Journal of Anatomical Sciences
Email: anatomicaljournal@gmail.com

J. Anat Sci 13(2)

Histological Developmental Horizons in the Pre-Natal Development of the Male Reproductive Organs of the One-Humped Camel *(Camelus dromedarius)*

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ABSTRACT

Dromedary or one-humped camel (*Camelus dromedarius*) belongs to the genus *Camelus*, and camel research is on the increase due to its importance as model for scientist in various discipline of biological science including reproduction. Here we examined the histological developmental horizons of the reproductive tract and accessory organs of fetal male *Camelus dromedarius* across three trimesters. A total number of fifteen (15) *Camelus dromedarius* male fetuses were collected from pregnant uteri (five in each trimester) and used for this study. Our results revealed an undifferentiated testis at the first trimester, developing sex cords appeared at the second trimester and marked tubular sex cords at the third trimester. The epididymis showed ducts lined with pseudostratified epithelium at the first trimester, enlarged ducts at the second trimester and larger ducts at the third trimester. The Vas deferens were frail at the first trimester, presented distinct muscular layers with a lumen at the second trimester and was lined by pseudostratified columnar at the third trimester. The prostate and bulbourethral glands were glandular. The mucosa of the penis was undifferentiated at the first trimester, a lumen with transitional epithelium was seen at the second trimester alongside with incomplete ectodermal lamina between the penis and preputial covering, which became wider at the third trimester. Our results provide new information on the histological features of the fetal reproductive organs in male *Camelus dromedarius*, an insight to reproductive management in this specie of mammal.

Keywords: *Camelus dromedarius*, fetal development, trimesters, male reproductive organs, histology

INTRODUCTION

Dromedary or one-humped camel (*Camelus dromedarius*) is one of the two species belonging to the genus *Camelus;* the other being the Bactrian or two-humped camel (*Camelus bactrianus*)[1]. The *Camelus dromedarius* has from decades been a very useful animal to humans, and more recently been described as a strategic animal for the exploitation of the desert and abhorrent arid and semiarid lands[2]. Reproductive efficiency of *Camelus dromedarius* is now receiving attention, targeted to better performance of this class of animal population[1, 3-6]. Precious report has shown that the reproductive performance of dromedary camels is relatively lower than in other domestic animals[1, 7], due mainly to its many complex genetic and natural constraints[8]. Interestingly, El-Hassanein[9], attributed this lower reproductive performance to the short breeding season and limited male libido of this specie. Of note, In Africa, the dromedary is reared mainly by the nomadic pastoralists, and forms a very important part of their livelihood and cardinal point in their economy, contributing significantly to the food security and sustainable development[10] hence the importance of their reproductive performance.

Although there is a lack of precise, detailed information about reproduction in the *Camelus dromedarius,* the male dromedary has been reported to have low mating efficiency throughout their reproductive lives[11]. Interestingly, most studies on sexual development of this species is post embryonic, and indicated that the male attains puberty at around three and a half to five ($3^1/_2$-5) years of age[11-15]. There is dwarf of information on the histological feature of embryonic developmental horizon of the male reproductive organs of this *Camelus dromedarius,* hence the aim of the present study. The knowledge on these histological features could be important in identifying critical periods of development of reproductive organs. The development stages of the male reproductive organ is of special interest in this study due to its role in the formation of gamete necessary for fertilization of the female ova resulting in progeny and continuity of life[16-18].

MATERIALS AND METHODS

Samples Collection, Sexing and Aging of Fetuses: A total number of fifteen (15) *Camelus dromedarius* male fetuses were collected from pregnant uteri and used for this study. All fetuses used were grossly normal and free from pathological lesions. The male fetuses were collected as fetal wastages from Kano Municipal Abattoir, Kano State. The pregnant uteri were carefully dissected out using surgical blades, scissors and forceps to display the fetuses. Fetuses were sexed using modified method of Ranjbar *et al*. [19]. In brief, the uteri were quickly dissected opened and fetuses were detached from fetal membranes. The male fetuses were then identified by the presence of an anogenital ridge spanning the length from the anus to the penis. The fetuses were then aged using the formula; X = 2.5 (Y + 21), where X = the developmental age in days; Y = crown – anus length measured in centimeters, and grouped into trimesters for histological developmental horizons description as described by El-Wishy *et al*. [20] and modified by Richardson's *et al*. [21] and Sonfada *et al*. [22]. The aged fetuses were then assigned five (5) each to three (first, second and third) trimester periods of *Camelus dromedarius* pregnancy.

Following aging, fetuses were dissected using the modified method of Habel[23] for collection of tissues. Briefly, the fetal skin was reflected from the abdominal wall from the preputial orifice, around the placenta and to the xiphoid region, and careful dissection was done to exposed the placenta. The placenta was carefully detached alongside with the bladder and kidneys for easy access to the reproductive organs. The testes, epididymis, vas deferens, ampulla, prostate and bulbourethral glands and penis were quickly dissected out and bisected into

two halves and immersion fixed in 4% neutral buffered formalin for histological examination.

