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Research Article
Temporal Changes in Macro and Microstructure and Seminiferous Epithelium of the Indigenous Sheep Testis from Birth to Puberty

Md. Sheikh Sadi and Md. Royhan Gofur

Department of Veterinary and Animal Sciences, University of Rajshahi, Rajshahi 6205, Bangladesh

Abstract

Background and Objective: Dynamic changes in testicular macro- and microstructures occur throughout sexual maturation. The present work was conducted to account for the temporal changes in the indigenous sheep testis during postnatal development. **Materials and Methods:** A total of twenty-one indigenous sheep, varying in ages from day 0-7 months, were divided into seven age groups (n = 3) at birth or day 0, 1 and 2 week, 1, 2.5, 5 and 7 month of postnatal age. **Results:** A rapid testicular development was observed after 2.5 months of age in indigenous lambs as determined by a significant ($p<0.05$) increase in testicular size. Centrally placed gonocytes migrated centrifugally towards the basement membrane with the progression of age and converted to spermatogonia and placed among the Sertoli cells at the periphery of the sex cords by the 2.5 months of age. Lumenization of seminiferous tubules and stratification of seminiferous epithelium, i.e., initiation of spermatogenesis in indigenous sheep were occurred at the 5th month of age. All types of the cell of spermatogenic lineage were found by 7 months of age. The presence of spermatozoa adhering to the adluminal border of the Sertoli cells as well as in the lumen of seminiferous tubules indicates the onset of puberty, i.e., the establishment of spermatogenesis, was to be established at 7 months of postnatal age in the indigenous sheep. **Conclusion:** Overall, this study represents the first analysis of the sequential changes in macro and microstructure and seminiferous epithelium of the indigenous sheep testis during postnatal development until puberty.

Key words: Biometry, histomorphometry, seminiferous epithelium, lumenization, postnatal development, puberty, indigenous sheep

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Corresponding Author: Md. Royhan Gofur, Department of Veterinary and Animal Sciences, University of Rajshahi, Rajshahi 6205, Bangladesh

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sheep with multi-facet utility (for mainly meat and also wool, skin, manure and to some extent milk) play a vital role in the Bangladesh Agrarian Economy. The size of the sheep population in the country is estimated to be 3.6 million and the size is increasing day by day[1]. Most of the sheep are the non-descript indigenous type with few crossbreds. The traits of sheep, like well adapted to hot and humid agro-climatic conditions, capable of lambing twice a year each with multiple births, make them popular with farm owners[2]. Indigenous non-descript sheep of Bangladesh is a valuable genetic resource, especially in their contribution to the overall genetic diversity and their stable production under rural condition even with changing climate. In recent years, some government institutes (Department of Livestock Services (DLS) and Bangladesh Livestock Research Institute (BLRI)) started to implement community-managed sheep breeding programs for their genetic improvements in some sheep potential areas of Bangladesh[3].

The testis is a primary reproductive organ for male mammals that produce sperm and secrete androgenic hormones and is also responsible to maintain secondary male characteristics[4]. The testis is composed mainly of the seminiferous tubules, the structural and functional unit of the testis, which consist of the highly specialized epithelium made up of germinal cells and supporting (Sertoli) cells[5]. Postnatal ontogenesis of the testis encompasses the period until the first wave of spermatogenesis is completed and set a framework for the future continuous sperm production[6,7]. The establishment of spermatogenesis, i.e., the onset of puberty as reflected by the first appearance of spermatozoa in the lumen of seminiferous tubules occurs when the seminiferous tubules are fully developed[8]. The pubertal age has a direct effect on the design of progeny testing as well as on the successful reproductive management of the domestic animals[9]. The process of spermatogenesis is different between primates and rodents. Spermatogenesis starts many months or years after birth in primates, whereas within a few days after birth in rodents[10]. It is not known when sex cords/seminiferous tubule development becomes complete in indigenous sheep. Moreover, good reproductive management of domestic animals requires detailed information about the onset of puberty and testicular maturation.

