DOI : 10.5958/2231-6736.2024.00022.8 *Vol 31 No 2, p 171-177*

HISTOLOGICAL STRUCTURE OF THE PROSTATE GLAND DURING ANTE AND POSTNATAL PERIOD OF ONTOGENESIS IN DROMEDARY CAMEL IN ALGERIA

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ABSTRACT

The current investigation was aimed to assess morpho-functional (histological) variations in the prostate of clinically healthy dromedaries during both ante- and postnatal phases of ontogeny. Thirty prostates from two distinct age cohorts (ante- and postnatal) were procured from the EL OUAD slaughterhouse (located in the eastern region of ALGERIA) for microscopic examination, with a focus on delineating the microscopic architecture of the prostate in this species. A range of staining methods, including Haematoxylin and Eosin, as well as silver nitrate impregnation, were employed to elucidate the normal histological framework and developmental trajectory of the prostate across different age groups. Subsequent statistical analysis was conducted to interpret the acquired data with significance set at $P<0.05$. Our findings revealed discernible patterns of structural and functional differentiation, specialisation and integration within the prostate gland parenchyma. Notable milestones in structural markers were identified, during the seventh month of the intrauterine phase and the eleventh and twelfth months of the foetal period. Epithelial cell secretory activity commenced during the animal's 36th month, while the formation of lobules and thickening of the capsule layer were observed during the terminal phase of gestation and the postnatal period, respectively. Concurrently, interlobular connective tissue expanded alongside epithelial cell formation and an increase in terminal capillary count, peaking during the pubertal phase of the animals. In summary, our results underscore the association between ante- and postnatal phases of ontogenesis and the initiation of puberty, shedding light on the intricate anatomical and histological dynamics of the dromedary prostate.

Key words: Anatomy, dromedary, histology, ontogenesis, prostate

The camel prostate, notably substantial and easily identifiable, comprises of two distinct elements: a compact and a diffuse segment and divides into three zones: central, peripheral and transition (Mahmud *et al,* 2016). Together, these segments form an L-shaped structure positioned dorsally to the pelvic urethra. Shaaeldin and Tingari (2019) found that the camel prostate is oval-shaped, encircling the proximal part of the urethra at the bladder neck, with ducts opening around the entire urethral circumference. Dorsally, the prostate splits into two lobes separated by a septum.

Ultrastructure of the prostate of camel has been studied previously (Ali *et al,* 1976; Soliman *et al,* 2010), illustrating it as a complex of branched tubule-alveolar glands whose ducts open into the prostatic urethra. Moreover, it is enveloped by a fibro-elastic capsule rich in smooth muscle cells, with septa from this capsule effectively segmenting the gland into lobes, albeit indistinct in adult male camels, a finding echoed in research on gazelles by Mohammed *et al* (2007).

While existing literature elucidates the anatomy, topography (Degen and Lee, 1982) and histological structure of the camel's prostate (Luo *et al,* 2016), there is a paucity of studies examining its prenatal and postnatal developmental phases. Building upon this gap, Biancardi *et al* (2017) emphasised the importance of morphological developmental analysis, linking it to systems theory and organ tissue regional concepts. Consequently, the aim of this study was to conduct a comprehensive investigation into the histological changes in the prostate gland of camel from prenatal to postnatal stages, elucidating critical developmental periods and their relationship with the onset of puberty.

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Materials and Methods

Ethical approval

The sampling and data collection in this study was performed in accordance to the ethical recommendations of the Algerian government.

The present research was conducted on camels from a slaughterhouse in El-Oued -a province in the southeast of Algeria- and spanned from July 2021 to May 2023. Thirty prostates were obtained from clinically healthy and unvaccinated male foetuses aged between 07 to 13 months, neonatally. They were taken from animals aged 12, 24, 36, 48 and 60 months postnatally, with foetal age verified through femur length measurement. The methodological framework adhered to in this study followed the protocols outlined by Kadim *et al* (2008) and Saber *et al* (2020).

The investigations took place at the Laboratoire des Sciences et Techniques du Vivant, Institut d'Agriculture et des Sciences Vétérinaires, Université de Souk Ahras, Algeria. Specimens selected to elucidate morpho-functional changes in prostates concerning age were preserved using a 10% neutral buffered formalin solution. Subsequently, some fixed organ fragments were embedded in paraffin to facilitate the creation of thin sections measuring 3-5 microns thick.

