The Pharma Innovation Journal 2022; SP-11(7): 505-508

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(7): 505-508

www.thepharmajournal.com
Received: 18-05-2022
Accepted: 22-06-2022

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Histological studies of seminiferous tubules in the testes of large white Yorkshire pig (*Sus scrofa*)

Seema Sikarwar, Rakesh Mathur and Ashok Dangi

Abstract
The present investigation was carried out on the testes 12 large white Yorkshire pig between the age of seven months to around one and half year for histological study. The testes were procured from the apparantly healthy animals. The seminiferous tubules formed the major portion of testicular parenchyma in each lobule of testis and were lined with the stratified Epithelium of spermatogenic cells and Sertoli cells. The basement membrane of each tubule along with framework of collagen, reticular and elastic fibres, which supported the germinal epithelium.
The average thickness of seminiferous tubules were 253.70±14.29 µm. The peri-tubular cells were made a layer around the seminiferous tubules. Sertoli cells were seen as fewer tall elongated cells and the average width of Sertoli cells were 7.90±0.61 µm.

Keywords: Pig, testes, parenchyma, seminiferous tubules, sertoli cells

1. Introduction
Piggery is the sector that directly plays an important role in the socio-economic status of the poor rural people, more particularly the tribal population of the country as it acts as an insurance coverage for the downtrodden and socially weaker section of the society (Sailo *et al.*, 2016) [23]. A pig is any of the animals in the genus Sus, within the even-toed ungulate family Suidae. The Large White breed of pig was developed in England in the late 1700s and has been rated as the leading breed of pigs in the world as Yorkshires in the USA and Canada, are direct descendants of the Large White. It is also known as the Yorkshire pig and the English Large White pig. Large White or Large White Yorkshire, breed of swine was produced by crossing the large indigenous white pig of North England with the smaller, fatter, white Chinese pig.
The knowledge of histology of testis is important for understanding normal physiology, histo-pathology, surgical anatomy and breeding aspects. This sphere always attracts the researchers for adding new information by their research which results to enrich and update the knowledge. Very scanty information on histology of testis in pig compared with other domestic animals. Hence, the target of this work was planned to highlight certain histological details on the testes of Large White Yorkshire pig. The seminiferous tubules of the testes are the source of spermatogenesis and they empty their contents by way of several straight tubes into the head of the epididymis.

2. Materials and Methods
The histological studies on the research samples were done in the Department of Veterinary Anatomy, College of Veterinary and Animal Science, RAJUVAS, Bikaner. The present study was conducted on 12 large white Yorkshire pig and samples were procured from the apparantly healthy animals from the local abattoir house, Bikaner. Small pieces of tissues (4-6 mm thickness) were collected from the 12 right testes and 12 left testes for the histological study.
Tissues were immediately fixed in10% formal saline for 48 hrs. Bouin's fluid for 12 hrs. And Zenker's fluid for 18 hrs. After fixation, the tissues were washed in running tap water for 6-10 hrs. The tissues were dehydrated in various ascending grades of alcohol. After proper dehydration, tissues were embedded in paraffin wax of melting point of 58-60 ºC. The tissues were sectioned serially at 5 µm thickness. Then the sections were mounted on albuminized slides and dried. Finally the sections were stained with the routine histological stains to demonstrate different components of the testes.

For the micrometrical observations, diameter of various parts of testicular tissues and cells was measured from random samples seen under a light microscope in high power (400X). The present study was conducted in the Department of Veterinary Parasitology, CVAS, RAJUVAS, Bikaner, Rajasthan.

The histological details of the testis across various areas were recorded on the basis of the paraffin sections stained with haemotoxylin & eosin and special staining methods, used in the current study. Micrometry was done by using an occular micrometer after calculating calibration with stage micrometer to measure the observations on mean parameters of various testicular tissues and cells. Following parameters which were used in the micrometrical study: thickness of the seminiferous tubules of testis and width of Sertoli cells.