Histological Examination of the Specimen: The protocol for histological preparations was according to the methods of Usende *et al.,*[24] and Igwenagu *et al.,*[25]. In brief, about 4–6 mm of testes epididymis, vas deferens, ampulla, prostate and bulbourethral glands and penis tissues were collected. The tissues were dehydrated in increasing concentrations of ethanol, cleared in xylene and embedded in paraffin. Sections of 5-6µm thickness were cut and serially collected on coated slides for staining with Haematoxylin and Eosin (H&E) and Period Acid Schiff (PAS) for light microscopy. Stained slides were viewed and age dependent histological descriptions were carried out with Amscope 40X 2500X Digital binocular photomicroscope fitted with a 10MP microscope digital camera.

RESULTS

Testes: In the 1st trimester of development, the fetal testis of *Camelus dromedarius* were undifferentiated histologically. However, mesenchyme cells were seen to line the epithelial layer (Fig. 1 A and B). At 2nd trimester, the testis had fibroblast cells and strings of developing sex cords with cells at the periphery (Fig.2 A).

The 3rd trimester testis of *Camelus dromedarius* were fully differentiated and had marked tubular and circular sex cords surrounded by numerous interstitial cells and collagen fibers. Most developing tubular sex cords were seen to be within the testicular cortex, while those at the periphery towards the albuginea are curved in shape (Fig.3 B and C). The sex cords were observed to have two types of cells, the small mesenchyme supporting cells and the large gonocytes. The small mesenchyme supporting cells were distinctly located at the border line of the basement membrane of each sex cord. These nuclei of these supporting cells appeared distinctly dark with PAS staining. The large gonocytes on the other hand have a pale nuclei and cytoplasm. Also, unique to the cells (the gonocytes) was their definite outline with weakly eosinophilic cytoplasm. Concerning the *tunica albuginea*, no distinct structure was seen at 1st trimester but in the 2nd trimester a thin *tunica albuginea* had developed. In the 3rd trimester, the thickness of the tunica albuginea had increased tremendously, and deeply to it was the *tunica vasculosa* or vascular layer developed with numerous fibroblasts and collagen fibres (noticed with PAS x400).

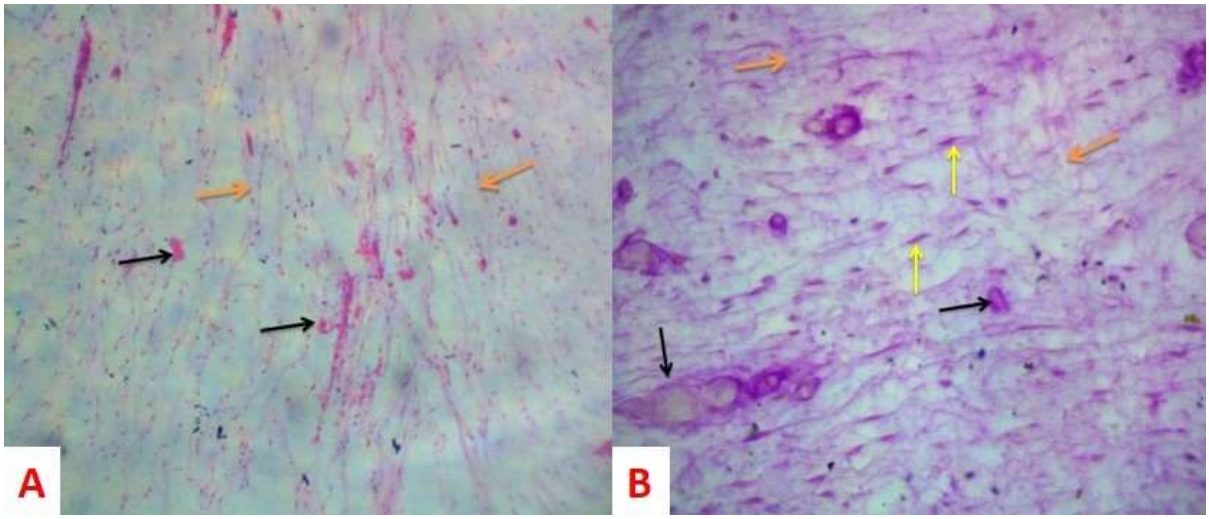

Figure 1a: Photomicrograph in cross section of 1st trimester *Camelus dromedarius* testes indicating blood vessels (black arrows), connective tissue stroma (orange arrow) H & E x40. B: Photomicrograph in cross section of 1st trimester *Camelus dromedarius* testes indicating blood vessels (black arrows), connective tissue stroma (orange arrow) and fibroblast (yellow arrow), PAS x40

