



ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(4): 1479-1482
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www.thepharmajournal.com
Received: 20-02-2023
Accepted: 25-03-2023

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Study of spermatogonial cells in Deccani Ram

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Abstract

The present study was performed on 30 apparently healthy Deccani rams of prepubertal (4-7 months of age), pubertal (9-12 months of age) and sexually mature (More than 1.5 years) groups. The testicular parenchyma was made up of seminiferous tubules and interstitial tissue. The seminiferous tubules of prepubertal group contained one or two cell layer thick spermatogonial cells, which were situated on basement membrane. In pubertal and sexually mature groups, it consisted of the tubular stratified epithelium *viz.*, type 'A' and type 'B' spermatogonia, primary spermatocytes, secondary spermatocytes, rounded and elongated spermatids and spermatozoa. The primary spermatocytes were also noticed in different developmental stage *viz.*, leptotene, zygotene, pachytene and diplotene.

Keywords: Testis, Deccani Ram, spermatogonial cells

Introduction

Sheep farming has contributed significant to the productivity, stability and sustenance of many farming systems. Sheep with its multi-facet utility for wool, meat, milk, skins and manure, form an important component of rural economy. Sheep production is considered as a main economic activity for maximizing income and providing better income to the rural poor of the tropical countries.

In male testicular size is the criteria from the physiological, genetic and practical perspective to improve the reproductive performance of related females (Walkley and Smith, 1980) [23]. The testis is the main organ responsible for the production testosterone and spermatozoa. The testosterone along with its stimulating effects on male reproduction induces a positive effect on body growth (Hafez, 1980) [13]. The testicular parenchyma is composed of seminiferous tubules and interstitial tissue. The spermatogonial cells of seminiferous tubules produces the sperms. The mere information is available on spermatogonial cells of sheep, particularly to Deccani ram, hence the experiment was carried out.

Material and Methods

The present work was carried out on spermatogonial cells in Deccani ram in different age groups. The age of animals has calculated from dentition (Hillson, 1986) [14]. These animals were divided into three groups according to their age.

Group I (Pre Pubertal)	4 to 6 months
Group II (Pubertal)	7 months to one year
Group III (Sexually mature)	1.5 years and above

The testes for this experiment were collected from 30 apparently healthy rams. The specimens were collected immediately after the slaughter of the animals from the local slaughter house nearby Shirwal. The histological work was carried out by taking the tissue pieces of 3-5 mm in dimensions from different regions of the testis and fixed in 10% neutral buffer formalin. These tissue pieces were treated with a routine method of dehydration in ascending grades of alcohol (ethanol), cleared in xylene and embedded in paraffin wax as per Drury *et al.* (1967) [8]. The paraffin blocks were sectioned at 3-5 μ m thickness. The prepared slides were stained with Harri's Hematoxylin and Eosin staining method (Mukharjee, 1992) [16] and observed under microscope.

Results and Discussion

In the prepubertal group, the testicular parenchyma was divided into several lobules and these lobules consisted of numerous seminiferous tubules. Each seminiferous tubule showed a distinct lumen, primitive Sertoli cells and large germ cells, which rested on the basement membrane.

The germ cells were present in one or two cell layers thick and contained mostly type 'A' spermatogonia. These cells had spherical nuclei with homogenous nucleoplasm. Similar findings were mentioned by Hafez (1980) [13], Odabas and Kanter (2008) [17] in ram lambs, Bashir *et al.* (2012^b) [5] in Bakerwali goat, Sarma and Devi (2012) [22] in Assam goat, Elzoghby *et al.* (2014) [9] in Ovine and Pathak *et al.* (2014) [19] in goat.

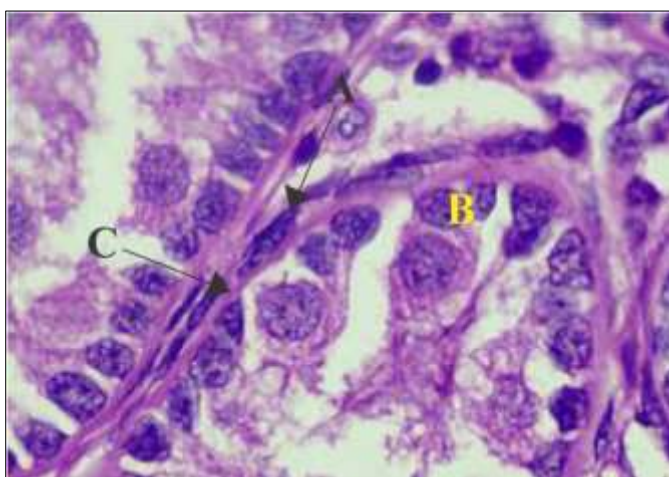


Fig 1: Microphotograph of testis from the pubertal group showing A- Myoid cells, B- Type A Spermatogonia, and C- Basement membrane of the seminiferous tubule. (HE—400X)

