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Scanning Electron Microscopic Studies on the Development of Epididymis and Vas Deferens in Non-descript Goat (*Capra hircus*) during Postnatal Period

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ABSTRACT

Background: The appearance and interaction of spermatozoa with the tubular epithelium is crucial for selection of non-descript goats for breeding purposes at the appropriate time. Further, various interventions can also be made for faster reproductive developments in the goats under study.

Methods: The present study was conducted on the testes of non-descript goats divided into three groups, *viz*. neonatal (within 1 month), prepubertal (from 1-18 months) and pubertal (above 18 months) with 10 healthy animals in each group. The collected samples of testis were processed for scanning electron microscopic study and subsequently, the samples were viewed and the photographs were taken in the facility available at Central Instrumentation Facility (CIF), OUAT, Bhubaneswar.

Result: It was found that the tunica vaginalis of both epididymis and vas deferens were having longitudinal and transverse folds with the depressions or troughs in between them. The epididymis and vas deferens were both lined by ciliated and non-ciliated types of cells. The ciliated cells showed tufts of cilia, where as the free surfaces of the non-ciliated cells were having microvilli. The luminal surface of the epididymal duct gave the appearance of a honeycomb due to the peculiar arrangement of stereocilia projecting from the columnar cells. Spermatozoa were found to be attaching with the epithelial cells of both epididymis and vas deferens, mostly in the pubertal goats. There was presence of apical protrusions on the non-ciliated cells in both epididymis and vas deferens. The luminal surface of vas deferens was raised at regular intervals giving wavy appearance with ridges and grooves. The present study would help in developing a baseline data on the scanning electron microscopic characteristics of different components of tunics of epididymis and vas deferens in non-descript goat at different stages of post-natal life under study.

Key words: Development, Epididymis, Goat, Non-descript, Post-natal, Scanning electron microscopy, Vas deferens.

INTRODUCTION

Goat is considered as the great future in changing livestock scenario (Ansari *et al*., 2017). The poor man's cow-goat has tremendous potential to be projected as the future animal for rural areas under the changing agro-climatic conditions and lack of forages (Jindal *et al*., 2011). Scanning electron microscopy (SEM) permits observation of structures in three dimensions. With other types of microscopy, these structures must be visualized by the reconstruction of serial sections (Johnson *et al*., 1978 and Singh *et al*., 2024). In capitalizing on this advantage, SEM has been employed to observe structures within the epididymis and vas deferens of the non-descript goat. The ductus epididymidis is extremely tortuous and coiled. Macroscopically, the epididymis is divided into a head, body and tail (Getty, 1975). It is surrounded by a thick tunica albuginea of dense irregular connective tissue covered by the visceral layer of the tunica vaginalis (Banks, 1993). Generally, the proximal parts of the duct (head and body) are involved in the maturation process of spermatozoa (Schimming *et al*., 2012; Rus *et al*., 2022 and Kour *et al*., 2023). The tail of the epididymis serves as their main storage place. Spermatozoa leaving the testis are both immotile and infertile, whereas spermatozoa leaving the epididymis have gained motility and fertility. During their passage through the ductus epididymis, spermatozoa undergo a series of morphologic and functional changes that lead to the

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acquisition of full fertilizing capacity by the time they reach the tail (Dellmann and Eurell, 1998 and Samuelson, 2007). The ductus epididymis, after a sharp bend at the end of the tail, gradually straightens (Getty, 1975) and acquires the histologic characteristics of the ductus deferens. In stallions and ruminants, the ductus deferens unites with the excretory duct of the vesicular gland to form a short ejaculatory duct, which opens at the colliculus seminalis into the urethra. The pseudostratified lining epithelium of the bovine ejaculatory duct contains engulfed spermatozoa. In boars, the ductus deferens and the excretory duct open separately into the urethra. In carnivores, the ductus deferens joins the urethra alone because the vesicular gland is absent. The terminal portion of the ductus deferens is one of the male accessory glands, regardless of whether it forms an ampulla (stallions, ruminants, dogs) or does not (boars, cats); it contains simple branched tubulo-alveolar glands in the propria-submucosa (Dellmann and Eurell, 1998 and Samuelson, 2007).

