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Ultrastructural Characterization of Testis in Non-descript Goat (*Capra hircus*)

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ABSTRACT

Background: The proposed experiment was aimed at locating the spermatogonial stem cell niches inside the testis and to characterise the development of germ cells of testis in the non- descript goats at the different stages of pre and post natal life, which could be useful in selecting the goats for breeding at appropriate time and for other interventions related to reproductive developments in the goats.

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: The present study revealed that the testis was surrounded by the tunica albuginea, a thin capsule of connective tissue, from which thin septum originated and extended into the inside of the organ. Among these septa, the seminiferous tubules were found. The seminiferous epithelium consisted of spermatogonial cells at different stages of development separated from each other by interstitial connective tissue. The interstitium was made up of randomly arranged collagen bundles. The intertubular vascular connective tissue contained Leydig cells mainly at wider locations where they were bounded by more than two seminiferous tubules. The mediastinal rete testis area contained many cavities of many intercommunicating channels supported by connective tissue pillars. The present study helped in developing a baseline data on the ultrastructural characteristics of seminiferous epithelium, rete restis, peritubular cells, Lyedig cells and other components of testis in non-descript goat at different post natal stages under study. The appearance of seprmatogonial cell lineage at the various post natal stages in non-descript goat would help in accessing the reproductive status of the animal.

Key words: Development, Goat, Non-descript, Post-natal, Scanning electron microscopy, Testis.

INTRODUCTION

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). In capitalizing on this advantage, SEM has been employed to observe structures within the testis of the non-descript goat. The testes are male gonads and helps in the production of spermatozoa (Banks, 1993). They are housed inside the scrotal sacs suspended by the spermatic cords. They are protected by the testicular tunic consisting of outer skin, tunica dartos, layers of testicular fascia, tunica vaginalis and tunica albuginea (Getty, 1975 and Singh *et al*., 2019). Each testis is divided into so many lobules and each lobule is occupied by the seminiferous tubules (Singh *et al*., 2020). The seminiferous epithelium is surrounded by connective tissue elements and peritubular tubular cells. The contraction of peritubular cells helps in the movement of immature spermatogonial cells from the periphery of the seminiferous tubules towards its lumen, where they are released as mature spermatozoa by the process known as Spermiation (Dellmann and Eurell, 1998 and Samuelson, 2007). Abundant research has been done on the gross and histolomorphology of testis in goat and other domestic animals. But a very scanty literature is available on the ultrastructure of testis in domestic animals and this area is quite explored till date. The ultrastructure

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of spermatogonial cells in the seminiferous epithelium of testis is vital for accessing the reproductive status of the animal. The present study was carried out to develop a baseline data on the ultrastructural characteristics of seminiferous epithelium, rete restis, peritubular cells, Lyedig cells and other components of testis in non-descript goat at different post natal stages under study.

MATERIALS AND METHODS

The present study was conducted from March, 2023 to July, 2024 on the testes 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. The age of the animal is known from the data book of the slaughter houses. The samples of testes 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 testes were also taken at the ultrastructural level by the inbuilt software programming system. The recorded data was subjected to routine statistical analysis (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The testis was surrounded by the tunica albuginea, a thin capsule of connective tissue (Fig 1 and Fig 2), from which thin septum originated and extended into the parenchyma of the testis. Among these septa, the seminiferous tubules were found (Baradi and Rao, 1980, Heyn *et al*., 1997, Mehraein and Negahdar, 2011, Villagra *et al*., 2018 and Kocakoglu *et al*., 2023).

The outer surface of tunica albugenia was folded. These folds were longitudinal and transverse with the depressions or troughs in between them. Ridges and throughs were also noticed in these folds at higher magnification (Fig 3). These ridges and folds may be attributed to the increase in surface area (Saleem *et al*., 2018). No microvilli, intercellular stomata or gaps were evident on the parietal layer (Baradi and Rao, 1980). The filamentous processes were observed in the mesothelial cells of tunica albugenia (Fig 4), which were likely to serve as attachment devices between mesothelial cells (Simionescu and Simionescu, 1977). These cells were subjected to mechanical stress as scrotal, inguinal and abdominal positions of the testis are achievable.

