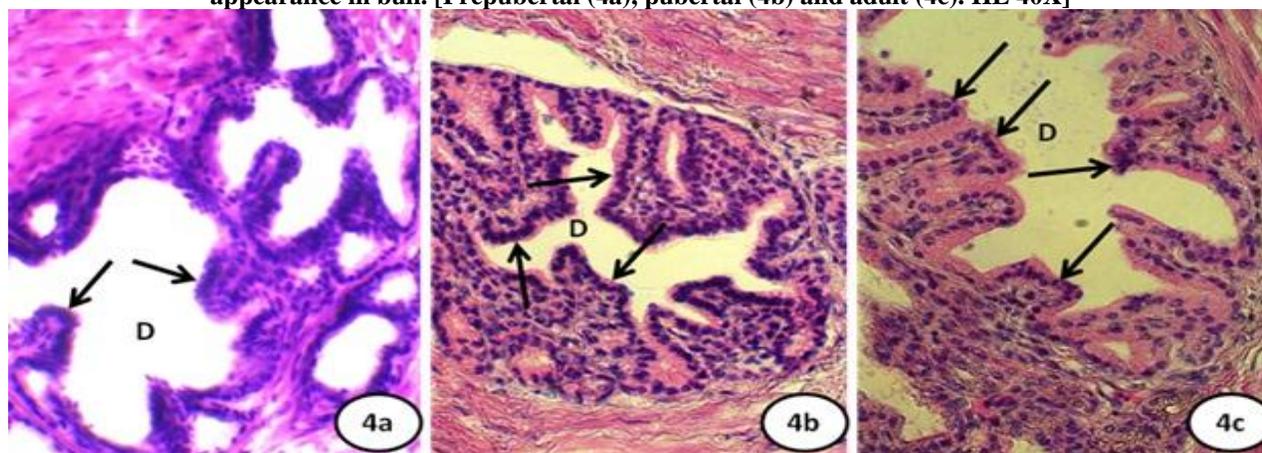


Table 1. Microscopic measurement (μm) of various component of prostate gland (Mean \pm SE) in indigenous bull of different age group (n=28)

S No.	Parameters (μm)	Pre pubertal	Pubertal	Post pubertal
1	Interlobular CT thickness	45.62 ^a \pm 4.10 ^a	29.62 ^b \pm 2.31	8.75 ^c \pm 1.03
2	Secretory end pieces			
	a) Diameter of luminated acini	32.37 ^c \pm 1.46	43.00 ^b \pm 2.27	56.75 ^a \pm 2.79
	b) Diameter of non luminated acini	27.87 ^b \pm 2.28	38.00 ^a \pm 2.28	43.75 ^{aa} \pm 2.71
	c) Epithelial height	11.01 ^b \pm 0.92	14.56 ^a \pm 0.63	16.62 ^{aa} \pm 0.59
3	Duct			
	a) Diameter of duct	27.87 ^c \pm 1.20	45.50 ^b \pm 2.13	51.75 ^a \pm 2.66
	b) Epithelial height	10.93 ^b \pm 0.67	13.82 ^a \pm 0.61	13.93 ^{aa} \pm 0.78

Means with different superscripts within each row for each parameter differ significantly.

DISCUSSION

Generally the prostate gland is present in all domestic animals. It is unpaired gland about the size of a chestnut located on the outside of the urethra just posterior to the excretory ducts of the vesicular gland (Frandsen and Spurgeon 1992, Dyce *et al.* 1987, and Nickel *et al.* 1973/17). In the present study, the prostate glands of bulls consisted of two portions, corpus prostate or external portion and internal or pars disseminata portion. The two portions were distinguished, compact external portion or corpus prostate was observed dorsal of the pelvic urethra at the level of the colliculus seminalis in the present study similar to the observation in bovine (Dellmann and Wrobel, 1976) and other domestic animals (Arthur 1975, Dellman and Brown 1987; Dyce *et al.* 2002). The corpus prostate was a white colored band of tissue observed below a fibrous cord which joined the two vesicular glands at the junction of urinary bladder with pelvic urethra. The corpus prostate was found as a band in all three groups of bulls. However, the corpus prostaticus does not occur in the small ruminants (Sisson, 1975; Dellmann & Wrobel, 1976). Further Kundu (1980) and Gupta & Singh (1982) and Gupta (1989) stated that. In prepubertal to post pubertal bulls, the corpus prostate was frequently observed in various samples as a distinct white color band below the cord joining the two vesicular glands. In post pubertal animals, it was observed approximately higher in raised area of the gland with dorsal depressing line, whereas in prepubertal bull dorsal surface was convex.

The pars disseminata portion was occupied along the entire wall of the pelvic urethra. In goats the internal portion of the gland occurred in the urethra from its junction with the urinary bladder to the level of the ischial arch (Archana *et al.*, 2011). In the present study, the cranial portion of the pelvic urethra, the glandular tissue was mostly present in the dorsal wall which is similar to the findings in buffalo (Sudhakar *et al.*, 1985). In the present study in the caudal segments of the pelvic urethra, the distribution of glandular elements was lack or less to the half of the pelvic urethra. On the other hand the glandular distribution was more in the lateral and ventral wall than the dorsal wall in case of buffalo (Sudhakar *et al.*, 1985).