The development of mammalian testis is a highly complicated and sophisticated process. Postnatal developmental studies on the male genital system at various ages at least until puberty, particularly the testis, are important to know the growth and development. Fragmentary reports on postnatal ontogenesis/development of testis of Ghezel sheep[8], Ouled Djellal sheep[11], D'Man sheep[12], etc., are available. Although the indigenous non-descript sheep of Bangladesh is a valuable genetic resource, no systemic study has been conducted on the postnatal development of testis of these indigenous sheep. This paper accounts for the temporal changes in macro-and microstructure and seminiferous epithelium of the indigenous sheep testis from birth to puberty. This work is the first in reporting the postnatal development of the testis in indigenous sheep until puberty which will provide valuable information to the anatomists, pathologists and theriogenologists.

MATERIALS AND METHODS

Animals and study area: Indigenous sheep varying in postnatal ages from day 0-7 months (the day of birth was regarded as day 0, d0) were reared with their dams under standard housing and feeding conditions. The sheep were divided into 7 age groups, namely, Group I: At birth or d0, Group II: 1 week, Group-III: 2 weeks, Group-IV: 1 month, Group V: 2.5 months, Group VI: 5 months and Group-VII: 7 months consisting of three animals in each group (n = 3). Birth records were kept accurately to calculate the age of the studied sheep. The present study was carried out on testes of twenty-one indigenous sheep (*Ovis aries*) for postnatal ontogenetic studies in the Histology Laboratory, Department of Veterinary and Animal Sciences, University of Rajshahi, Bangladesh from July, 2020 to December, 2021. Animal procedures were performed following the guidelines set by the Institutional Animal, Medical Ethics, Biosafety and Biosecurity Committee of the University of Rajshahi, Bangladesh (Memo No. 293(13)/320/IAMEBBC/IBSc).

Biometrical and histomorphometric studies: The sheep were sedated by intramuscular injection of Sedil at 0.6 mg kg^{-1} b.wt. (Sedil® injection containing 5 mg diazepam mL^{-1}, Square Pharmaceuticals Ltd., Dhaka, Bangladesh) and subsequently anaesthetized locally by injecting the local anaesthetic, Jasocaine at 4 mg kg^{-1} b.wt. (Jasocaine® injection containing 20 mg 2% Lidocaine Hydrochloride mL^{-1}, Jayson Pharmaceuticals Ltd., Dhaka, Bangladesh) at the upper part of the scrotum. After induction of proper level of anaesthesia, the testes were collected by cutting at the lateral side of the scrotum and the left testes were subsequently fixed in 10% formalin solution for microscopic study. Right testes were used for the gross biometrical study. The measurements of the length and breadth of the right testis were taken with the help of a vernier-calliper and the readings were noted against a

centimetre scale. The weight of the right testis was measured with a triple beam balance and the readings were recorded in grams. All the formalin fixed-left testes were processed for paraffin sections. Sections were cut at 5 µm thickness using a sliding microtome (Thermo, Germany) and then stained with routine hematoxylin and eosin stain according to Gofur *et al.*[13] for microscopic study. Stained sections were examined under 10×, 20× and 40× magnifications of a compound microscope. The images of the tissue sections were grabbed by using a photographic microscope system (Digital camera model: LC-20, Labomed, Inc., USA fitted with a microscope, Model MBL-2100, Krüss, Germany).

Statistical analyses: Statistical analyses were performed with Microsoft Excel and statistical software available online (https://astatsa.com/OneWay_Anova_with_TukeyHSD/). All values represent Mean±SE. Differences in gross and microscopic values among the different postnatal ages were evaluated by One-way Analysis of Variance (ANOVA), followed by Tukey's HSD *post hoc* analysis. The $p<0.05$ were considered significant.