Abundant research has been done on the gross and histomorphology of epididymis and vas deferens in goat and other domestic animals. But a very scanty literature is available on their scanning electron microscopic details in domestic animals and this area is quite unexplored till date. The ultrastructure of the ciliated and non-ciliated cells with their interaction with the mature spermatozoa is vital for accessing the reproductive status of the animal. The present study was carried out to develop a baseline data on the scanning electron microscopic characteristics of different components of tunics of epididymis and vas deferens in non-descript goat at different stages of post-natal life under study.

MATERIALS AND METHODS

The present scanning electron microscopic study was conducted on the epididymis and vas deferens of non-descript goats at various stages of post-natal life. The samples were collected from the nearby local slaughter houses in and around Bhubaneswar. The animals were divided into three groups, *viz*. neonatal (within 1 month), prepubertal (from 1-18 months) and pubertal (above 18 months) with 10 healthy animals in each group (Banerjee, 2019). The age of the animal is known from the data book of the slaughter houses. The samples of epididymis and vas deferens were processed for scanning electron microscopic study (Scanning Electron Microscope, Make: Hitachi and Model: S-3400N) as per the standard methods of Sahu *et al.* (2021). The samples were subsequently viewed and the photographs were taken in the facility available at Central Instrumentation Facility (CIF), OUAT, Bhubaneswar. The measurements of various parameters of epididymis and vas deferens were also taken at the scanning electron microscopic level by the inbuilt software programming system. The recorded data were subjected to routine statistical analysis (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The present study focused on the scanning electron microscopic details of the tunics of epididymis and vas deferens in non-descript goats at different stages of postnatal life under study.

Scanning electron microscopy of epididymis

The epididymis was covered with a tough layer of fibrous tissue called as tunica vaginalis. The septae originated from the tunica vaginalis and penetrated deep into the epididymis dividing the organ into a non-regular form resembling lobules (Sharma *et al*., 2012). In the connective tissue of the septae or in between the lobules, few small blood vessels were also observed (Singh, 1989; Schimming *et al*., 2001; Mohamed, 2005 and Sharma *et al*., 2012). The outer surface of tunica vaginalis was folded. These folds were longitudinal and transverse with the depressions or troughs in between them. Ridges and passages were also noticed in these folds at higher magnification. These ridges and passages were seen in all the age groups (Fig 1), but quite well developed in the pubertal goats (Fig 2). These ridges and folds might be attributed to the increase in surface area (Sharma *et al*., 2012). The ducts comprised of lining epithelium, peritubular stroma and intertubular stroma. The peritubular stroma known as propria-submucosa surrounded the epithelium and consisted of concentrically arranged smooth muscle fibers separated by connective tissue fibers (Fig 3 and Fig 4). The epididymal lumen contains spermatozoa and fluid, whose composition is constantly changed as the fluid moves from the initial to the terminal segment (Robaire and Viger, 1995). The intertubular stroma was present in between the ducts and mainly comprised of inter-woven connective tissue fibers (Fig 4). The present findings were in agreement to the observations of Schimming *et al*. (2001) in dog and Sharma *et al*. (2012) in buffalo foetus.

The epididymis was lined by ciliated and non-ciliated types of cells (Fig 5, Fig 6 and Fig 7), which was similar to

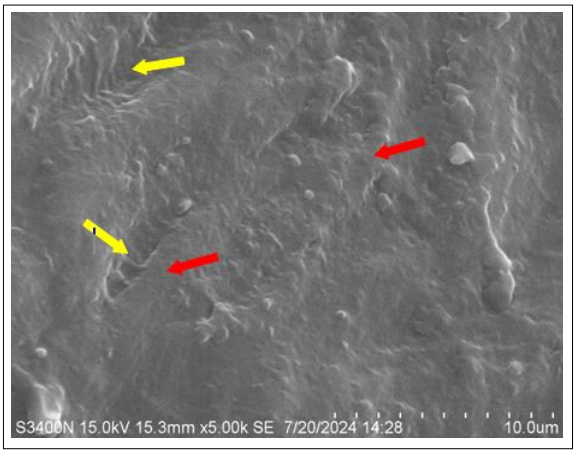

Fig 1: Photograph showing the folds or ridges (red arrows) and passages (yellow arrows) in tunica vaginalis of epididymis in pre-pubertal non-descript goat.