Present study revealed in all three groups of bulls that the pars disseminata portion did not extend to the penile urethra. But in adult buffalo the extension of pars disseminata was beyond the ischial arch whereas in calves it was present only up to the middle third to the pelvic urethra (Chandramouly, 1971).

In our present study, the disseminate portion of the prostate gland occurred throughout the length of the pelvic urethra. It was enclosed by the connective tissue capsule composed of collagen, elastic, reticular fibers and smooth muscle cells. The fibro muscular capsule was surrounded by the skeletal muscle (*M. urethralis*). This observation was similar to that of buffalo bull (Sudhakar *et al.*, 1985). In bull (Kainer *et al.* 1969) and mature bovine bull (Trotter, 1959) the capsule and urethralis muscle was also found in the pars disseminate portion. The capsule composed of collagen and reticular connective tissue fibers and smooth muscles. The connective tissue trabeculae extending from the capsule entered into the parenchyma of gland forming interlobular connective tissue and divided the gland into lobes and lobules as stated in goats (Archana *et al.*, 2011) and buffalo (Sudhakar *et al.*, 1985). Histologically, they were similar in disseminate portion and corpus prostaticus except in histometric parameters.

The secretory end pieces were luminated and non luminated distributed within the wall of the pelvic urethra for pars disseminate and body of the corpus prostate which is similar to the findings in buffalo (Sudhakar *et al.*, 1985). Both types of secretory end pieces were lined by simple cuboidal epithelium with rounded nuclei in prepubertal bulls, simple low columnar epithelium with large rounded nuclei in pubertal bulls and simple high columnar epithelium with oval or flattened basally placed nuclei in the post pubertal bulls indicated the activity of the gland. Although, the lining epithelium of the pubertal and post pubertal bull contain secretory granules in the cytoplasm of the cells. In pre-pubertal age the glandular alveoli were observed mainly mucous type with only a few serous ones seen in the peripheral part of the pars disseminate prostate of the adult bulls. With the advancement of age, the number of luminated secretory end pieces increased over the non luminated secretory end pieces. In adult buffalo majority of the alveoli were serous only a few mucous

alveoli (Chandramouly, 1971). But in another observation in buffalo (Sudhakar *et al.*, 1985) noted that the characteristic serous and mucous components of the gland became evident at 4 years of age. The gland was predominantly serous in nature in the cranial portion of the pelvic urethra. In the middle region the mucous lobules were present under the capsule another sides of the pelvic urethra surrounding the serous lobules. In the caudal region the mucous lobules were predominant. These observations were not similar to the present study in bulls. This glandular distribution might be the species variation.

In pubertal bull the number of secretory end pieces increased in comparison to pre-pubertal animals. The luminated acini dominated over the non-luminated acini. This observation revealed that the size of the secretory acini increased as well as with their epithelial height also increased along with the advancement of age and reached maximum at adult stage. In buffalo the maximum measurement of prostate gland observed at 6 years of age and after that remain more or less static (Sudhakar *et al.*, 1985).

The lining epithelium of the duct was simple columnar or pseudostratified epithelium. The lumen of the prepubertal prostatic duct were very clear indicated no secretion or the gland not reaching in secretory stage. But in the lumen of the pubertal and post pubertal prostatic duct showed secretion indicated that the gland became secretory stage. The lining epithelium of the duct became enfolded from prepubertal to post pubertal stage. The number and height of the enfolded increased with the advancement of age. It may be due to the functional development of the ductal epithelium. On the other hand, the luminal diameters were observed decreasing with the increasing mucosal enfolded with an age. In buffalo the ducts of the pars disseminate prostate had wider lumen

having lined by stratified cuboidal epithelium with indistinct cell membranes (Sudhakar *et al.*, 1985). The ducts were opened into the lumen of the urethra having transitional epithelium nearer the opening in the present study of all three groups of bull. This observation was similar to that of in buffaloes (Chandramouly 1971) and bull (Kainer *et al.*, 1969; and Dellmann and Wrobel 1976).

CONCLUSION

Compact corpus prostate was observed dorsally and the pars disseminate portion was occupied along the entire wall of the pelvic urethra. The disseminate portion enclosed by the connective tissue capsule composed of collagen, elastic and reticular fibers and smooth muscle cells. Interlobular connective tissue decreased with the advancement of age. The secretory ends pieces were lined by simple cuboidal epithelium with the rounded nucleus, simple low columnar epithelium with the large rounded nucleus, simple high columnar epithelium with the oval or flattened basally placed nucleus in prepubertal, pubertal and adult bulls respectively. The height of the lining epithelium increased with the advancement of age. In ducts, height and number of the typical enfolded were increased with age. The average diameters of the ducts and the height of the epithelium increased with the advancement of age. The findings revealed that the gross and histometric changes were associated with the advancement of age.

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