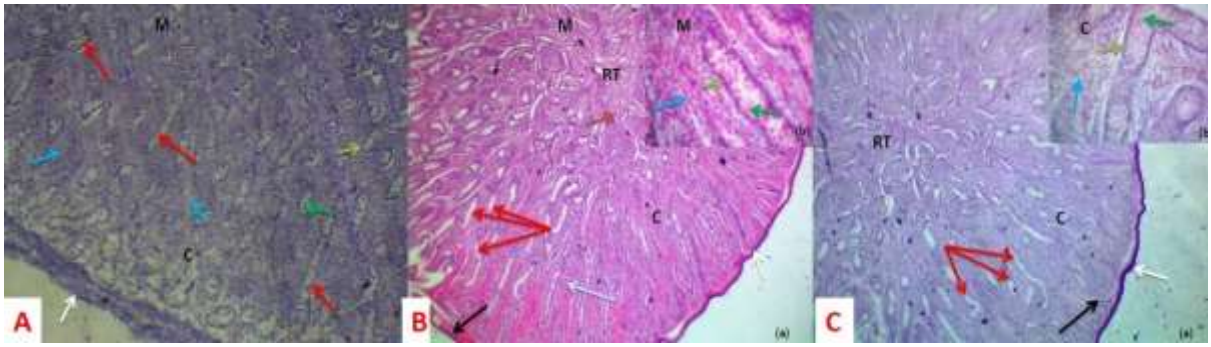

Figure 2a: Photomicrograph in cross section of 2nd trimester *Camelus dromedarius* testes indicating sex cords (red arrow), supporting cell (light green arrows), connective tissue stroma (blue arrow), gonocyte (dark green arrow), Tunica albuginea (white arrow) and C-cortex (a) H & E x100

B: Photomicrograph in cross section of 3rd trimester *Camelus dromedarius* testes indicating sex cords (red arrow) , supporting cell (light green arrows), connective tissue stroma (blue arrow) , gonocyte (dark green arrow) , C-cortex, RT- rete testes (a) H & E x40 (b) H & E x400

C: Photomicrograph in cross section of 3rd trimester *Camelus dromedarius* testes indicating sex cords (red arrow) , supporting cell (light green arrows), connective tissue stroma (blue arrow) , gonocyte (dark green arrow) , C-cortex, RT- rete testes (a) PAS x40 (b) PAS x400

Epididymis: Like the testes, the fetal epididymis of *Camelus dromedarius* was undifferentiated at the 1st trimester (Fig.3 A). At the 2nd trimester, the epididymal ducts were observed to comprise of a lumen enveloped with a *tunica albuginea*. The epididymal capsule at this stage of development was thick with inner vascular layer containing numerous blood vessels. In the 3rd trimester, the epididymis of *Camelus dromedarius* was seen to have connective tissue emanating from the albuginea to divide the epididymal parenchyma into lobules with numerous ducts (of notes, ducts were seen also at the 1st and 2nd trimesters). The ducts were lined with pseudostratified epithelium. Interestingly, the sizes of these ducts varied across the trimesters showing developmental progress as the fetus age. Numerous undifferentiating smooth muscle cells, collagen fibers, fibroblasts and blood vessels were also seen within the connective tissue stroma (Fig.3 B and C) in the 3rd trimester epididymis of *Camelus dromedarius*.

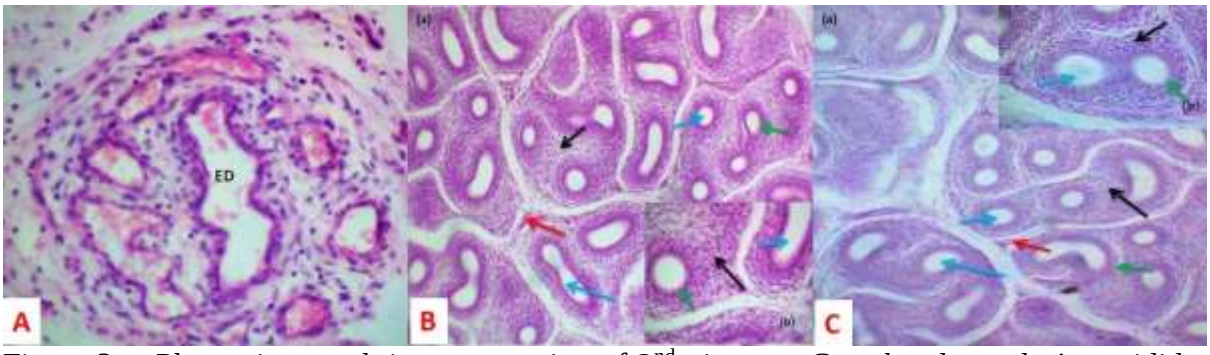

Figure 3a: Photomicrograph in cross section of 2nd trimester *Camelus dromedarius* epididymis indicating EP- Epididymal duct. H & E x400

B: Photomicrograph in cross section of 3rd trimester *Camelus dromedarius* epididymis indicating venules (red arrow), columnar epithelium (light green arrows), connective tissue stroma (black arrow) , lumen of seminiferous tubule (blue and green arrows) , (a) H & E x40 (b) H & E x400.