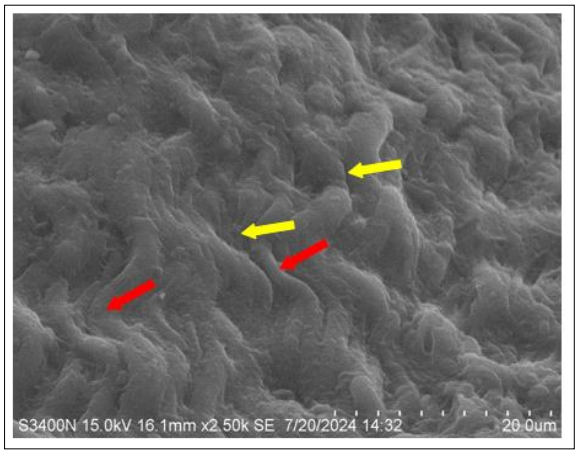

Fig 2: Photograph showing well developed folds or ridges (red arrows) and passages (yellow arrows) in tunica vaginalis of epididymis in pubertal non-descript goat.

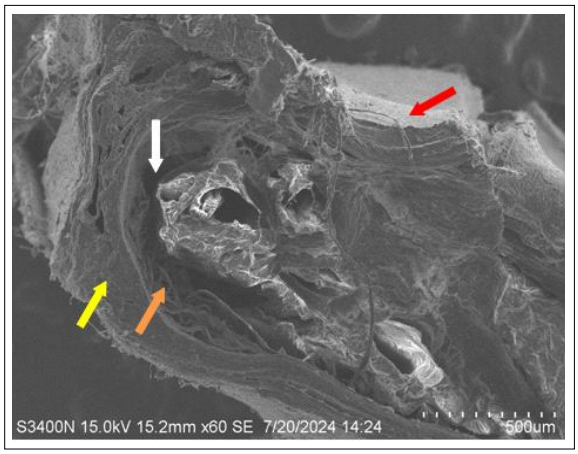

Fig 3: Photograph showing the tunica vaginalis (red arrow), tunica muscularis (yellow arrow), propria-submucosa (orange arrow) and lumen (white arrow) of epididymis in pre-pubertal non-descript goat.

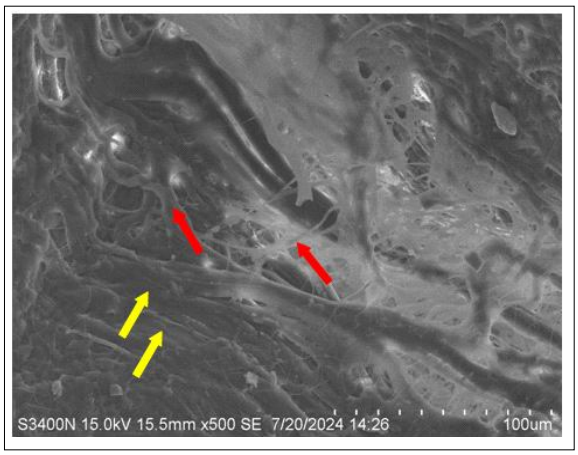

Fig 4: Photograph showing the concentric layers of smooth muscles in tunica muscularis (yellow arrows) and intertubular connective tissue fibers (red arrows) of epididymis in pre-pubertal non-descript goat.