3. Result and Discussion

3.1 Seminiferous tubules

The seminiferous tubules were tubular structures located in the lobules, which were formed by the trabeculae and were lined by stratified epithelium of spermatogenic cells and Sertoli cells (Fig. 1, 2). These observations were in accordance with the findings of Trautmann and Fiebiger (1957) [26] in domestic animals, Miller (1965) [21] in dog, Dellmann and Brown (1986) [11] in domestic animals, Bank (1993) [4] in domestic animals, Bacha and Bacha (2000) [3] in domestic animals, Bansal *et al.* (2009) [5] in guinea pig, Babu (2012) [2] in pig, Shukla *et al.* (2013) [24] in Chamurthi horses, Singh (2013) [25] in Marwari sheep and Hanumant (2016) [19] in goat. The basement membrane known as limiting membrane of each tubule along with framework of collagen, reticular and elastic fibres, which supported the germinal epithelium as similar as the reports of Trautmann and Fiebiger (1957) [26] in domestic animals. On contrary, Dellmann and Brown (1986) [11] viewed the convoluted seminiferous tubules were surrounded by a basal lamina composed by a layer of collagen and elastic fibers in boar. Whereas, Gofur *et al.* (2008) [17] in bull, Bashir *et al.* (2012) [6] in adult Bakarwali goat, Adhikary *et al.* (2014) [1] in Indigenous bull viewed that the seminiferous tubules were comprised of spermatogonia, primary or secondary spermatocytes, round spermatids, elongated spermatids and spermatozoa as observed in present study (Fig. 3, 4). Sertoli cells were irregularly columnar cells that extended from the basal lamina to the lumen of the seminiferous tubules (Fig. 2, 3). On contrary, Mohammed *et al.* (2011) [22] described that it was difficult to determine the secondary spermatocytes in the seminiferous tubules in Indigenous male goat.

The average thickness of seminiferous tubule was 253.70 ±29.27 µm and varied between 168.00 µm to 332.50 µm. Whereas, Yaseen (2009) [27] observed less thickly (150.63±4.30 µm) in Marwari goat and Singh (2013) [25] (144.89±4.46 µm) in Marwari sheep.

3.2 Peri-tubular cells

The peri-tubular cells (contractile elements) made a layer around the seminiferous tubules (Fig. 2, 3). Similar findings were reported by Dellmann and Brown (1986) [11] in domestic animals, Eurell and Frappier (2006) [14] in domestic animals and Bacha and Bacha (2000) [3] in domestic animals. This observation was in disagreement with the findings of Bersford (1977) [7] stated in mammalian species that the seminiferous tubules had a substantial support of the basal lamina plus two alternating layers of myoid cells; Dellmann and Carithers (1996) [12] in domestic animals and Dellmann and Brown (1986) [11] in boar mentioned that myofibroblasts formed several layers around seminiferous tubules; Fawcett (1994) [15] mentioned that in larger species such as ram, boar and bull, the seminiferous tubules were ensheathed in multiple layers of adventitial cells.

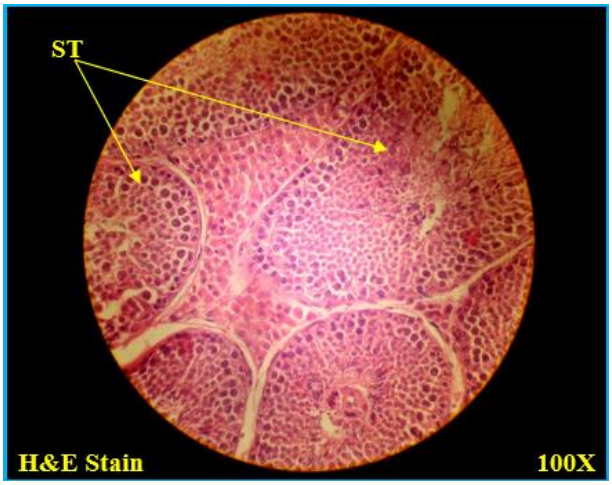

Fig 1: Cross section of testis showing seminiferous tubules (ST).