For histological examination of the prostate's structure, staining with haematoxylin and eosin, the protocols of Cardiff *et al* (2014) and Abrahamsson *et al* (1989) were employed. Additionally, frozen sections of prostate tissue underwent staining with silver nitrate impregnation to highlight proteins prominently expressed on silicone.

Stained histological sections were examined and morphometry and microphotography were conducted using a MOTIC DM-52 light microscope, mono, digital (eyepiece x4, objective x10/0.25, x40/0.65).

Statistical analysis was carried out using standard "statist SF" software, with data analysis performed using SPSS Version 26.0.2019 (SPSS, Armonk, NY: IBM Corp). Data were expressed as means ± SD (standard deviation) and subjected to statistical analysis using Duncan's test, with statistical significance set at $P<0.05$.

Results

Histological analysis of neonatal camel prostates revealed a thin prostatic capsule that was mainly composed of collagen and which is surrounded by isolated cells and isolated bundles of internal smooth muscle. Each tubuloalveolar gland was encapsulated in fibrous connective tissue that contained delicate bundles of smooth muscle fibres. This glandular organisation takes the form of lobes subdivided into lobules by thin collagenous partitions, with scattered smooth muscle cells in this arrangement (Fig 1).

Examination of the thickness of the prostatic capsule revealed a significant variation ($P < 0.01$) with the age of the subjects studied. At the 7th month of gestation, capsule thickness reached 54.32 ± 0.67 µm, increasing significantly to 76.32 ± 1.96 µm at the end of gestation. During the postnatal period in the spring, the thickness of the prostatic capsule in newborns measured 85.1 ± 1.04 µm ($P <0.05$). At the age of 60 months, this measurement reached a value of 98.6 ± 1.27 µm.

Concerning the secretory ducts, the application of silver nitrate to the histological sections revealed three distinct colours, indicating different cellular structures. At low magnification, the presence of multiple lumens suggests the presence of grouped empty spaces, with two forms of these lumens observed in foetal prostates.

The intra-lobular ducts were lined with tall columnar secretory cells The interlobular ducts and the main excretory ducts were lined with stratified transitional epithelium visible in histological sections in neonatal prostates. Light brown staining indicated the presence of reticular tissue which was concentrated around urethral areas rich in this type of tissue.

Moreover, the intensity of the proliferative process in the connective tissue of the prostate varied, creating a distinctive architecture of the acini, leading to active proliferation of the mucous glands, a feature only observed in animals aged two years and over, while the acinar cells of the intercalary glands showed less active proliferation, the proliferative activity of the acinar cells of the main glands was lower during the prenatal period. These results justify the rejection of the term "prostatic activity" (Fig 2).

At higher magnification, the glandular epithelium appeared with a white lumen and a pale eosinophilic layer bordering the prostate glands. Papillary projections, sometimes with a connective or vascular axis, bordered the prostate glands and were seen in this lumen. Although this feature was absent in the prostates of camel foetuses, it was visible in those of newborns, particularly the older ones (Fig 3).

The development of true acini in the glandular epithelium is exceptionally rare, beginning only from the age of 24 months according to the researcher's observations.

The secretory endings revealed that large fibromuscular septa originate in the muscle bundles, distinctly delimiting the lobules of the gland, particularly in neonates. The branches of these septa, also fibromuscular, give rise to intra-lobular collecting ducts, lined with a two-layered pseudostratified epithelium, at the heart of each lobule. Numerous small ducts branch into tubular outgrowths which terminate in tubular endings.

In fact, it was noted that myoepithelial cells were absent and these septa, which were seen as detached filaments in the lobules, form a delicate intra-lobular stroma surrounding the alveoli that was constituting the secretory endings. These endings, aligned in the active secretory gland, were characterised by simple cylindrical cells with compressed nuclei. Each alveolus was surrounded by a capillary network within a sparse framework of loose connective fibres. There were variations in the stages of secretion. While some alveoli have glandular cells with dark pink cytoplasm, others had less dilated cytoplasm due to secretion.

The body of the prostate remaines inactive in late-gestation foetuses with groups of undilated intra-lobular ducts without tubular secretory endings. It was also revealed from the sections of the pelvic part of the urethra that the transitional epithelium was similar to that of the body.