RESULTS

Gross biometrical changes in indigenous sheep testis during the postnatal development: To study the relative gross biometrical values of indigenous sheep testis during the postnatal development, length, breadth and weight of the testes of day 0, 1 and 2 week, 1, 2.5, 5 and 7 months old indigenous sheep were measured. The representative figures of postnatal developing testis of indigenous sheep of different age groups were presented in Fig. 1 and their biometrical values (length, breadth and weight) were presented in Table 1. The length, breadth and weight of testis were increased with the advancement of the age of lambs. The biometrical values of the testes were varied in different postnatal age groups. Up to 1 month of age, the testicular development was slow indicated by insignificant biometrical values within age groups (d0 vs. 1 w vs. 2 w vs. 1 m) and then a rapid testicular development was observed indicated by a significant difference in biometrical values within age groups (1 vs. 2.5 vs. 5 vs. 7 m) until puberty ($p<0.05$). The testicular length and breadth were 7-8 times at puberty than that of at birth whereas, the weight was around 200 times higher at puberty than that of at birth of lambs.

Table 1: Biometrical values (Mean±SE) of the testis in indigenous sheep at different postnatal ages

Postnatal ages	Length (cm)	Breadth (cm)	Weight (g)
At birth or day 0 (d0)	0.92±0.067^{a}	0.54±0.077^{a}	0.26±0.022^{a}
1 week (1 w)	1.11±0.083^{a}	0.67±0.082^{a}	0.38±0.031^{a}
2 weeks (2 w)	1.43±0.063^{a}	0.85±0.085^{ab}	0.69±0.042^{a}
1 month (1 m)	1.69±0.088^{ab}	1.02±0.061^{ab}	1.15±0.086^{ab}
2.5 months (2.5 m)	1.95±0.11^{abc}	1.32±0.13^{b}	2.69±0.18^{b}
5 months (5 m)	3.83±0.16^{d}	2.74±0.10^{c}	14.61±0.34^{c}
7 months (7 m)	7.17±0.21^{e}	4.59±0.17^{d}	50.23±0.79^{d}

Values with different superscripts in a column indicate significant differences among postnatal ages ($p<0.05$)

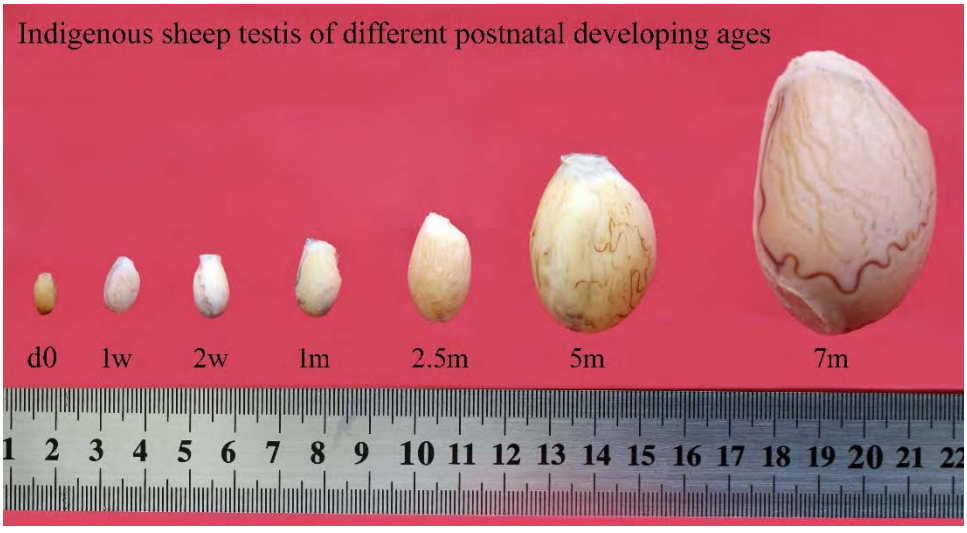

Fig. 1: Gross photographs of testes of indigenous sheep of different postnatal ages from birth to puberty
d0: At birth or day 0, 1 w: 1 week, 2 w: 2 weeks; 1 m: 1 month, 2.5 m: 2 months and a half, 5 m: 5 months and 7 m: Seven months of postnatal age

Table 2: Histomorphometric values (Mean±SE) of the testis in indigenous sheep at different postnatal developing ages