C: Photomicrograph in cross section of 3rd trimester *Camelus dromedarius* epididymis indicating venules (red arrow), columnar epithelium (light green arrows), connective tissue stroma (black arrow), lumen of seminiferous tubule (blue and green arrows), (a) PAS x40 (b) PAS x400.

Vas deferens and Ampulla: The vas deferens of *Camelus dromedarius* fetus was very frail in structure and difficult to study in the 1st trimester but careful examination revealed a vas deferens with no distinct muscular layers on a transverse section. The lumen presented a stellate shaped with a basal membrane. In the 2nd trimester the vas deferens had distinct muscular layers (Fig. 4 A) which had become much developed at the 3rd trimester. The muscular layer of the vas deferens at the 3rd trimester was covered with a pseudostratified columnar epithelium on a stellate lumen (Fig. 4B). A lamina propria was also observed alongside with a muscular layer bearing interwoven smooth muscle fibers.

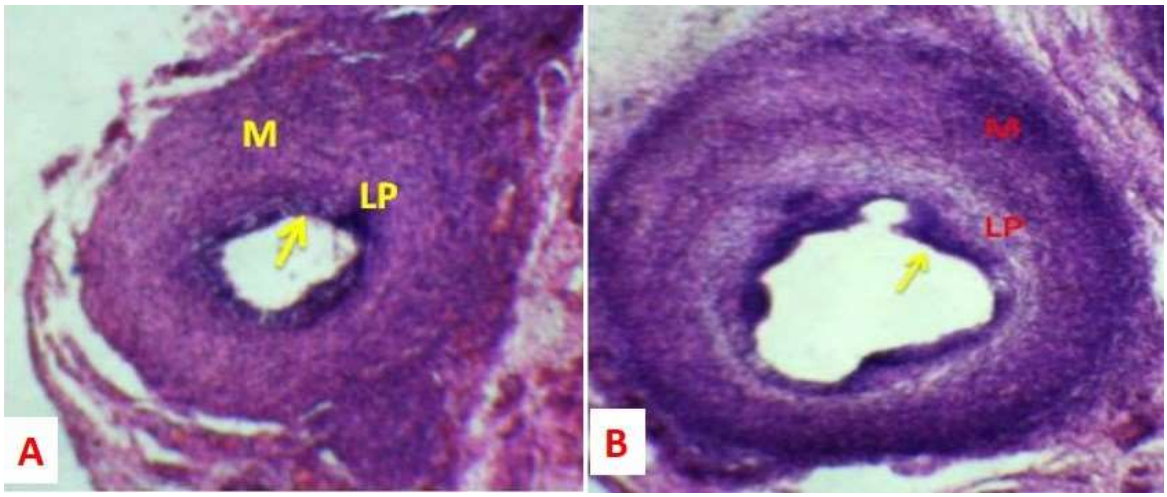

Figure 4a: Photomicrograph in cross section of 2nd trimester *Camelus dromedarius* vas deferens indicating epithelium (yellow arrow), M- Muscularis and LP- Lamina propria H & E x40

B: Plate 4.62: Photomicrograph in cross section of 3rd trimester *Camelus dromedarius* vas deferens indicating epithelium (yellow arrow), M- Muscularis and LP- Lamina propria H & E x40

The ampullae on the other hand were not well developed at the 1st and 2nd trimesters (Fig. 5 A and B). At the 3rd trimester, a developing ampullae was seen as a longitudinal fold projecting into the lumen (Fig.5 C)

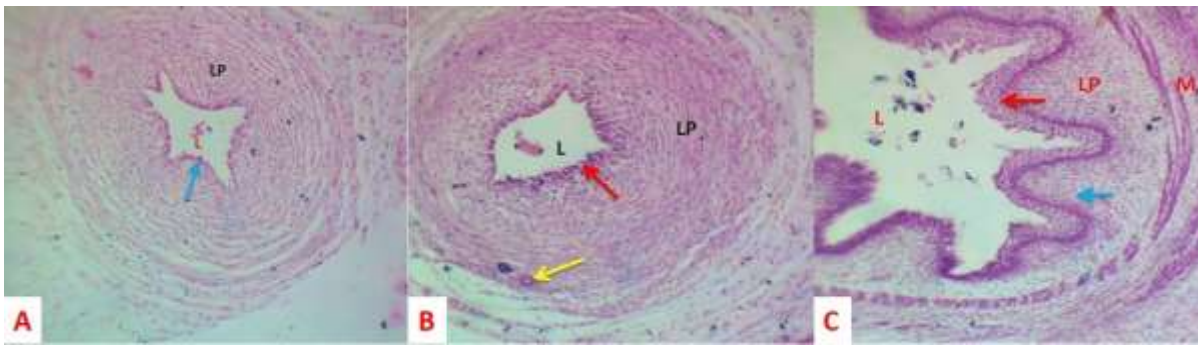

Figure 5a: Photomicrograph in cross section of 1st trimester *Camelus dromedarius* ampulla indicating epithelium (blue arrow), L- lumen and LP- Lamina propria H & E x100.