As far as the glandular epithelium is concerned, from the 7th month of gestation, the prostate takes the form of solid cell buds on the urethral epithelium. By the 8th month, some tubules are ducted, but most remain without a lumen. At this stage, the prostate is lined with a morphologically identical simple epithelium, frequently expressed by large pyramidal cells with flattened nuclei at the base. These characteristics persist in the prostate of the 9-month-old embryo.

The excretory ducts were initially lined with a two-layer epithelium, replaced by a transitional epithelium near the point of entry into the urethra. These changes occurred at a stage when the appearance of the prostate approaches that of a normal adult camel. The number of glandular tubules increases, accompanied with an increase in intertubular connective tissue.

Examination of the glandular epithelium's height of neonatal prostate glands reveals significant fluctuations throughout the postnatal period, with a minimum height at 12 and 60 months. Between 24 and 48 months, the height of the glandular epithelium was relatively uniform in the central, peripheral and transitional zones. From 36 months, the epithelium in the central zone became significantly higher than that in the other two zones. At 48 months, the thickness of the lobule mucosa in postnatal study animals differed slightly by about 2 to 3 µm. The epithelial height of all three layers of the prostate in 48-month-old animals was greater, suggesting a decrease in epitheliocyte height. In the 60-month-old animals, the surface area of the lobule was significantly increased, reaching around 1.5 times its initial size.

Concerning the lumen and number of glands, lobular formations of different sizes were observed in the prostatic part of 12-month-old foetuses. In newborns aged 60 months and over, a significant increase in prostate volume was observed, accompanied with a dilatation of the terminal sections of the prostate. The septa became thin and narrow. As such, the smooth muscle fibres became thinner, leading to a visible reduction in the thickness of the interlobular septa and a slight increase in the height of the epithelium of the terminal sections of the prostate was seen.

Observation of the interlobular connective tissue in foetal prostates showd that it appeared around the 9th month of gestation, with an initial thickness of 26.61 ± 3.21 µm, increasing to 45.41 ± 3.15 µm at the end of gestation. The height of the epithelium of the secretory endings in the foetal prostates reached 4.57 ± 2.45 µm at the 9th month of gestation and increased to 16.58 ± 2.67 µm at the end of gestation.

The diameter of the secretory ducts reached 13.57 ± 2.07 µm at the 9th month of gestation and doubled to reach 16.58 ± 2.67 µm at the end of gestation. It doubled to reach 26.12 ± 3.47 µm at the end of gestation, with the thickness of the epithelium increasing by 4.68 ± 3.47 µm to 12.35 ± 3.78 µm. The diameter of the illuminated acini was 21.65 ± 3.87 µm at the 9th month of gestation and increased to 38.25 ± 2.74 µm at the end of gestation. The diameter of the non-illuminated acini was 14.57 ± 2.36 µm at the 9th month of gestation and increased to 32.47 ± 2.74 µm at the end of gestation. It was also noted that the lumen of the glands decreases significantly from 12 to 36 months and remained relatively constant up to 60 months. This trend, correlated with previous data in the tables on glandular content at different ages, suggests an increase in the number of glands over time.

Examination of the number of glands in the prostates of camels at different stages of development revealed their presence after birth, with a slight increase at two months, followed by a significant decrease towards the third year of life, to reach minimum values (Table 1).