Postnatal ages	Thickness of tunica albuginea (μm)	Diameter of seminiferous tubules (μm)
At birth or day 0 (d0)	112.88±6.11[a]	31.78±2.14[a]
1 week (1 w)	125.63±8.13[ab]	37.67±2.82[a]
2 weeks (2 w)	135.50±8.01[ab]	44.33±3.22[a]
1 month (1 m)	149.88±12.17[ab]	54.11±3.48[ab]
2.5 months (2.5 m)	170.13±11.81[b]	71.44±4.17[b]
5 months (5 m)	206.75±10.74[c]	114.22±6.44[c]
7 months (7 m)	312.38±13.58[d]	176.22±8.21[d]

Values with different superscripts in a column indicate significant differences among postnatal ages ($p<0.05$)

Changes in microstructure and seminiferous epithelium of the indigenous sheep testis during the postnatal development: The microscopic studies revealed the sequential changes in the thickness of tunica albuginea and diameter of seminiferous tubules and also in the seminiferous epithelium at different ages during the postnatal development of indigenous sheep testis. During the testicular postnatal development, a gradual increase in the thickness of tunica albuginea was observed with the advancement of postnatal age of indigenous sheep. Though the thickness of tunica albuginea was varied in different postnatal age groups, the significant differences ($p<0.05$) among the postnatal age groups were observed from 2.5 months of age and onward (Table 2). Tunica albuginea was increased in thickness more than 2.5 times at the 7th month of postnatal age than that at birth (day 0).

At birth (day 0 old lamb), the testicular parenchyma consisted of seminiferous tubules interspersed within the interstitial tissue. The tubular diameter was 31.78±2.14 μm (Table 2) and the tubules were mostly spherical with a few convoluted types. No lumen was observed within the tubules, thus, these tubules were called the sex cords. The interior of the sex cords was filled with acidophilic ground substances. These solid sex cords contained peripheral Sertoli cells (resting on the basement membrane) with a mostly ovoid-shaped nucleus and light cytoplasm and large central germ cells (gonocytes) with the centrally placed spherical nucleus and acidophilic cytoplasm (Fig. 2a). A distinct basement membrane was observed around each sex cord which was surrounded by one or more layers of peritubular myoid cells (Fig. 2a). A large number of stromal or interstitial cells including Leydig cells were present in the interstitial spaces (stroma) among the sex cords or tubules (Fig. 2a).

With the advancement of the age of lambs, the sex cords or seminiferous tubules showed a growing trend in their diameter. The diameter of seminiferous tubules of a pubertal ram was 176.22±8.21 μm which was about 5.5 times broader than that of day 0. Though the diameter of seminiferous tubules was varied in different postnatal developing age groups, the significant differences ($p<0.05$) among postnatal age groups were observed from 2.5 months of age and onward (Table 2). Moreover, a decreased intertubular (stromal) space along with the increase in tubular convolution was observed with the advancement of postnatal age, hence, a gradual decrease of stromal cells was also observed during the postnatal development of indigenous sheep testis.

The structure of seminiferous tubules of up to 2.5 months of age was almost similar to that of day 0, but the centrally placed gonocytes started migrating centrifugally towards the basement membrane of the seminiferous tubules with the progression of age and which was later termed as spermatogonia placed among the Sertoli cells at the periphery of the tubules conforming the basal cell layer of the seminiferous tubules by the 2.5 months of postnatal age (Fig. 2b-e). The presence of the lumen in the centre of the seminiferous tubules was observed at 5 months of postnatal age and onward (Fig. 2f). At 5 months of age, the seminiferous tubules were lined by stratified epithelium comprising the spermatogonia and primary spermatocytes in most tubules and also secondary spermatocytes in a few tubules among Sertoli cells (Fig. 2f). All types of cells of spermatogenic lineage were found in the seminiferous tubules of 7 months aged indigenous sheep testis, especially the spermatozoa in the lumen of the seminiferous tubules as well as attached to the Sertoli cells at their ad luminal border (Fig. 2g). Few detached degenerated/apoptotic cells were found in the lumen of some seminiferous tubules of both 5 and 7 months aged testis. Histological findings showed that indigenous sheep attained puberty at 7 months of postnatal age indicated by the abundance of spermatozoa in the lumen of seminiferous tubules, along with elongated spermatids and spermatozoa adhered with Sertoli cells at their ad luminal border. The overall developmental patterns of seminiferous tubules in the testis of indigenous sheep during the postnatal development until puberty are presented in Fig. 3.