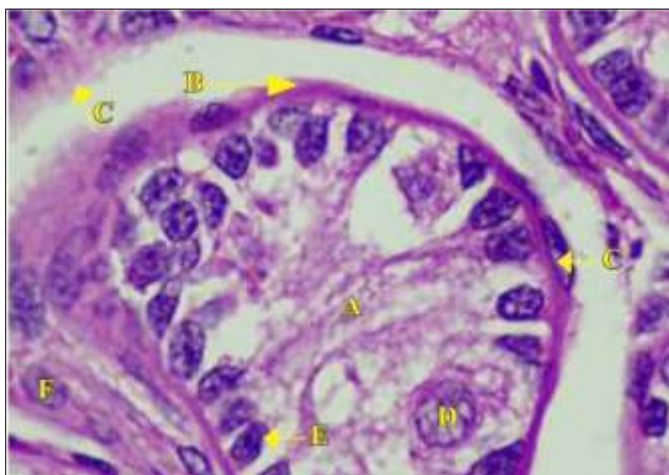


Fig 2: Microphotograph of the testis from the pubertal group showing A- Lumen of the seminiferous tubule, B- Basement membrane of the seminiferous tubule, C-Fibroblast, D- Fibroblast, E- Primary Spermatocytes (HE- 400X)

In the pubertal and sexually mature groups, the seminiferous tubules showed distinct lumen, Sertoli cells and the tubular stratified epithelium *viz.*, type 'A' and type 'B' spermatogonia, primary spermatocytes, secondary spermatocytes, round spermatids, elongated spermatids and spermatozoa. The germinal epithelium rested on the basement membrane of the tubule. The type 'A' spermatogonia were located close to the basement membrane with their successive

descendants progressive towards the lumen of the seminiferous tubule. These findings were in accordance with the results mentioned by Dellmann and Brown, (1986) [7] in domestic animals, Clemmons *et al.* (1995) [6] in equines, Onyango *et al.* (2000) [18] in goat Gofur *et al.* (2008^b) [11] in bull, Bashir *et al.* (2012^b) [5] in Bakerwali goat and Saida and Djahida (2014) [21] in ram.

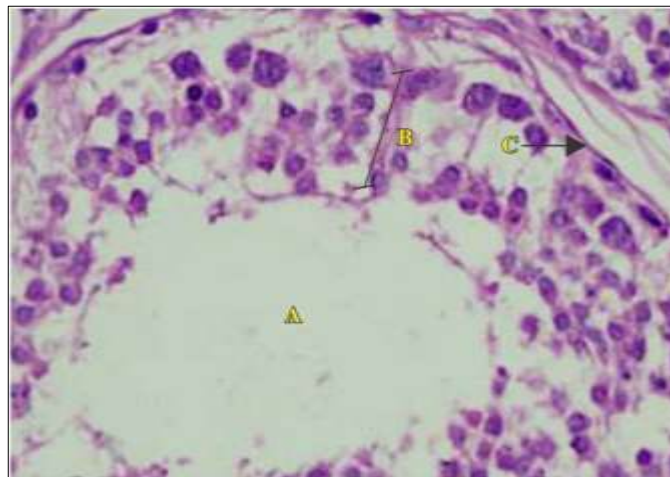


Fig 3: Microphotographs of testis from pubertal group showing A- Lumen of seminiferous tubule, B- Spermatocytes, C- Basement membrane of seminiferous tubule (HE- 400X)

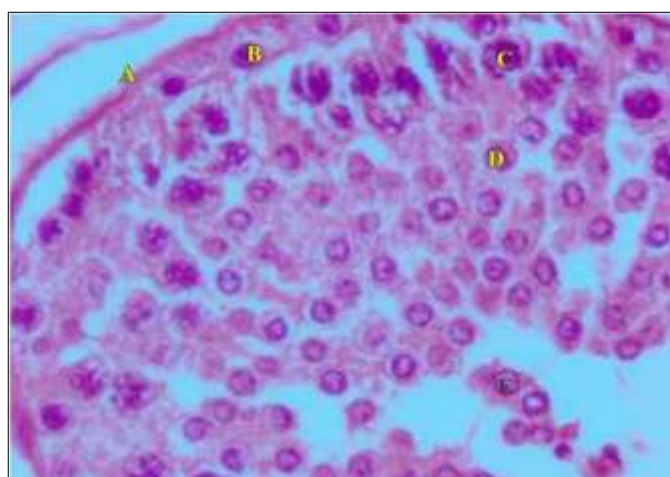


Fig 4: Microphotograph of the testis from pubertal group showing A- Basement membrane of seminiferous tubule, B- Spermatogonia, C- Primary Spermatocytes, D- Secondary Spermatocytes, E- Rounded spermatids (HE- 400X)