B: Photomicrograph in cross section of 2nd trimester *Camelus dromedarius* ampulla indicating epithelium (red arrow), blood vessel (yellow arrow), L- lumen and LP- Lamina propria H & E x100.

C: Photomicrograph in cross section of 3rd trimester *Camelus dromedarius* fetus ampulla indicating epithelium (red arrow), connective tissue (blue arrow) M- muscularis, L- lumen and LP- Lamina propria H & E x100

Prostate gland: The *Camelus dromedarius* fetal prostate have a body filled with glands and the disseminating part and there were age dependent morphological differences. In the 1st trimester the prostate appeared poorly differentiated. However, at the 2nd trimester, the prostate appeared markedly differentiated with a developed capsule comprising collagen fibers and fibrous connective tissue. The glands were clearly seen within the body of the prostate. Within these developed glands, were connective tissue stroma with interstitial cells and spindle shaped smooth muscle fibre. The lumen of most glands was filled with cells and no patency at this stage. The epithelial cells were seen to take up most part of the gland. The prostatic urethra was also seen to have stratified squamous epithelium (Fig. 6 A and B). The 3rd trimester was seen to have gland bigger in size with open lumen. The prostatic urethra was wider with numerous transitional epitheliums on the mucosa. Also, the lamina propria was seen with connective tissue stroma (Fig.6 C and D).

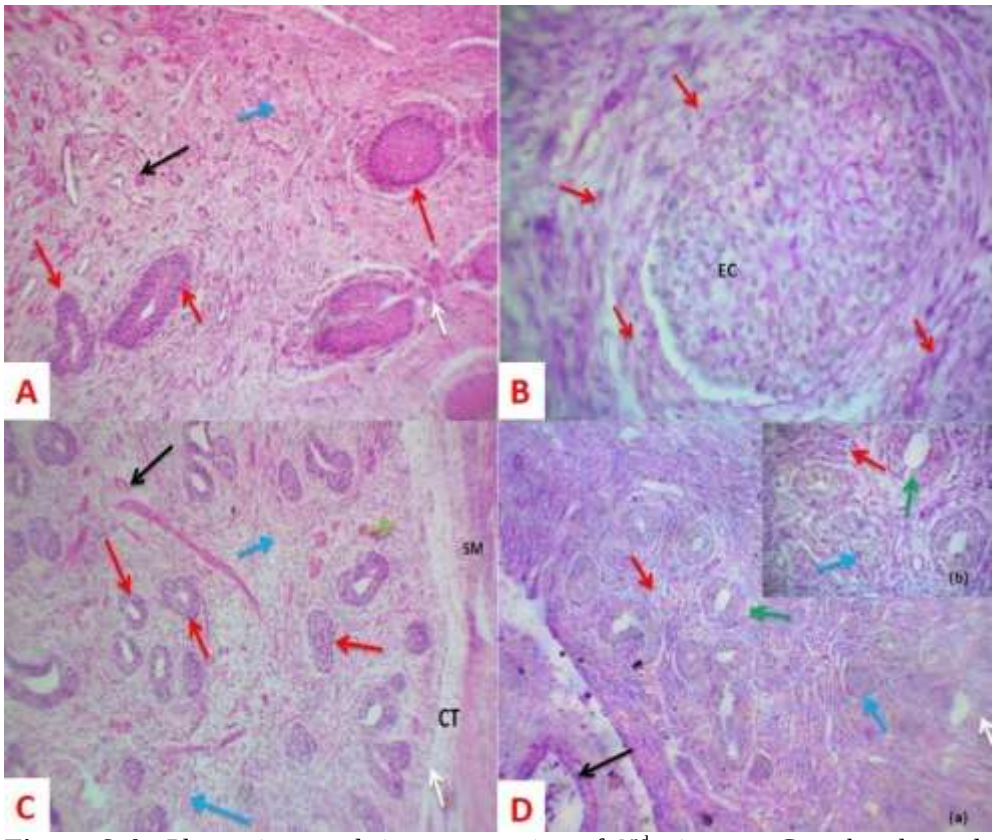

Figure 6 A: Photomicrograph in cross section of 2nd trimester *Camelus dromedarius* prostate gland indicating glands (red arrow), smooth muscle (white arrows), arteriole (black arrow), connective tissue stroma (blue green arrow), H & E x40

B: Photomicrograph in cross section of 2nd trimester *Camelus dromedarius* prostate gland indicating Spindle shaped myofibers (red arrow), EC- epithelial cells in a squamous form. PAS x400

C: Photomicrograph in cross section of 3rd trimester *Camelus dromedarius* prostate gland indicating glands (red arrow), arteriole (black arrow), connective tissue stroma (blue arrow) , SM- smooth muscle, CT- fibrous connective tissue, H & E x40

D: Photomicrograph in cross section of 3rd trimester *Camelus dromedarius* prostate gland indicating glands (red arrow), arteriole (black arrow), connective tissue stroma (blue arrow) , SM- smooth muscle, CT- fibrous connective tissue, (a) PASx40 (b) PASx400

Bulbourethral gland: The bulbourethral gland of *Camelus dromedarius* fetus was paired. Each gland was observed to be enveloped in a thick capsule (Fig.7 A).