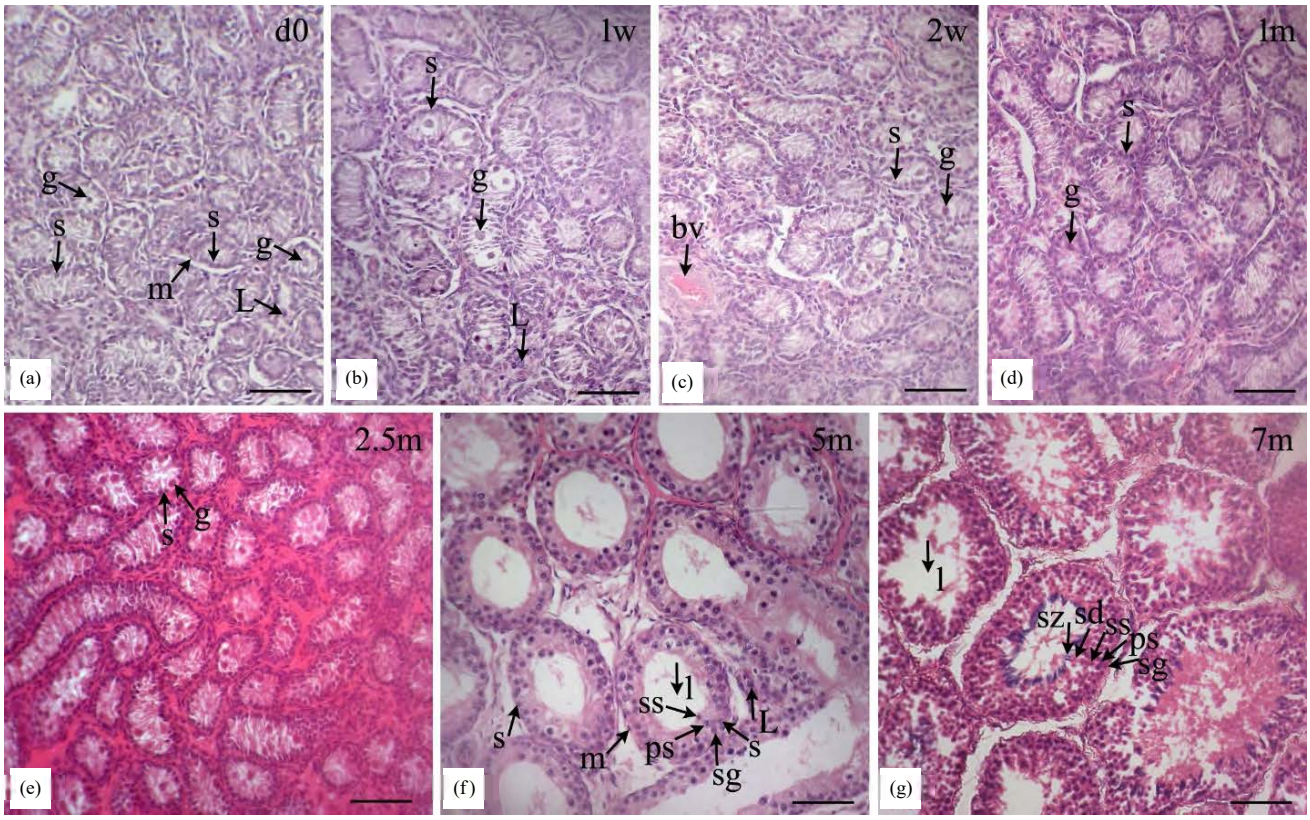

Fig. 2(a-g): Histological micrographs show the development of seminiferous tubules of indigenous sheep during postnatal development