The Spermatogenic cell includes spermatogonia, spermatocytes and spermatids. The basal portion of the seminiferous tubule contained spermatogonia, while the middle contained spermatocytes and surface region content spermatids. The type 'A' and type 'B' spermatogonia were distinguished in the present study on the basis of the distribution pattern of chromatin material and the size of spermatogonia. These findings were in agreement with Bashir *et al.* (2012^b) [5] in Bakerwali goat and Sarma and Devi, (2012) [22] in Assam goat. The type 'A' spermatogonia were a large, oval and elliptical shape, which was found close to the basement membrane of the seminiferous tubule. They had large and oval nuclei with uniform chromatin material. One or more nucleoli also observed randomly within the nucleus. The long axis of these cells was parallel to the basement

membrane. These findings were in accordance with the findings of Bashir *et al.* (2012^a)^[4] in Bakerwali goat, Pathak *et al.* (2014)^[19] in goat and Ahmed (2016)^[1] in Naemi rams. The type 'B' spermatogonia were comparatively smaller than type 'A'. Their shape was rounded and the nucleus was spherical. The chromatin material was usually observed as clumps and adhered to the nuclear membrane. The nucleoli were usually one or more and mostly observed at the center of the nucleus. Similar observations were noted by Sarma and Devi (2012)^[22] in Assam goat and Ahmed (2016)^[1] in Naemi rams.

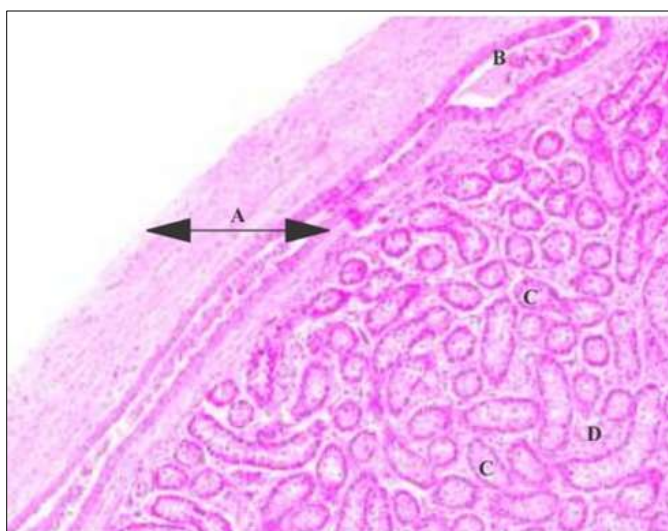


Fig 5: Microphotograph of testis from prepubertal group showing A- Tunica albuginea, B- Blood Vessels, C- Seminiferous tubules, D- Interstitial tissue (HE- 40X)

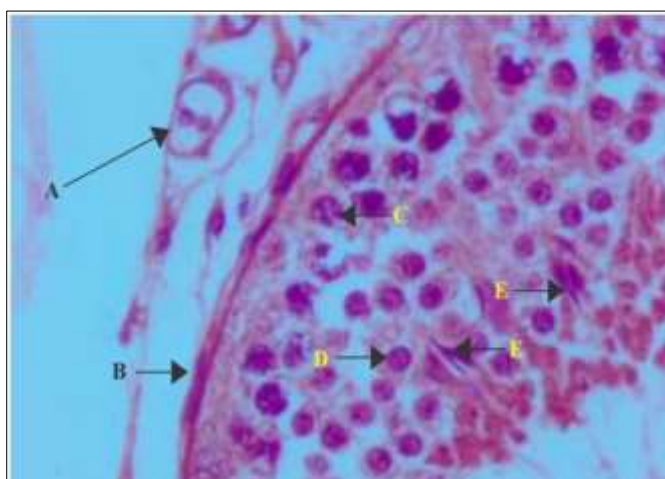


Fig 6: Microphotographs of testis from sexually mature group showing A- Leydig cells, B- Basement membrane of seminiferous tubule, C- Primary spermatocytes, D- Secondary Spermatocytes and E- Elongated spermatids (HE- 1000X)

The type 'B' spermatogonia showed mitotic activity and produced primary spermatocytes. These primary spermatocytes were observed in various stages *viz.*, leptotene, zygotene, pachytene and diplotene. These observations were in agreement with the findings of Banks, (1993)^[3] in domestic animals, Onyango *et al.* (2000)^[18] in goat, Gofur *et al.* (2008^b)^[11] in bull.