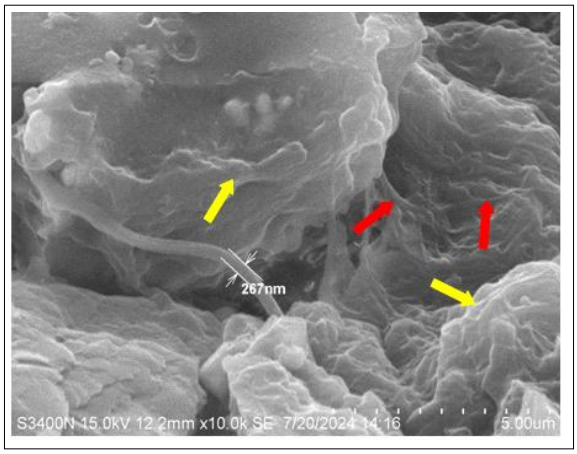

Fig 5: Photograph showing the ciliated cells with stereocilia (red arrows) and non-ciliated cells (yellow arrows) in the epithelium of epididymis in neonatal non-descript goat.

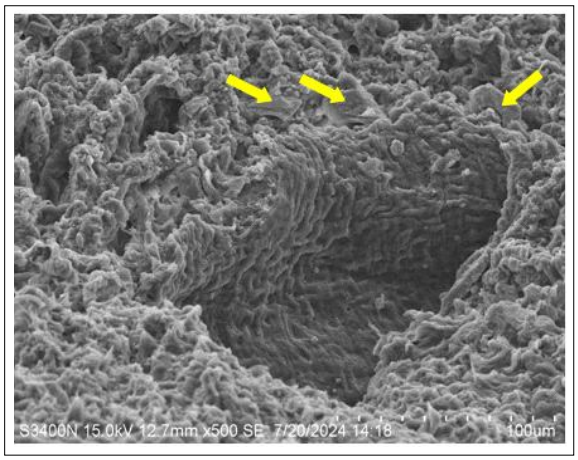

Fig 6: Photograph showing the tufts of stereocilia on the apical surfaces of populated ciliated cells with few non-ciliated cells (yellow arrows) in the epithelium of epididymis in neonatal non-descript goat.

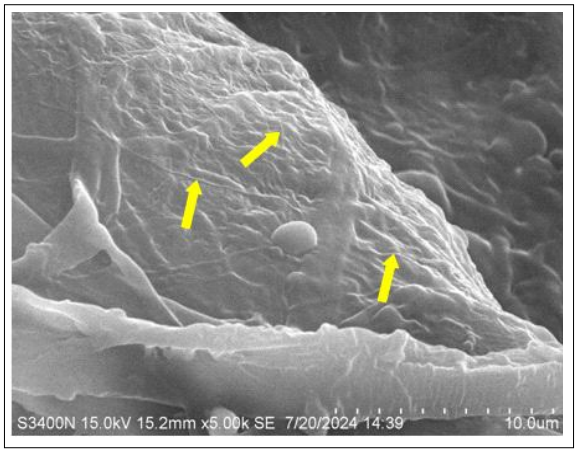

Fig 7: Photograph showing the tufts of stereocilia (yellow arrows) on the apical surfaces of populated ciliated cells in the epithelium of epididymis in pubertal non-descript goat.

the finding of Saleem *et al*. (2018) in the local Hill fowl of Uttarakhand. The ciliated cells showed tufts of cilia (Fig 6), where as the free surfaces of the non-ciliated cells were lined by microvilli (Fig 8). The present observations were in agreement with the findings of Budras and Saucer (1975) in cock, Bakst (1980) in male chicken and turkey and Saleem *et al*. (2018) in the local Hill fowl of Uttarakhand. However, Aire and Soley (2000) in ostrich observed several non-ciliated cells showing single long cilium which projected into the ductal lumen. The luminal surface of the epididymal duct gave the appearance of a honeycomb (Fig 9) due to the peculiar arrangement of stereocilia projecting from the columnar cells (Murakami *et al*., 1975). The stereocilia were not uniform in length and distribution as in the case of microvilli of the intestinal absorptive cells, but left a funnel-shaped space (Fig 10) widely opening to the lumen on the center of each cell (Murakami *et al*., 1975). Individual stereocilia were in general long and slender with almost constant in diameter. The diameter of stereocilia was measured as (267±5.14) nm, (1.35±0.06) µm and (1.71±0.04) µm in neonatal, pre-pubertal and pubertal non-descript goats from base to the tip respectively. Their outer surface seems smooth and without a particular structure. Frequently, small globules were found to adhere to the tip or the shaft of the stereocilia, which might be the artifacts produced during preparation. The functional significance of the brush border was unclear, but probably it conveyed the luminal content forward (Orsi *et al*., 1998).