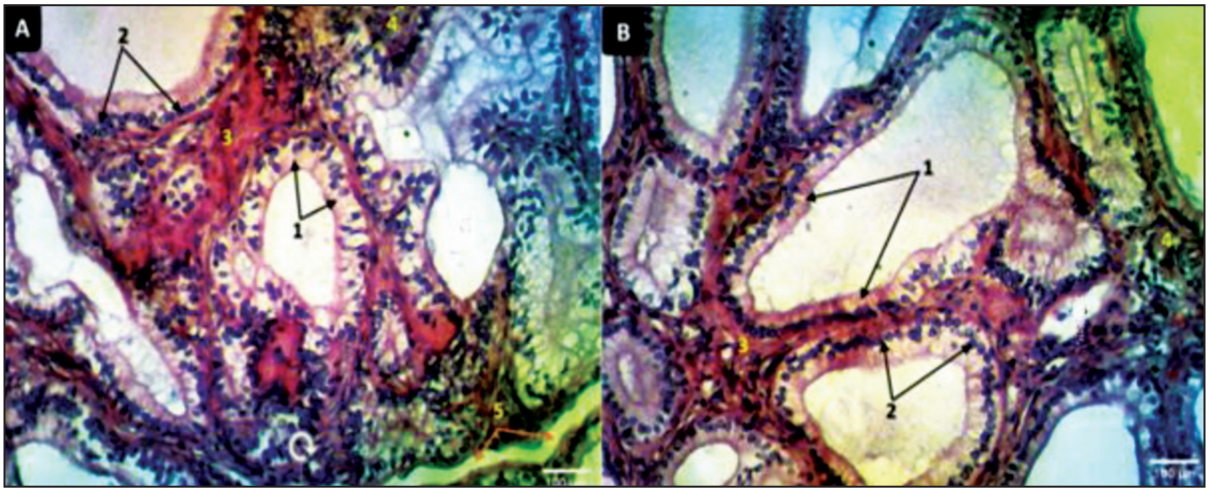

Fig 1. Histological section of the dromedary prostate gland in the postnatal period, **A:** 12th month after birth, **B:** 60th month after birth, stained with Hematoxylin and Eosin; 1-acinar cells; 2- connective tissue; 3-intraluminal secretions; 4-stroma; 5-duct. X 100.

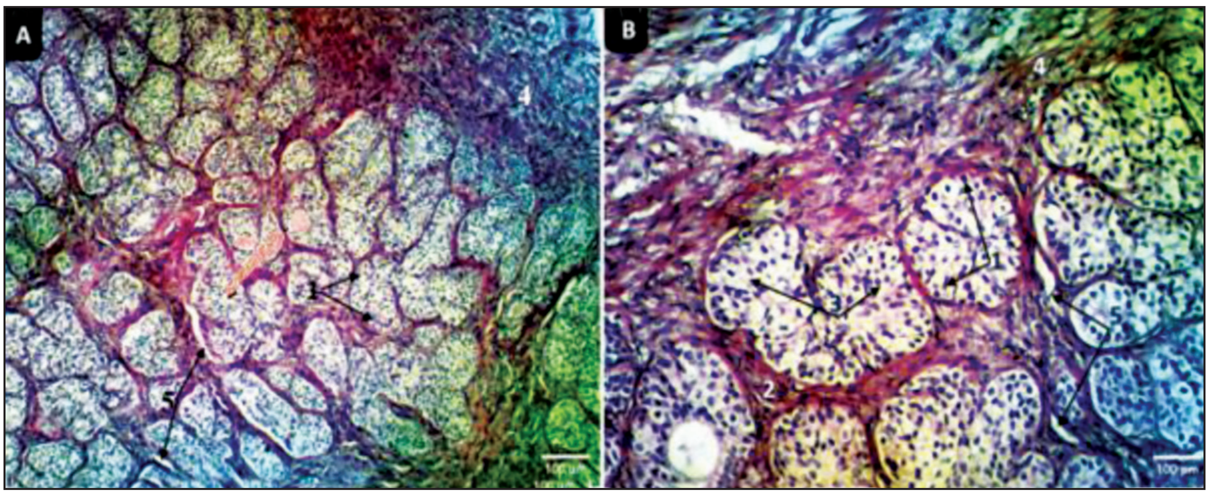

Fig 2. Histological section of the dromedary prostate gland during the antenatal period, **A:** 7th month of gestation, **B:** 11thh month of gestation, Hematoxylin and Eosin staining; 1-acinar cells; 2-connective tissue; 3-intraluminal secretions; 4-stroma; 5-duct. X100.