Seminiferous tubules appeared in the form of cylinders which were longitudinally arranged (Fig 5 and Fig 6). Two types of free space continuous with each other were produced: open, triangular interstices between three adjacent cylinders (Fig 6) and flat, biconcave interstices between two adjacent cylinders (Clark, 1975 and Saleem *et al*., 2018). The seminiferous epithelium consisted of spermatogonial cells at different stages of development separated from each other by interstitial connective tissue (Pires *et al*., 2012 and Saleem *et al*., 2018). The seminiferous epithelium comprised of mainly spermatogonia and primary spermatocytes in neonatal goats (Fig 7), where

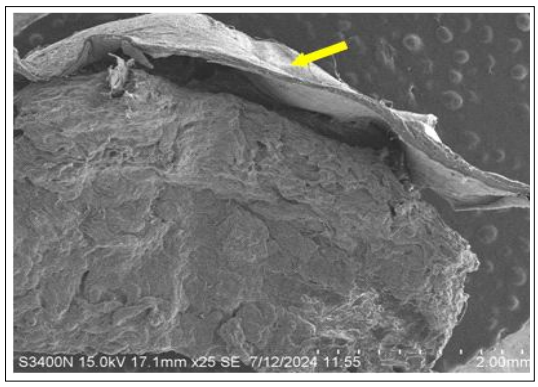

Fig 1: Photograph showing the tunica albugenia and testicular tissue in prepubertal non-descript goat.

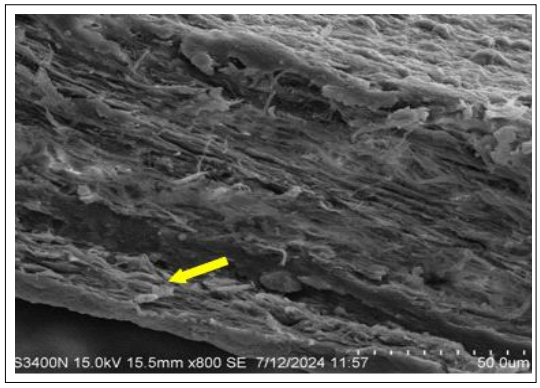

Fig 2: Photograph showing the dense irregular connective tissue component of tunica albugenia in prepubertal non-descript goat.

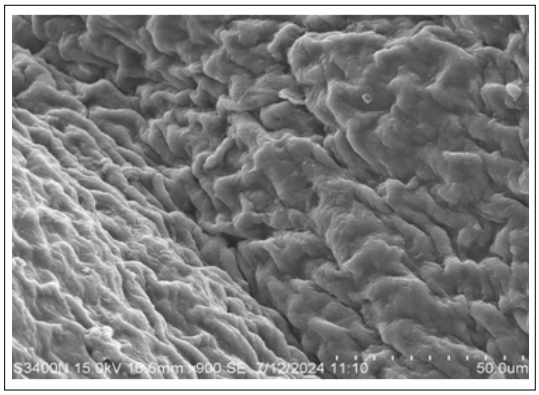

Fig 3: Photograph showing the ridges and throughs on the surface of tunica albugenia in prepubertal non-descript goat.

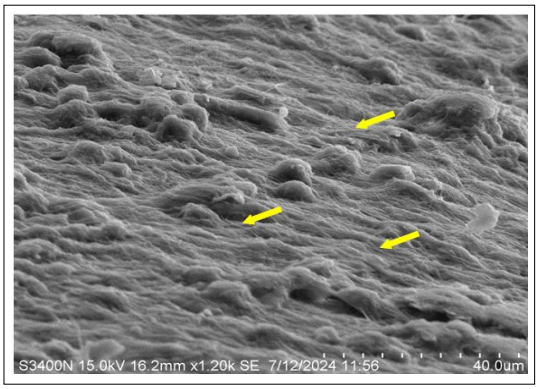

Fig 4: Photograph showing the filamentous processes of tunica albugenia in prepubertal non-descript goat.

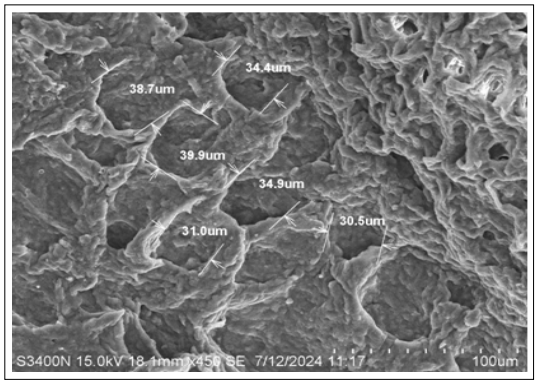

Fig 5: Photograph showing the cylindrical seminiferous tubules in neonatal non-descript goat.