The luminal surface of epithelium especially the non-ciliated cells of the ducts was studded with apical protrusions and few circular areas giving cobbled appearance (Fig 11) in the prepubertal and pubertal goats (Saleem *et al*., 2018). These areas might represent the regions from which the apical protrusions had become detached (Sharma *et al*., 2012). The presence of apical protrusions on the luminal surface of the epithelium in the prepubertal and pubertal animals was indicative of the apocrine secretory activity of the epididymis at these stages (Paunescu *et al*., 2014). While the process of vesicle shedding via plasma membrane budding was originally thought to represent an artifact caused by poor fixation, these vesicles are now considered to play significant roles in the communication between neighboring cells (Cocucci *et al*., 2009).

Variable numbers of spermatozoa were present in the lumen over the stereocilia, mostly in the pubertal group (Fig 12). The heads of the spermatozoa invaded deep into the epithelium of the duct, especially to the non-ciliated cells indicating a close physical interaction between spermatozoa and the epithelial cells (Paunescu *et al*., 2014), but it was contradictory to the observations of Murakami *et al*. (1975) in Japanese Monkey, Schimming *et al*. (2001) in dog and Saleem *et al*. (2018) in local Hill fowl of Uttarakhand. This strong bond was vital in sperm maturation in the epididymis, allowing the transfer of new proteins from epithelial cells to the sperm cells. The extracellular vesicles might be originating either from the fusion of multivesicular bodies

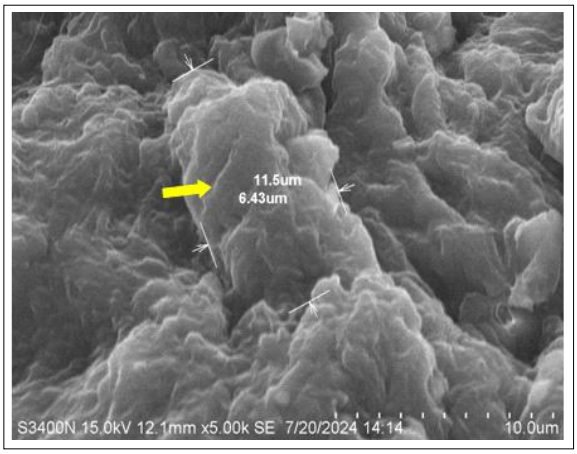

Fig 8: Photograph showing the microvilli on the apical surface of non-ciliated cell (yellow arrow) in the epithelium of epididymis in neonatal non-descript goat.

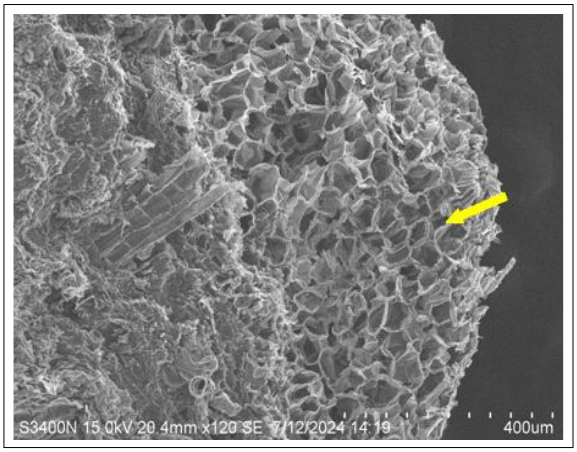

Fig 9: Photograph showing luminal surface of the epididymal duct giving the honeycomb appearance due to the peculiar arrangement of stereocilia projecting from the ciliated cells in neonatal non-descript goat.