Sections were stained with hematoxylin and eosin stains. Seminiferous tubules or sex cords at birth or day 0 (d0) to 2 months and a half (2.5 m) contained only Sertoli cells (peripherally placed) and gonocytes (centrally placed)/prespermatogonia (placed among the Sertoli cells) with no lumen. A distinct lumen was present in the tubules of 5 and 7 months. Seminiferous tubules with stratified seminiferous epithelium, containing the spermatogonia, primary spermatocytes and occasionally secondary spermatocytes were observed at 5 m of postnatal age. Seminiferous tubules with all types of cells of the spermatogenic lineage including spermatozoa adhered with Sertoli cells at their ad luminal border and in the lumen was observed at 7 m of postnatal age indicating the onset of puberty in indigenous sheep occurred at this age. d0: At birth or day 0, 1 w: 1 week, 2 w: 2 weeks, 1 m: 1 month, 2.5 m: 2 months and a half, 5 m: 5 months, 7 m: 7 months of postnatal age, g: Gonocyte, s: Sertoli cell, sg: Spermatogonia, ps: Primary spermatocytes, ss: Secondary spermatocytes, sd: Spermatids, sz: Spermatozoa, m: Peritubular myoid cell, L: Stromal cells including Leydig cells, l: Lumen, bv: Blood vessel and scale bar 50 µm

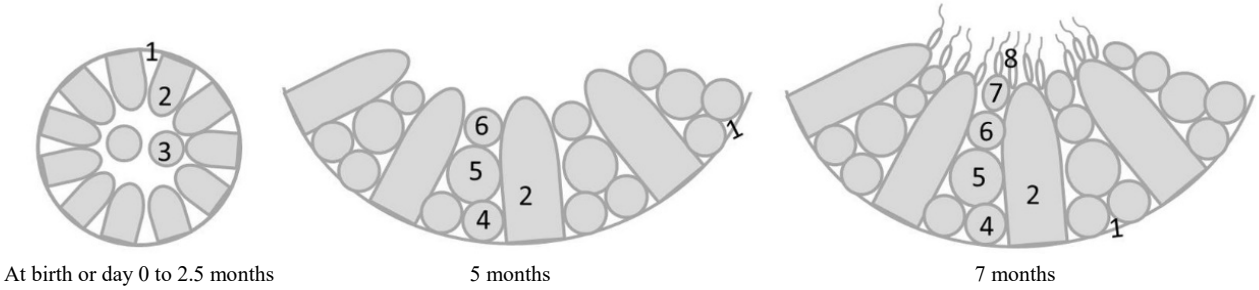

Fig. 3: Schematic drawings illustrating the overall postnatal developmental patterns of seminiferous tubules in the testis of indigenous sheep

1: Basement membrane, 2: Sertoli cell, 3: Gonocyte, 4: Spermatogonia, 5: Primary spermatocyte, 6: Secondary spermatocytes, 7: Spermatids and 8: Spermatozoa

DISCUSSION

The postnatal period, which extends from birth to the period of puberty, is one of the most important stages of reproductive life that will allow the ram to acquire reproductive abilities, i.e., the ability to produce sperm[14]. Postnatal studies are important to know the growth and development of the male genital organs[9]. Postnatal testicular development is critical in the establishment of future fertility. For monitoring the testis for normality and assessing sperm production potential, biometry and histomorphometry of the testis are important components. In particular, testicular size or weight predicts the present and future sperm production in animals[15]. The gross biometrical values of indigenous sheep testis of the present study were gradually increased with the advancement of age which was in agreement with previous studies[12,16] in different breeds of sheep. Results showed that testicular development of indigenous sheep was slow in the early postnatal period and more rapid after 2.5 months of postnatal age until puberty. The value of testicular parameters (weight, length and breadth) increases at a greater rate around after 9-10 weeks after birth, because of an association with remarkable changes in length and diameter of seminiferous tubules[12,17]. The trend of postnatal testicular growth is almost similar to in Ouled Djellal lambs observed by Belkhiri *et al.*[11], D'Man ram lambs observed by Boukenaoui *et al.*[12] and Kivircik (Western Thrace) ram lambs observed by Koyuncu *et al.*[16]. Low serum testosterone concentrations and high androstenedione concentrations are responsible for the initial slow phase of testicular growth and vice versa is for the later rapid growth phase of testis in animals[18].