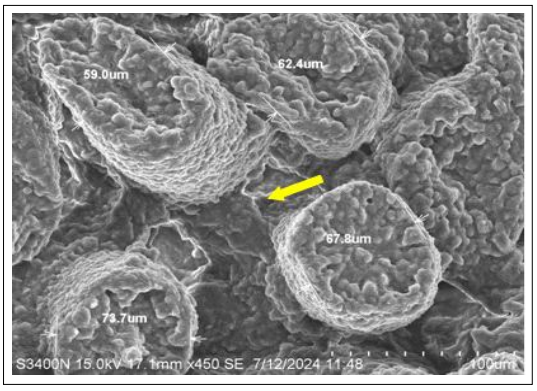

Fig 6: Photograph showing the cylindrical seminiferous tubules with triangular spaces in between in pubertal non-descript goat.

as the spermatids were visible in pre-pubertal and pubertal non-descript goats (Fig 8).

The spermatogonia cells, those initiate the development of the germ line, were isolated oval cells arranged on the tubule basal membrane, in contact with a Sertoli cell (Villagra *et al*., 2018). The primary spermatocytes were rounded cells placed adjacent to the spermatogonial cells (Fig 6 and Fig 8). The secondary spermatocytes were also rounded cells, but were comparatively smaller in size than that of the primary spermatocytes (Fig 8). The early spermatids were oval shaped cells, which were located towards the lumen of the seminiferous tubules along with the elongated late spermatid cells (Fig 8). The present findings were in line with the findings of Villagra *et al*. (2018) in frogs. These cells undergo a process called spermiogenesis, the last stage of spermatogenesis, which involves a complete reorganization of the nucleus (Villagra *et al*., 2018).

Our observations show that, until the spermatogenesis process is completed, all the cells of the germ cell line are in close relationship with at least one Sertoli cell (Choudhary *et al*., 2015 and Villagra *et al*., 2018). The sertoli cells were tall columnar cells with few cell populations in the germinal epithelium (Fig 8). They were extended from the basal membrane of the seminiferous epithelium up to the lumen of the tubule. The Sertoli cells, which nurture maturing germ cells, play an important role in the process of spermatogenesis. Although a number of studies in many mammals have been made on their morphological

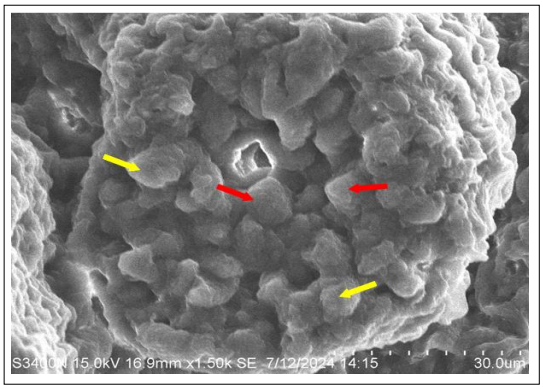

Fig 7: Photograph showing the germinal epithelium of seminiferous tubules with spermatogonia (yellow arrows) and primary spermatocytes (red arrows) in neonatal non-descript goat.

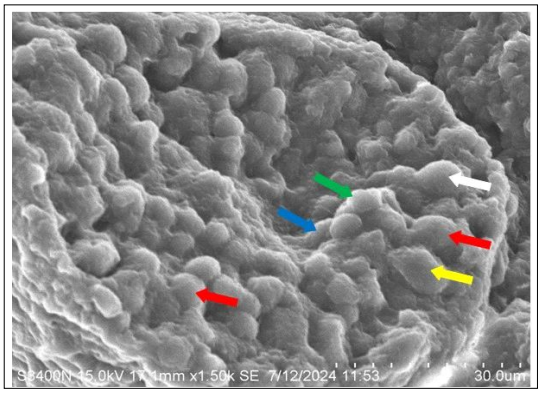

Fig 8: Photograph showing the germinal epithelium of seminiferous tubules with spermatogonia (red arrows), primary spermatocyte (yellow arrow), sertoli cell (white arrow), early spermatid (green arrow) and late spermatid (blue arrow) in pubertal stage.