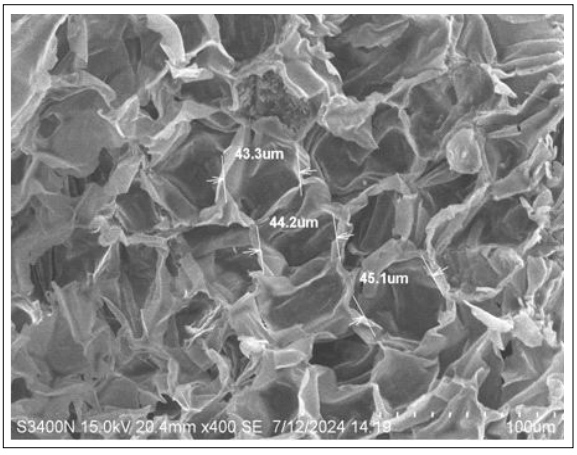

Fig 10: Photograph showing funnel shaped spaces (left by sterocilia) opening into the lumen of epididymis in neonatal non-descript goat.

with the apical membrane followed by the release of exosomes, or from the budding of larger vesicles from the plasma membrane (Belleannee *et al*., 2013; Sullivan and Saez, 2013). The presence of a very dense array of stereocilia in principal cells precluded the detection of any exocytotic or fusion event that would take place at the level of the plasma membrane (Paunescu *et al*., 2014). The morphology of certain sperm cells suggested a distinct kind of interaction with the epididymal epithelium. Increasing the magnification reveals concentric rings on the sperm midpiece (Villalpando *et al*., 2000), as well as small, vesicle-like structures on the surface of the cytosolic droplet located at the mid-principal piece junction (Paunescu *et al*., 2014).

Scanning electron microscopy of Vas deferens

The vas deferens was covered with a tough layer of fibrous tissue known as tunica vaginalis. The outer surface of tunica vaginalis was folded. These folds were longitudinal and transverse with the depressions or troughs in between them. Ridges and passages were also noticed in these folds at higher magnification in all the age groups, but quite well developed in both pre-pubertal and pubertal non-descript goats (Fig 13). These ridges and folds might be attributed to the increase in surface area.

The luminal surface of ductus deferens of local Hill fowl showed numerous longitudinal folds. They were raised at regular intervals giving it a wavy appearance (Fig 14) with ridges and grooves (Saleem *et al*., 2018). Every groove presents in the midline a straight ridge due to the overlapping of the adjacent cell membranes (Orlandini *et al*., 1979). The higher SEM magnification showed that the lamina epithelialis was made up of irregular polygonal cells with indistinct cell boundaries (Saleh *et al*., 2020). The apical surface of the epithelial cells appeared slightly dome-shaped (Fig 14). The present finding was in agreement with the report given by Saleem *et al*. (2018) in local Hill fowl of Uttarakhand.

The epithelium of the vas deferens comprised of ciliated and non- ciliated types of cells. The ciliated cells showed tufts of cilia (Fig 15), where as the free surfaces of the non-ciliated cells were lined by microvilli (Fig 16). The present finding was in line with the reports of Saleem *et al*. (2018) in local Hill fowl of Uttarakhand, Saleh *et al*. (2020) in Dromedary camel and Kocakoglu *et al*. (2023) in long horned beetle. Individual stereocilia are in general long and slender with almost constant in diameter from base to the tip. Their outer surface seems smooth and without a particular structure. The functional significance of the brush border was unclear, but probably it conveyed the luminal content forward. It might also help in the movement of the sperms and secretion in and out of the glandular alveoli, located in the lamina propria of the vas deferens (Saleh *et al*., 2020). The luminal surface of epithelium, especially the non-ciliated cells of the duct was studded with apical protrusions (Fig 17) in the prepubertal and pubertal goats (Brueschke *et al*., 1974). The presence of apical protrusions on the luminal surface of the epithelium was indicative of the apocrine secretory activity of the vas deferens at these stages. Further,

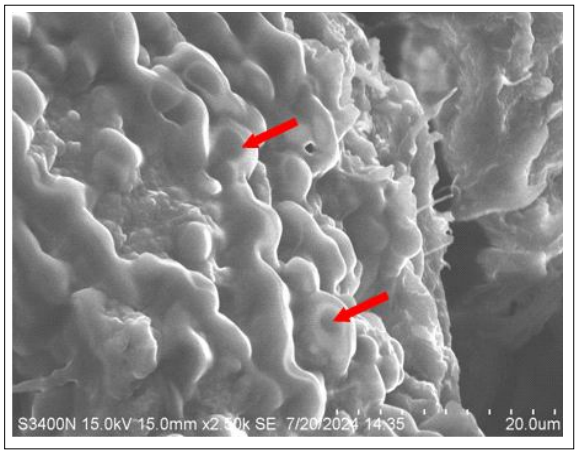

Fig 11: Photograph showing apical protrusions (red arrows) with 'cobbled appearance' on the non-ciliated cells in the epithelium of epididymis in pubertal non-descript goat.