The gland was seen to have numerous smooth muscles at the 2nd trimester surrounding the tubule alveolar glands that were developing. Numerous fibro-muscular fibers of skeletal muscle type were seen in between the glands at the 3rd trimester (Fig. 7 B and C).

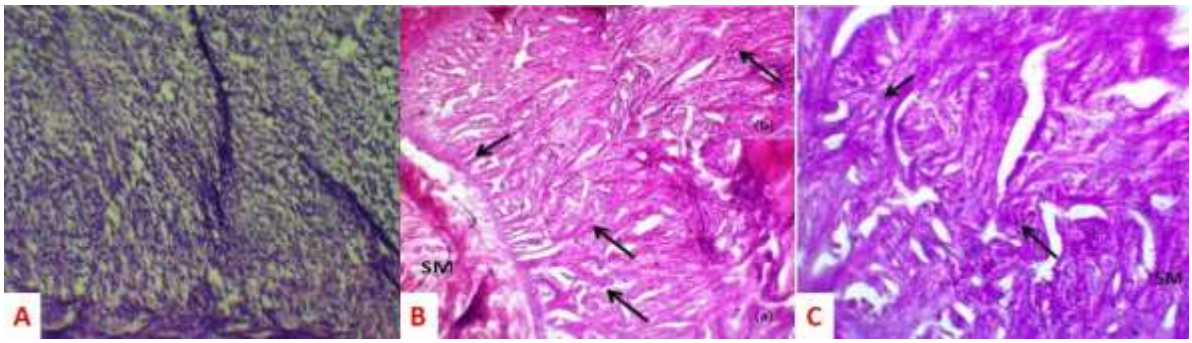

Figure 7a: Photomicrograph in cross section of 1st trimester *Camelus dromedarius* bulbourethral gland indicating glands (black arrow) and SM- smooth muscle H & Ex10

B: Photomicrograph in cross section of 3rd trimester *Camelus dromedarius* bulbourethral gland indicating glands (black arrow) and SM- smooth muscle (a) H & Ex40 (b) H & E x400

C: Photomicrograph in cross section of 3rd trimester *Camelus dromedarius* bulbourethral gland indicating glands (black arrow) and SM – smooth muscle, PAS x400

Penis: The penis of *Camelus dromedarius* fetus was seen to have a urethra with transitional epithelium. The mucosa had undifferentiated cells. The penis is enveloped in a tunica albuginea and divided histologically into body and glans penis at the 1st trimester (Fig. 8A). The glans penis in the 2nd trimester was note to have a developed lumen. The lining of the urethral epithelium was transitional. The lamina propria contained the corpus spongiosum, corpus cavernosum, cavernous spaces, blood vessels and connective tissue.

It was enveloped in a thick tunica albuginea. An incomplete circle, an ectodermal lamella was also observed. The lamella was seen to have a space in the overlap for the frenulum which is the connective tissue between the penis and the prepuce (Fig. 8 B and C). The 3rd trimester had a transitional epithelium in the urethra of glans penis, with an increase in number and size of cavernous spaces within the lamina propria. The ectodermal lamella was wider and the space created by the frenulum was also increased (Fig. 8D) comparing the 3rd to the 2nd trimester.