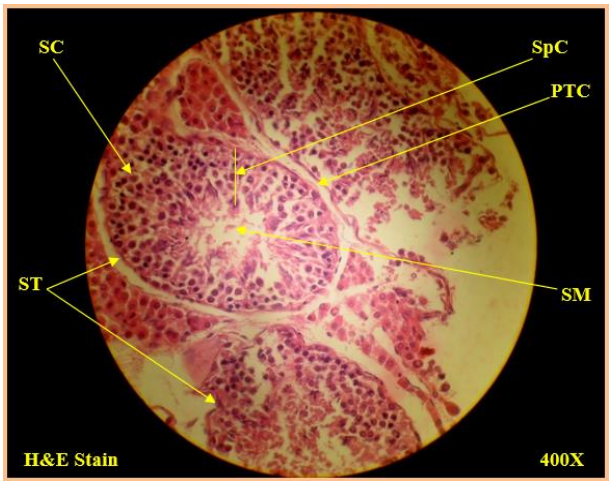

Fig 2: Cross section of testis showing Seminiferous tubules (ST), Sertoli cells (SC), Spermatogenic cells (SpC), Peri-tubular cells (PTC) and Sperm mass (SM)

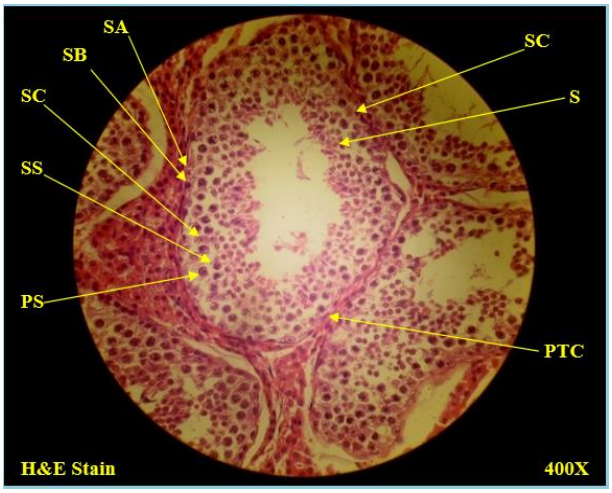

Fig 3: Cross section of testis seminiferous tubules showing Spermatogonia type A (SA), Spermatogonia type B (SB), Sertoli cells (SC), Secondary spermatocytes (SS), Primary spermatocytes (PS), Spermatids (S) and Peri-tubular cells (PTC)

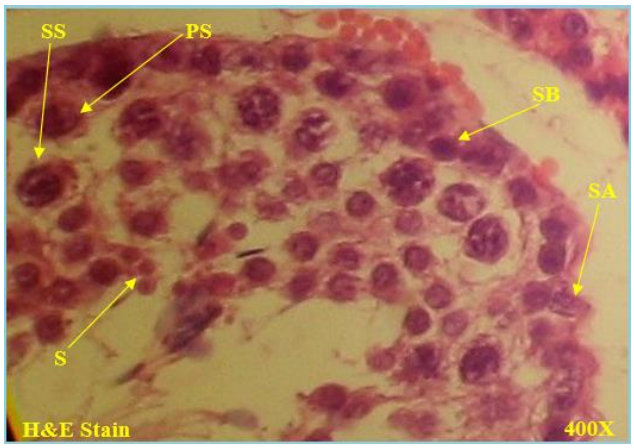

Fig 4: Cross section of testis showing Spermatogonia type A (SA), Spermatogonia type B (SB), Primary spermatocytes (PS), Secondary spermatocytes (SS) and Spermatids (S)