The gross biometrical values of the testes varied among the different postnatal age groups. Moreover, no significant difference was observed in testicular matrices from birth or day 0-1 month of age ($p>0.05$) and the testicular matrices significantly increased from 2.5 months to until puberty (7 months) ($p<0.05$) in indigenous sheep. The gross morphological values of the present study are inconsistent with the findings of some parameters in Kivircik (Western Thrace) ram lambs by Koyuncu *et al.*[16]. The results of the present study also collaborated with the results of da Luz *et al.*[19] in buffalo bulls. Some inconsistency with previous studies was also found, testicular length and weight were lower than that observed in Ouled Djellal lambs by Belkhiri *et al.*[11], D'Man ram lambs by Boukenaoui *et al.*[12], Blackbelly sheep by Herrera-Alarcón *et al.*[17]. The variations might be due to differences in breed, nutritional level, the physical condition of the examined sheep, agro-climatic condition, housing and other managemental or experimental procedures.

Microscopically, the tunica albuginea covers the testis and the testicular parenchyma is mainly composed of seminiferous tubules within the stroma/interstitial tissue. Primordial germ cells/gonocytes migrate from the yolk sac into the primary sex cords which eventually differentiate into testicular cords (seminiferous cords) and finally to seminiferous tubules lined by seminiferous epithelium[20]. The thickness of the tunica albuginea followed a gradually increasing trend during the postnatal development until puberty in indigenous sheep. A similar finding was also found in Assam goats[21]. However, Gofur *et al.*[13] reported a gradual decrease in thickness of the tunica albuginea in post-pubertal indigenous bull, indicating the tunica albuginea increases in thickness until puberty and/or maturity and then decrease at a certain point and maintains its thickness in animals.

A similar increasing trend was also found in the diameter of the seminiferous tubules with the advancement of age, as recorded in Ghezel ram lambs[8] and also in Assam goat[21] and Tokara goat[22]. The diameter of the seminiferous tubules found in the study was almost similar to that in Ghezel ram lambs[8] at puberty (7 months of age). An increase in the area of occupancy in the testis by seminiferous tubules indicates testicular growth. In neonatal kid testes, the interstitial tissue/stroma (area/volume) was much higher (65%) than the parenchyma (35%), whereas in pubertal animals it maintained this ratio as 13:87[23]. This indicated a faster growth rate of parenchyma to meet the (spermatozoa) requirement of breeding animals at puberty. This greater parenchyma is achieved by the drastic increase in the diameter of seminiferous tubules around the time of puberty. The present study recorded a significant increase in tubular diameter from 2.5 months of postnatal age and more precisely, between 2.5 and 5 months old sheep and between 5 and 7 months old sheep indicated a rapid growth of the tubules at the onset of spermatogenesis and before the onset of puberty in indigenous sheep, respectively. In contrast to the gradual increment of parenchymal occupancy by seminiferous tubules, we observed a gradual decrease in the number of stromal or interstitial cells. A gradual decrease in intertubular space along with a decreased number of stromal cells was also recorded in Gaddi goats[23], Assam goats[21] and also in ICR mice[20] during their postnatal development.

From birth to 2.5 months of postnatal age, the sex cord or seminiferous tubules contained only two types of cells (Sertoli cells and germ cells or gonocytes). At birth, Sertoli cells were placed peripherally of the sex cord, i.e., resting on the basement membrane and large spherical gonocytes were placed centrally. The gonocytes were started migrating centrifugally towards the basement membrane of the sex cords with the advancement of age and placed among the Sertoli cells at the periphery of the sex cords (at this stage, these cells were termed prespermatogonia) comprising the basal cell layer in almost all of the seminiferous tubules by the 2.5 months of postnatal age of indigenous sheep. Nazari-Zenouz *et al*.[8] observed a similar pattern of centrifugal movement of gonocytes and placement of prespermatogonia among Sertoli cells in Ghezel ram lambs and Sarma and Devi[24] in Assam goats. Lumenization of the seminiferous tubules was first observed at 5 months of postnatal age in indigenous sheep. Nazari-Zenouz *et al*.[8] also observed lumenization in seminiferous tubules at 4 months of postnatal age in Ghezel ram lambs. However, the age of lumenization of the seminiferous tubules was reported to be variable in domestic animals.