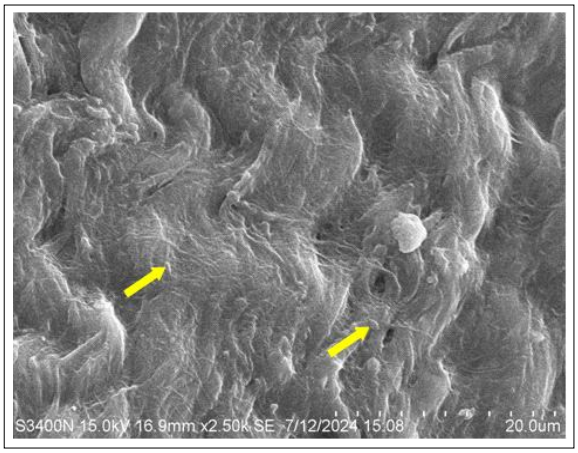

Fig 12: Photograph showing heads of spermatozoa (yellow arrows) anchored into the non-ciliated cells of epithelium in the epididymis of pubertal non-descript goat.

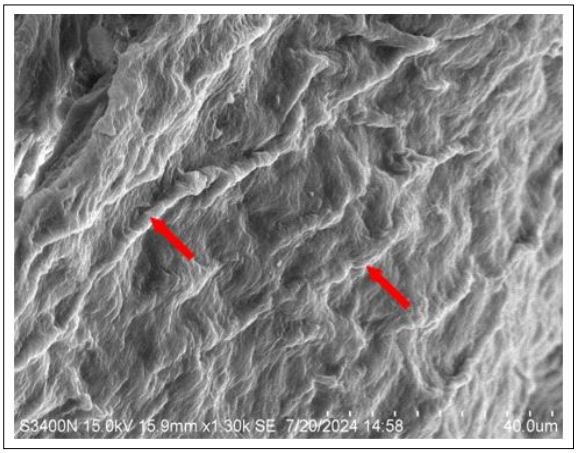

Fig 13: Photograph showing well developed folds or ridges (red arrows) and passages in tunica vaginalis of vas deferens in pre-pubertal non-descript goat.

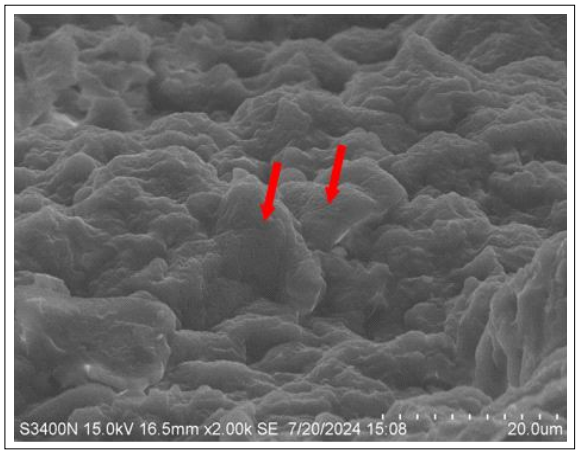

Fig 14: Photograph showing dome shaped epithelial cells (red arrows) with numerous longitudinal folds (wavy appearance) in the luminal surface of vas deferens in neonatal non-descript goat.