characteristics using light and transmission electron microscopy (Elftman 1963; Ploen and Ritzen, 1984; Ekstedt *et al*., 1986), scanning electron microscopic configuration was never accurately described, probably because of its complicated morphological features as well as its close attachment to germ cells. In addition, the three-dimensional structure of the Sertoli cell in the rat and monkey was also reconstructed from electron micrographs of semiserial sections (Wong and Russell, 1983; Weber *et al*., 1983; Russell *et al*., 1986). Moreover, Hamasaki and Murakami (1981) described in detail certain processes of the rat Sertoli cell by scanning electron microscopy. The results of this work demonstrated that there is a close relationship between germ cells and Sertoli cells during spermatogenesis, a fact with important functional significance. It is generally accepted that the shape of the Sertoli cell continually changes in association with the progress of spermatids formation (Elftman, 1963). Russell and co-workers (Wong and Russell, 1983; Russell *et al*., 1986) classified the configuration of the Sertoli cell into Type A and Type B. The cell observed in this study corresponds to their Type A. In the basal portion of the seminiferous epithelium, the germ cells seem to be situated in compartments enclosed by adjacent Sertoli cells. Furthermore, they were located in successive recesses as viewed from the basal aspect. These recesses were formed by continuous Sertoli cells, presumably being equivalent to the so-called basal compartment. The tight junctions and desmosomes among somatic cells indicate the existence of a hemato-testicular barrier in charge of maintaining the differences in the composition of the germinal and interstitial compartments. This barrier would give rise to an adequate medium for the development of the germ line cells, as suggested by Yoshida (2016), protecting them from pathogens and toxins that cannot pass through the intercellular junctions. Another function of Sertoli cells is to act as a structural support for germ cells (Gavrila and Mircea, 2001; Griswold and McLean, 2006 and Kopera *et al*., 2010).

The interstitium was made up of randomly arranged collagen bundles (Heyn *et al*., 1997). The arrangement of the interstitial cells of the testis in relation to the capillaries and lymphatic channels, in particular, was easier to visualize in SEM (Connell, 1976). The intertubular vascular connective tissue contained Leydig cells, which were polyhedral or rounded in shape located mainly at wider locations where they were bounded by more than two seminiferous tubules (Fig 9). The present observation was similar to the reports given by Ezeasor (1985) in goat and Villagra *et al*. (2018) in frog. The cells were generally scanty, mostly occurring singly close to vascular elements. The cells were generally oval-elongated in shape (Ezeasor, 1985 and Villagra *et al*., 2018). The peritubular cells in the seminiferous tubules were mostly occupied by the spindle shaped myo-fibrocytes having contractile activity (Fig 10). The regular contraction of these cells, propels the mature germ cells towards the lumen of the seminiferous tubules from their periphery for spermiation.

The mediastinum rete was an elongated epithelial-connective tissue structure, which followed the major axis of the testis (Messing 1877, Klein and Noble-Smith 1880; Hill, 1906; Benoit, 1926; Lasserre and Armingaud, 1934; Schulte, 1937; Short *et al*. 1967; Setchell, 1970 and Dym, 1974). The mediastinal rete testis area contained many cavities of intercommunicating channels supported by connective tissue pillars (Fig 11). conical columns were observed separating the channels of rete testis (Roosen-Runge and Holstein, 1978). The present findings were in agreement with the reports of Burgos *et al*. (1979) in monkey, Nykanen (1980) in rat and Goyal and Williams (1987) in goat. Delicate ridges were present on the walls of the rete, possible overarching subepithelial blood vessels. These ridges were quite prominent in the pubertal period (Fig 12).

The rete and the seminiferous tubules were usually separated by only a thin layer of dense connective tissue (Fig 13). Thus, the seminiferous tubules caused modifications in the contour of the inner rete walls (Nykanen, 1980). Blood vessels with RBC were also

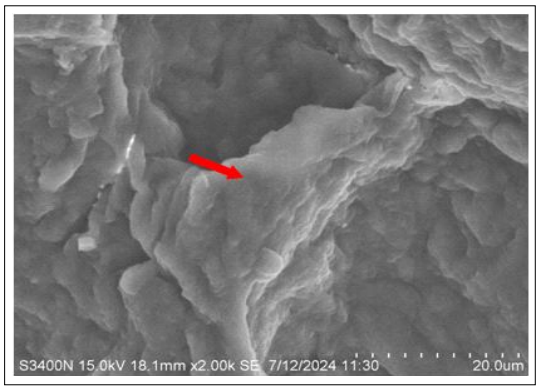

Fig 9: Photograph showing the Lyedig cell in between the seminiferous tubules in neonatal non-descript goat.

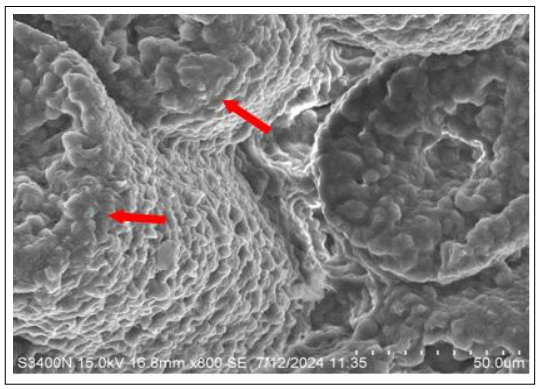

Fig 10: Photograph showing the spindle shaped peritubular myo-fibrocytes around the seminiferous tubule in pubertal non-descript goat.