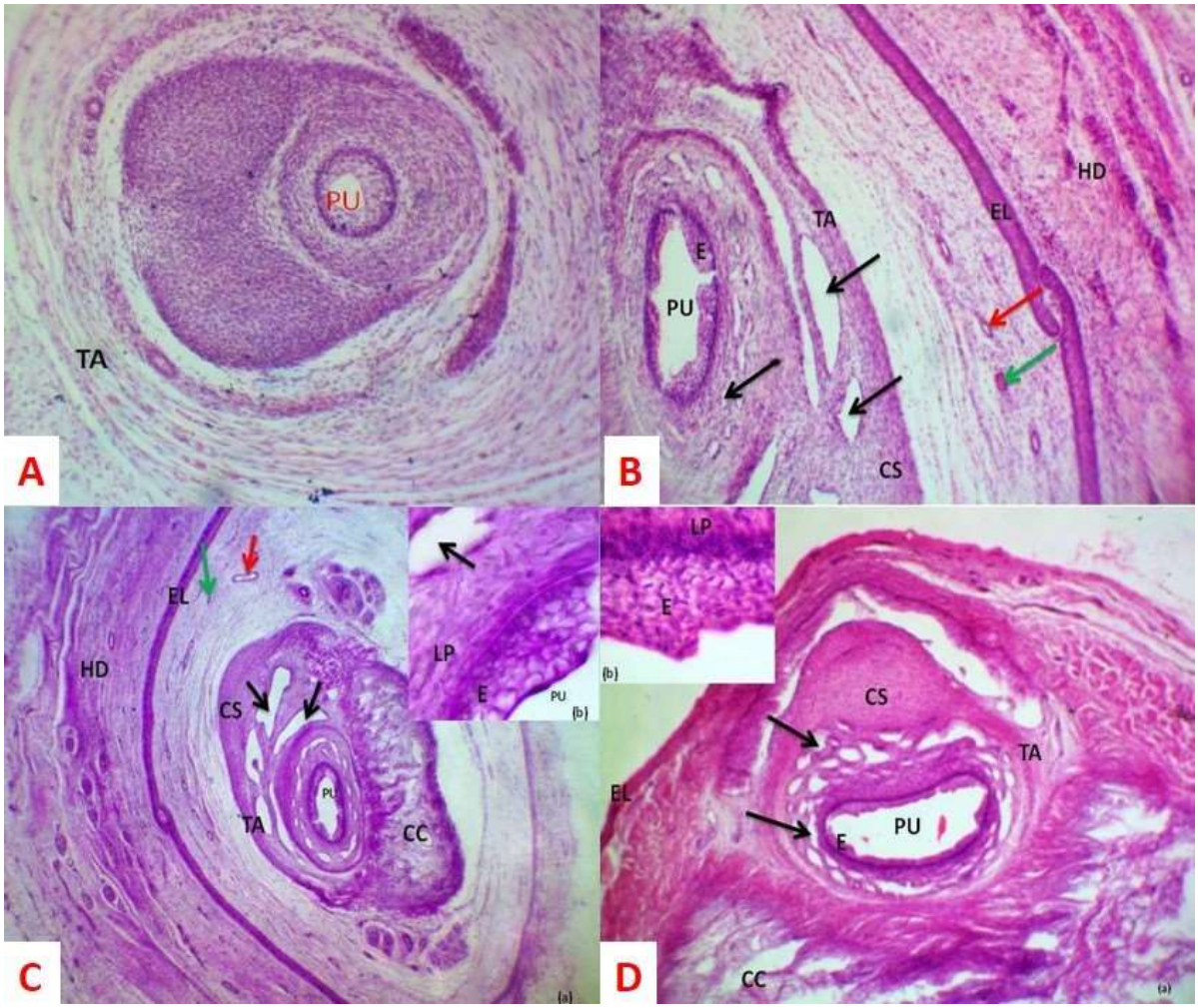

Figure 8a: Photomicrograph cross section of 1st trimester *Camelus dromedarius* glans penis indicating PU- penile urethra and TA- tunica albuginea H&E x100.

B: Photomicrograph cross section of 2nd trimester *Camelus dromedarius* glans penis indicating cavernous space (black arrow), artery (red arrowa0, vein (green arrow), PU- penile urethra, E- epithelium, TA- tunica albuginea, CS- corpus spongiosum, EL- ectodermal lamella and HD- hypodermis, H&E x400

C: Photomicrograph cross section of 2nd trimester *Camelus dromedarius* glans penis indicating cavernous space (black arrow), artery (red arrow), vein (green arrow), PU- penile urethra, E- epithelium, TA- tunica albu4inea, CS- corpus spongiosum, CC- corpus cavernosum, LP- lamina propria, EL- ectodermal lamella and HD- hypodermis, (a) PAS x 40 (b) PAS x400.

D: Photomicrograph cross section of 3rd trimester *Camelus dromedarius* glans penis indicating cavernous space (black arrow), PU- penile urethra, E- epithelium, TA- tunica albu4inea, CS- corpus spongiosum, CC- corpus cavernosum, LP- lamina propria, (a) H&E x40 (b) H&E x100

DISCUSSION

In this study, we described the histological developmental horizons of the male reproductive organs of the *Camelus dromedarius* fetus. A general observation was that the histologic development of these organs was age dependent. We reported herein that the 1st trimester of *Camelus dromedarius* fetal testes was undifferentiated, and that mesenchyme cells were seen to line the epithelial layer. Similar findings have been reported in Gaddi sheep fetal testis[26]. We also showed that the 2nd trimester fetal testes had more fibroblast cells and strings of developing sex cords with cells at the periphery. This was similar to the findings of Singh *et al*. [27] and Farooqui *et al*. [28] in buck goat. Of note, this germinal epithelium produced the primary sex cords which later form medullary cords and subsequently differentiated to the testicular cord[29] and this corroborate well with our current findings studying the different development stages of the *Camelus dromedarius* fetus.

Our results indicated that the 3rd trimester testis had marked tubular and circular sex cords surrounded by numerous interstitial cells and collagen fibers similar to findings of Shukla[26], in Gaddi sheep. Interesting, most tubular sex cords in this present study were seen within the testicular cortex, and the few at the periphery (towards the albuginea) were curved in shape. We also reported that the sex cords of *Camelus dromedarius* fetal testes were of two types of cells. This was similar to the observation made in goat[27-28], cattle[30] and pig[31].