The leptotene primary spermatocytes were rounded and had spherical nuclei at centre and the cytoplasm was lightly

stained. The cell showed a coarse dense filamentous network of chromatin material all over the nucleoplasm with indistinct nuclear envelope. The nucleoli were deeply stained and occasionally notched. Similar observations were noted by Bashir *et al.* (2012^b)^[5] in Bakerwali goat and Sarma and Devi (2012)^[22] in Assam goat.

The zygotene primary spermatocytes were marked by darkly stained nuclei and the cytoplasm was pale stained. The nucleus was spherical and the chromatin material was filamentous and decondensed. These findings were in agreement with the reports of Ahmed (2016)^[1] in Naemi rams.

The pachytene primary spermatocytes were large spherical cells with a uniform spheroid nucleus. They showed a coarse filamentous arrangement of chromatin substance with irregular interstices in the nucleoplasm. The nucleoli were small, lightly stained and nuclei membrane was indistinct. Similar observations were noted by Bashir *et al.* (2012^b)^[5] in Bakerwali goat and Sarma and Devi (2012)^[22] in Assam goat and Reddy *et al.* (2016)^[20] in the domestic pig.

Diplotene primary spermatocytes were the largest size in the series of primary spermatocytes. They were found in round shape with the spherical nucleus. These cells showed indistinct nuclear membrane, loosely arranged chromatin material and the nucleoli were rarely seen. These observations were in agreement with the findings of Banks (1993)^[3] in domestic animals, Bacha and Bacha (2000)^[2] in domestic animals and Kishore *et al.* (2012)^[15] in ram.

The secondary spermatocytes were produced by the meiotic division of the parent spermatocyte. These secondary spermatocytes were undergoing meiosis soon after formation and give rise to spermatids. These were hardly observed in the histological section of the present investigation. This finding was in agreement with the reports of Ahmed (2016)^[1] in Naemi rams. The secondary spermatocytes were smaller than primary spermatocytes and larger than spermatids. They were rounded with the spherical nucleus. Centrally the fine filamentous network of chromatin was observed in the nucleus. The cell showed a distinct nuclear membrane and indistinct nucleoli. The cytoplasm was scanty with a distinct cytoplasmic rim. Similar findings were mentioned by Banks (1993)^[3] in domestic animals, Bashir *et al.* (2012^b)^[5] in Bakerwali goat, Kishore *et al.* (2012)^[15] in ram and Sarma and Devi (2012)^[22] in Assam goat and Singh *et al.* (2020)^[24] in Large White Yorkshire pig.

Secondary spermatocytes immediately after formation transformed into spermatids. These spermatids were observed rounded (early) and elongated (late) in shape. The round spermatids were the smallest cells in the size as compared to other spermatogenic cells. They possessed spherical nuclei at the center and thin peripheral cytoplasmic rim. The intensely stained irregularly aggregated chromatin substance with surrounding nucleolus was found in the nucleoplasm. The nuclear envelope was distinct and thin. Rounded spermatids were observed in groups and toward the lumen of the seminiferous tubule. Similar findings were recorded by Onyango *et al.* (2000)^[18] in goat, Gofur *et al.* (2008^b)^[11] in bull and Gopikrishna *et al.* (2017)^[12] in adult ram.

The late spermatids had elongated nuclei with condensed chromatin material and stained deeply. They showed a small head and long tail, which protruded into the lumen of the tubule. Their head was directed toward the basement membrane and was usually found attached to the Sertoli cells.

Few late spermatids were observed with detached cytoplasmic masses. These observations were in accordance with the findings of Bashir *et al.* (2012^b)^[5] in Bakerwali goat, Kishore *et al.* (2012)^[15] in ram, Sarma and Devi (2012)^[22] in Assam goat, Genedy *et al.* (2019)^[10] in Egyptian water buffalo and Reddy *et al.* (2016)^[20] in the domestic pig.

The spermatozoa were usually found attached to the luminal border of the Sertoli cell and in the lumen of the seminiferous tubule after release from the Sertoli cell. The spermatozoa had elongated oval head and long tail. Similar results were mentioned by Onyango *et al.* (2000)^[18] in goat testis, Gofur *et al.* (2008^b)^[11] in bull. Reddy *et al.* (2016)^[20] in domestic pigs, Genedy *et al.* (2019)^[10] in Egyptian water buffalo and Soori *et al.* (2019)^[25] in Lori sheep of Iran.

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