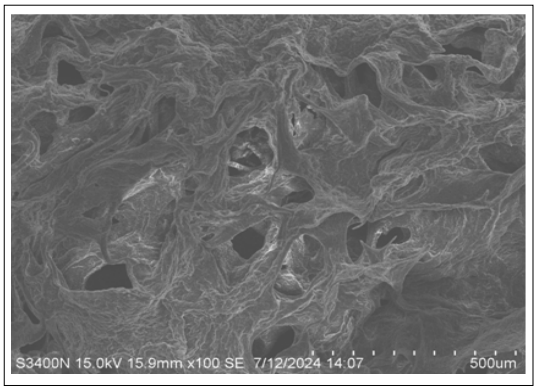

Fig 11: Photograph showing intercommunicating channels of mediastinal rete testis in prepubertal non-descript goat.

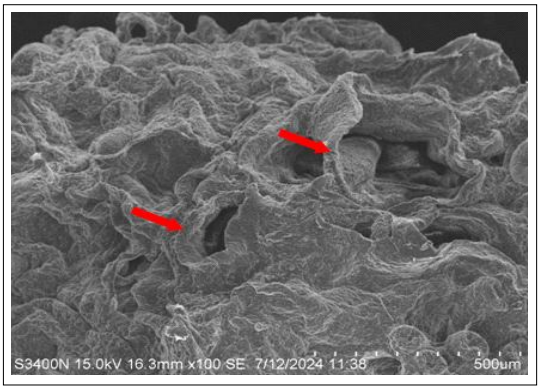

Fig 12: Photograph showing prominent delicate ridges separating the intercommunicating channels of mediastinal rete testis in pubertal non-descript goat.

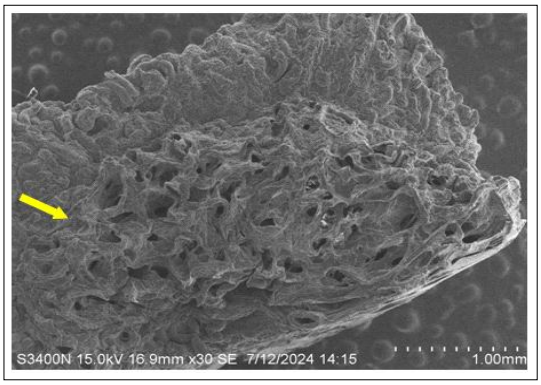

Fig 13: Photograph showing the connective tissue area separating the seminiferous tubules and rete testis in prepubertal non-descript goat.

observed in the areas of rete testis. The rete cavities were nearly devoid of spermatozoa (Nykanen, 1980). The rete testis, regardless of its location, was lined by flat cuboidal cells (Fig 14). The present report was in line with the observation of Goyal and Williams (1987) in goat. The epithelial cells had "cobble-stoned" appearance (Nykanen, 1980). The epithelial cells possess few microvilli and a

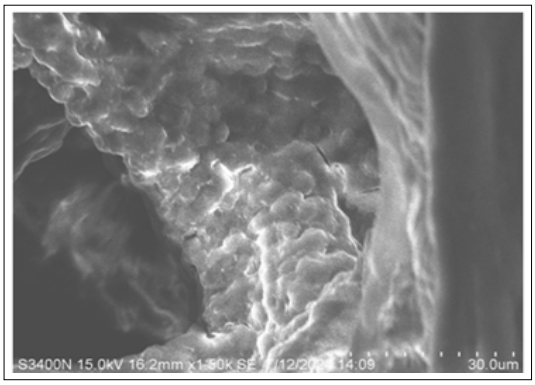

Fig 14: Photograph showing flat cuboidal bulging cells lining the rete testis in prepubertal non-descript goat.

centrally located flagellum at the luminal border (Leeson, 1962 and Goyal and Williams, 1987). The epithelium of the mediastinum rete was lying on a layer of connective tissue, which was usually quite thin (Nykanen, 1980). The subepithelial loose connective tissue had a fine structure typical of this type of tissue and might have a role in the fluid physiology of the rete testis (Nykanen, 1980).

CONCLUSION

The present study helped in developing a baseline data on the ultrastructural characteristics of seminiferous epithelium, rete testis, peritubular cells, Lyedig cells and other components of testis in non-descript goat at different post natal stages under study. The results presented in this work provide data for the knowledge of the reproductive biology of non-descript goat, setting up bases for further studies of molecular mechanisms oriented towards a better understanding of gonadal functions. The appearance of seprmatogonial cell lineage at the various post natal stages in non-descript goat 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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