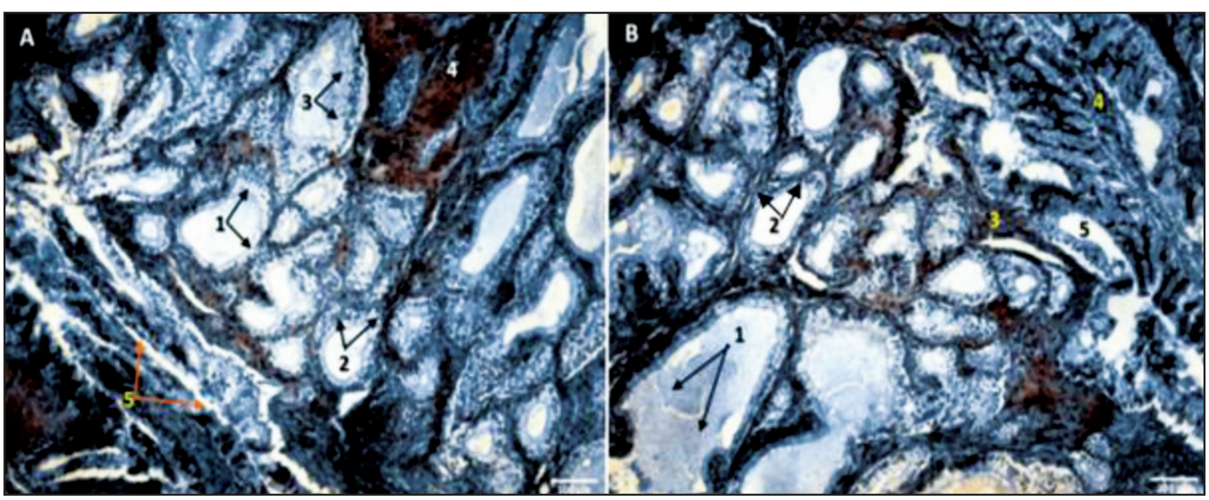

Fig 3. Histological section of the dromedary prostate gland during the antenatal period, **A:** 7th month of gestation, **B:** 11th month of gestation, silver nitrate staining; 1-acinar cells; 2-connective tissue;3-intraluminal secretions; 4-stroma; 5-duct. X 100.

Discussion

The histological description of the developing prostate during the prenatal and postnatal period in animals has been studied extensively. The present research focuses on the analysis of prostate development in the dromedary. As an organ, the prostate is surrounded by a fibromuscular capsule. Previous studies on boars (Sakairi *et al,* 2003) have reported that the gland is covered externally by striated urethral muscles and lined internally by an intermittent layer of smooth muscle. In contrast, studies on bovines (Abou-Elmagd and Wrobel, 1989) described the capsule surrounding the gland as a dense, irregular connective tissue containing numerous smooth muscle cells and striated muscles. Our research findings are consistent with those of Gibson *et al* (2013), who observed that the prostate surrounds the urethra at its origin from the urinary bladder. This finding may be related to the need to produce a large amount of seminal fluid to ensure reproduction under dry conditions. The smooth muscle cells present in the capsule of the prostate could help to control the flow of seminal fluid towards the urethra.

Previous research on sheep by Lopes *et al* (2011) showed that the interlobular ducts were characterised by a low cylindrical epithelium lining. In contrast, the gland ducts in the current study were found opened into the urethral canal, with the excretory ducts exhibiting a single cylindrical epithelium lining. In addition, we observed a transition in the epithelial composition of the prostate, where single columnar epithelium becomes transitional epithelium in the terminal segments of the ducts. This may be related to the difference in environmental conditions, prostate epithelial cells may be more sensitive to desiccation in a desert climate. In this case, the presence of transitional epithelium in the terminal segments of the prostate ducts could help to protect the epithelial cells from desiccation.

Conversely, Hafez and Hafez (2001) reported the presence of predominantly serous glands with some mucosal acini in the disseminated pars of camels, which is also consistent with current observations, with columnar pseudostratified epithelium lining the disseminated pars. The solid and luminal secretory ends of the pars disseminata remained undifferentiated until three years of age. In this research, similar results were found at this stage of foetal development as the prostatic epithelium showed distinctive features. The results also indicated that the formation of the main definitive structural features of the prostates as peripheral reproductive organs occur in camels during the foetal period of stepwise ontogeny. Similarly, results from Xia *et al* (1990) mentioned the same suggestions for other mammals. However, the prostatic body does not appear in pigs in textbook descriptions. In that regard, Lossi *et al* (2016) reported that the disseminated Pars appears in the early postnatal period, but a narrower band of white glandular tissue with a glandular mass was found in camels. It is reported that this band has a condensed structure and is deeply buried in the muscle tissue of the urethra during the foetal period of the animal with a progressive increase in the degree of structure and differentiation of the different organ components. Consequently, the lobular structure of mammalian prostates is formed during the foetal period and the base of each component of these organs is a distinct gland. The number of which in some prostates is genetically determined (Angelsen *et al,* 1997). The body of the prostate is a glandular mass surrounded by a distinct fibrous capsule and exposed by the urethral muscle. The fibroreticular capsule of the gland also contains abundant smooth muscle cells. Similar findings have also been described by the authors and this remark has been found in prostates of mouse (Oliveira *et al,* 2016).