Stratification of the seminiferous epithelium was observed in the seminiferous tubules at 5 months of postnatal age and stratified epithelium comprised the spermatogonia, primary spermatocytes and occasionally secondary spermatocytes in some seminiferous tubules. Nazari-Zenouz *et al*.[8] reported the initiation of spermatogenesis at around the 4 and 5 months of postnatal age in Ghezel ram lambs as spermatocytes were first seen in the seminiferous epithelium at this age. The major growth (in size) of testis occurs after the onset of spermatogenesis[25]. A drastic and significant increment in diameter of seminiferous tubules around the onset of spermatogenesis at 5 months of postnatal age of indigenous sheep was also found.

All types of the cell of spermatogenic lineage were found in the seminiferous tubules of 7 months old testis indicating the development of seminiferous tubules was complete at this age. The present study reported the first appearance of spermatozoa attached to the ad luminal border of the Sertoli cells as well as in the lumen of the seminiferous tubules at 7 months of postnatal age indicating the onset of puberty occurred at this age in indigenous sheep of Bangladesh. By histological examination, the first appearance of spermatozoa in testis had been reported at 5 weeks of age in mice[10], 24 weeks in Assam goats[24] and 28 weeks in Dagestan rock sheep[26] and Ghezel ram lambs[8]. The age of puberty in males differs in animals and even breeds to breed of the same species and puberty is indicated by the appearance of spermatozoa in the lumen of the seminiferous tubules in the testis or in the ejaculated semen[27]. Histological findings of the present study reported that indigenous sheep attained puberty at 7 months of age indicated by the abundance of spermatozoa in the lumen of seminiferous tubules, suggesting the indigenous young ram can fertilize females at about 7 months of age, but this age may differ depending on the size of the body, individual, race, diet and the season of birth. Nazari-Zenouz *et al*.[8] also reported that Ghezel ram lambs attain puberty by 7 months of postnatal age. The precise time of completion of the first wave of spermatogenesis and the first appearance of spermatozoa in testis is crucial in the study of spermatogenesis and the determination of the age of puberty in animals. In the present study, the first appearance of spermatozoa in the lumen of seminiferous tubules of 7 months aged indigenous sheep was found. As the present study was made at 5 and 7 months of age, the possibility of the first appearance of spermatozoa after 5 months and prior or at 7 months of age could not be ascertained in the present work. A further detailed study is needed to determine the exact time of completion of the first wave of spermatogenesis and the first appearance of spermatozoa in the testis of indigenous sheep.

CONCLUSION

Indigenous non-descript sheep of Bangladesh is a valuable genetic resource, yet no detailed and systemic study has been conducted on the postnatal ontogenesis of testis of this sheep. The present study accounts for the sequential changes in the testis of indigenous sheep during postnatal development from birth to puberty. The presence of spermatozoa in the tubular lumen as well as adhering to the ad luminal border of the Sertoli cells indicates the onset of puberty, i.e., the establishment of spermatogenesis, was to be established at 7 months of postnatal age in the indigenous sheep. This study is the first to record the age-based changes in macro-and microstructure and seminiferous epithelium of the indigenous sheep testis during the postnatal development from birth until puberty. Further study is needed to determine the time of completion of the first wave of spermatogenesis in indigenous sheep.

SIGNIFICANCE STATEMENT

The study notices a rapid testicular development in indigenous lambs after 2.5 months of age during the postnatal development that will be beneficial in progeny testing of indigenous sheep. Lumenization of seminiferous tubules and

stratification of seminiferous epithelium, i.e., initiation of spermatogenesis in indigenous sheep were started at the 5th month of postnatal age. All types of the cell of spermatogenic lineage were found by 7 months of age. The onset of puberty, i.e., the establishment of spermatogenesis, was observed to be established at 7 months of postnatal age in the indigenous sheep. The findings of this study provide valuable information to the anatomists, pathologists and theriogenologists that will help in further studies, especially to determine the time of completion of the first wave of spermatogenesis in indigenous sheep.

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