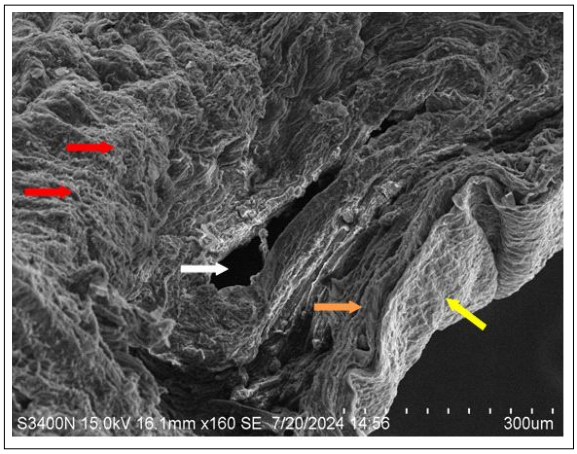

Fig 15: Photograph showing tunica vaginalis (yellow arrow), tunica miscularis (orange arrow), lumen (white arrow) and tufts of cilia (red arrows) on the apical surface of ciliated cells in the epithelium of vas deferens in pre-pudertal non-descript goat.

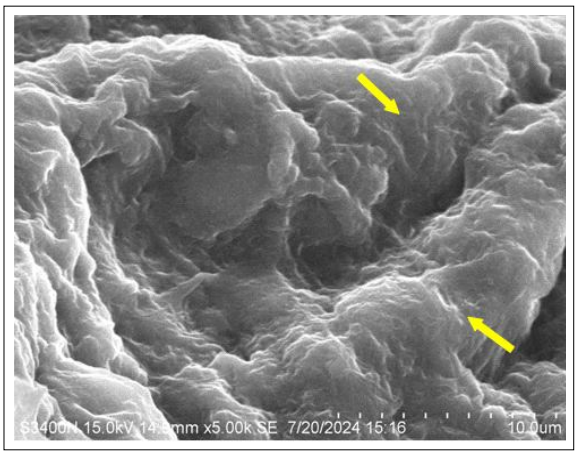

Fig 16: Photograph showing non-citiated cells lined by microvilli in the epithelium of vas deferens (yellow arrows) in pubertal non-descript goat.

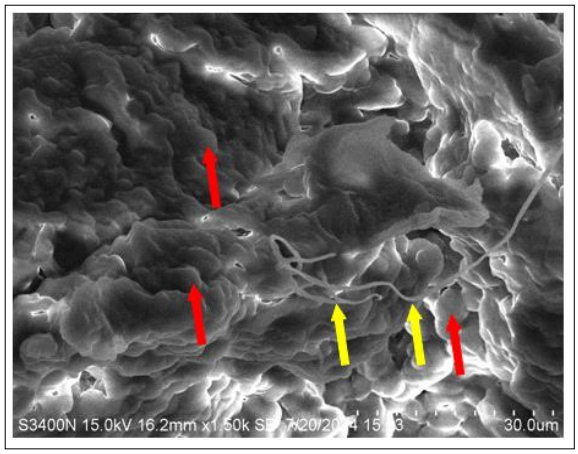

Fig 17: Photograph showing apical protrusions on the non-ciliated cella of epithelium (red arrows) with presence of spermatoza (yellow arrows) in the lumen of vas deferens in pubertal non-descript goat.

the apical surface of the non-ciliated cells was having microvilli. The microvilli might increase the surface area for water reabsorption and luminal fluids concentration (Saleh *et al*., 2020). The spermatozoa were demonstrated in contact with the surface epithelium of the vas deferens (Fig 17), mostly in the pubertal group (Saleem *et al*., 2018 and Saleh *et al*., 2020). The presence of a large amount of the spermatozoa in tubular lumen was indicative of storage of spermatozoa for a time before ejaculation (Perera, 1974 and Ali *et al*., 1978).

CONCLUSION

The present study would help in developing a baseline data on the scanning electron microscopic characteristics of epithelium, lamina propria, tunica muscularis and tunica serosa of epididymis and vas deferens in non-descript goat at different postnatal stages under study. The appearance of ciliated and non-ciliated cells of epididymis and vas deferens, the orientation of cilia along with the appearance and attachment of mature spermatozoa to the epithelial cells of both epididymis and vas deferens would help in accessing the reproductive status of the animal. The same could be exploited in the selection of goats at specific stages of life for breeding purposes.

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Conflict of interest

There is no conflict of interest among the authors.

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