While interlobular connective tissue decreases in quantity with age, the remaining glandular

Table 1. Morphometric parameters of the prostate in a mature one-humped camel (x ± SD, n =8).

Months	Interlobular connective tissue thickness	Secretory tips			Secretory ducts	
		Diameter of illuminated acini	Diameter of non-illuminate acini	Height of epithelium	Duct diameter	Height of epithelium
Antenatal period						
9-10	26,61±3,21	21,65±3,87	14,57±2,36	4,57±2,45	13,57±2,07	4,68±3,14
11-12	45,41±3,15	38,25±2,74	32,47±3,74	16,58±2,67	26,12±3,47	12,35±3,78
Postnatal period						
0-12	33,45±2,54	36,14±3,18	42,68±2,24	15,57±2,47	43,14±3,14	11,24±2,11
48-60	11,34±3,41	49,54±2,54	45,73±3,22	21,24±3,28	49,47±3,14	18.24±3,87

SD: Standard Deviation of the mean

components increase. This result may be linked to the increased production of male sex hormones androgens, which stimulate the growth and differentiation of the glandular cells of the prostate. It should be noted that the main excretory duct of the gland is lined with stratified cuboid epithelium. In comparison, the epithelium in the prostate of rams is characterised by a simple cuboid to cylindrical structure with the presence of some basal cells (Odabaş and Kanter, 2008). This is probably due to lower androgen levels in this species. Rams also have a shorter reproductive cycle than dromedaries, which may also contribute to the difference in epithelium. The ducts were more abundantly branched with age and terminated in more luminal secretory ends. Initially, they were mainly mucous, but serous components were added by differentiation of the non-luminous secretory tips later in foetuses and neonates as confirmed by Krill *et al* (1999). Rodger and Hughes (1973) reported the presence of numerous tubular compounds in the prostate. Thus, the free surface of the glandular epithelium is clearly delineated and the epithelium is bilayered. Again, the proportion of glandular tissue to connective tissue is roughly the same in the late foetus. Nevertheless, the epithelial cells are larger, although still slightly smaller than in camel foetuses. In this study, we noted that whereas the prostatic ducts start to appear from the 7th month of gestation, they develop and come into place in cattle between 1 and 15 days after birth and 2 weeks after birth according to Groot and Biolatti (2004).

Studies performed on Bengal goat prostates by Gofur (2019) showed that only luminous acini were considered for micrometry, although some non-luminous acini were observed in the prostatic body of day-old goats. In contrast, the diameter of the acini found with a value of 44.98a±1.31 µm and that of our results, maximum sums of 49.54±2.54 µm for luminous acini and 45.73±3.22 µm for non-luminous acini of 48-60 month old neonates, the height of the epithelial cells found in the same breed was 12.89±0.28 µm, whereas in our research, we found a sum of 18.24±3.87 µm in neonates aged 48-60 months. We conclude that these indices vary according to the animal species by its body structures and its state of evolution.

The most important development of the prostate parenchyma growth took place during the prepubertal phase and spanned from birth to 18 months. These results are consistent with the observations made by Higgins and Gosling (1989) regarding the growth of parenchymal components in human newborns, which persisted until the age of 12 months before reaching a static phase until 60 months.

Conclusion

Histological differentiation and complete formation of prostatic tissue have been observed in the dromedary throughout its life, from the ante-natal period to adulthood, in three main phases:

_Embryonic phase (7th month of gestation): formation of the first epithelial cords and glandular tubules, lined with an undifferentiated stratified epithelium.

_ Neonatal phase (8th month-end of gestation): development of the parietal and caudal parts of the gland. The parenchyma is represented by epithelial cords derived from the mucosa of the urogenital canal.

_ Postnatal phase (1st-3 years): maturation of the gland, with increase in size, glandular and connective tissue and decrease in muscular tissue.

Acknowledgement

The authors would thank the stuff of the laboratory of histology in the Institute of Agriculture and Veterinary Sciences, University of Souk Ahras, Algeria for their comments that greatly improved the manuscript.

Competing interest statement

The authors state that there is no competing interest in this research.

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