One important histological feature of the supporting cells is that they have dark stained nuclei, when stained with PAS. Although this is an important feature but it is not a surprise as similar observations have been reported by Bacha and Bacha[32] in boar, and Shukla[26] in Gaddi sheep. Concerning the second cell type, the large gonocytes, we reported herein that they presented a pale nuclei and weakly eosinophilic cytoplasm with definite outline. These observation were similar to the findings of Santamarina and Reece[30] in buffalo, Farooqui *et al*.[28] in goat, Bacha and Bacha[32], in boar, and Shukla[26] in Gaddi sheep. Furthermore, the sex cords have received different nomenclature from different authors. Patten[33], Copenhaver[34] (1978) and Singh *et al*. [27] designated primordial germ cells (gonocytes) as the large cells and Farooqui *et al*.[28] termed the small cells as mesenchymal cells. In this report, we have choose to use small mesenchyme supporting cells and the large gonocytes based on our histological morphological observations of the cells.

Also, our current findings that tunica albuginea of *Camelus dromedarius* fetal testes in the 2nd trimester was thin and invaginate into the testicular parenchyma dividing it into lobules supports earlier reports of Shukla[26]. In his report (Shukla[26]) the tunica albuginea of Gaddi sheep fetal testes at 3rd trimester became thicker forming the tunica vasculosa and this corroborate well with our current report on the *Camelus dromedarius* fetal testes at 3rd trimester.

We also showed from the report herein that the fetal epididymis of *Camelus dromedarius* enveloped the tunica albuginea and increases in thickness across the three trimesters. The thick capsule was seen to have an inner vascular layer with blood vessels. This is similar to the findings of Shukla[26] in Gaddi sheep. The sizes of the ducts varied across the three trimesters under study and we showed that these ducts were lined with pseudostratified epithelium. The findings were similar to the observations of Singh[35] in buffalo calf and Mohamed[36] in bovine fetuses and Shukla[26] in Gaddi sheep fetuses.

We reported herein that the histological observation of the vas deferens of *Camelus dromedarius* fetus in the 1st trimester was not feasible as it was frail in structure. Vitthalrao[37] reported similar findings in

fetal sheep. We however reported that the vas deferens has lumen with not too distinct muscular layers in the 2nd trimester and that at the 3rd trimester, the muscular layer was more distinct with a pseudostratified columnar epithelium. Similar findings have been reported by Vitthalrao[37] in sheep.

We also showed herein histologically, that the prostate gland appeared poorly differentiated at the 1st trimester. Further development revealed glands that have no patency at the 2nd trimester. This was similar to the report of Cunha *et al*.,[38] in human fetus with branching and canalization of glands occurring at 12weeks of gestation. This is not in accordance with the study of human fetal prostate at 17 weeks of age (Ernst *et al*.,[39]), wherein the ducts of the gland had a stellate shape. Still on the 2nd trimester, the smooth muscle fibre cells were spindle in shape, similar to the report of Ernst *et al.*,[39] on the stroma of human fetal prostate at early mid trimester. The present report at the 3rd trimester revealed a prostatic ureter with a mucosal transitional epithelium layer.

The most important finding regarding the developmental strides of the bulbourethral gland of fetal *Camelus dromedarius* was that numerous fibro-muscular fibers of skeletal muscle type were seen in between the glands at the 3rd trimester. This is similar to the report of Eroschenko[40] who suggested that the skeletal muscle fibers are in the bulbourethral gland because the gland is located in the urogenital diaphragm.

Concerning the penis, our findings that the penis of fetal *Camelus dromedarius* had urethral process with transitional epithelium bearing a mucosa with undifferentiated cells is interesting. We also showed that at different stages, the penis was enveloped in a tunica albuginea. This is in corroboration with the findings of Farooqui[41] in goat and Vitthalrao[37] in sheep. Also, we reported herein that the glans penis in the 2nd trimester presented a developed lumen and that the lining of the urethral epithelium was transitional while the lamina propria contained the corpus spongiosum, corpus cavernosum, cavernous spaces, blood vessels and connective tissue. This is in accordance with the findings of Farooqui[41] in goat and Vitthalrao[37] in sheep. The incomplete circle ectodermal lamella we reported in this study together with the space in the lumen that overlapped the frenulum agrees with the findings of Atyia[42] in postnatal camels. Our report is the first describing these findings in prenatal camels. Interesting is the fact that this ectodermal lamella was wider, and the space created by the frenulum increased in 3rd trimester implicating developmental strides.

CONCLUSION

In summary, we report here developmental changes in the testes, epididymis, vas deferens, ampulla, prostate and bulbourethral glands and penis of the fetal *Camelus dromedarius* based on histological results and highlight trimester-based differences in the development of these tissues. We also in compared our findings with other mammals and noticed agreements in developmental strides. Our results provide new information on the histological features of the fetal reproductive organs in male *Camelus dromedarius*, an insight to reproductive management in this specie of mammal.

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