3.3 Spermatogenic cells

In this study, the spermatogenic cells were spherical or ovoid, which lined the seminiferous tubules extending from base towards the lumen (Fig.2). These were observed in groups between Sertoli cells. Similar findings were in accordance with reports of the Trautmann and Fiebiger (1957) [26] in domestic animals, Burgos *et al.* (1970) [9] in the domestic animals, Dellmann and Brown (1986) [11] in domestic animals, Kishore (2006) [20] in sheep and Gaykee *et al.* (2008) [16] in Neelgai, Yaseen (2009) [27] in Marwari goat and Singh (2013) [25] in Marwari sheep. However, Bacha and Bacha (2000) [3] in domestic animals, Babu (2012) [2] in pig, Dhyana *et al.* (2016) [13] in domestic pig reported that the spermatogenic cells were small and round in shape. These cells were placed at the periphery of the seminiferous tubules in single layer and were small and round shaped cells with large, darkly stained nucleus. The epithelial lining of the spermatogenic cells were composed of spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids in different stages of development and differentiation (Fig. 3, 4). These observations were similar with the reports of Hanumant (2016) [19] in goat.

3.3.1 Spermatogonia

In Large White Yorkshire pig, spermatogonia were the most immature cells among the spermatogenic cells. These cells were placed adjacent to the basement membrane of the seminiferous tubules in single layer (Fig. 3, 4). These findings were similar as the reports of Hanumant (2016) [19] in goat. In present study, two types of spermatogonia were recognized. The type A spermatogonia were usually oval shaped and located near the basal lamina and the type B spermatogonia were more spherical and begin to move away from the basal lamina. These were having large round and ovoid darkly stained nucleus with chromatin material (Fig. 3, 4). These findings were similar as the findings Bashir *et al.* (2012) [6] in adult Bakerwali goat.

3.3.2 Primary spermatocytes

Primary spermatocytes were largest among the spermatogenic cells in the seminiferous tubules. They lie just inner to the spermatogonia cells (Fig. 3, 4). These findings were similar as reported by the Hanumant (2016) [18] in goat.

3.3.3 Secondary spermatocytes

The secondary spermatocytes were observed smaller than the primary spermatocytes and were rarely seen. The primary spermatocytes were divided into the two smaller secondary spermatocytes that laid internal to the primary spermatocytes (Fig. 3, 4). These observations were similar as the report of Copenhaver *et al.* (1978) [10] in human.

3.3.4 Spermatids

The spermatids were smaller cells in comparison to primary or secondary spermatocytes. Spermatids were grouped in the luminal compartment of the seminiferous tubules and were closely associated with the Sertoli cells. Spermatids were smallest among spermatogenic series with large central spherical or oval nucleus and were located towards the lumen (Fig. 3, 4). Each secondary spermatocytes divided to formed two spermatids. The observations were as the report of Copenhaver *et al.* (1978) [10] in human.

3.4 Sertoli cells

The Sertoli cells were fewer tall elongated cells sandwitched between spermatogenic cells which were arranged radially from basement membrane to the lumen of seminiferous tubules (Fig. 2, 3). The nuclei of Sertoli cells were large and ovoid in shape with characteristic infolding of its lateral surface. Spermatozoa were embedded at the apex of Sertoli cells. These findings were in accordance with the observations were reported by Trautmann and Fiebiger (1957) [26] in domestic animals, Burgos *et al.* (1970) [9] in domestic animals, Dellmann and Brown (1986) [11] in domestic animals, Eurell and Frappier (2006) [14] in domestic animals and Dhyana *et al*. (2016) [13] in domestic pig.

Whereas, Bacha and Bacha (2000) [3] in domestic animals, Babu (2012) [2] in pig and Hanumant (2016) [19] in goat described the Sertoli cells as triangular or oval in shape. However, Mohammed *et al.* (2011) [22] observed pyramidal shaped Sertoli cells with ovoid nuclei in Indigenous male goat; Bashir *et al.* (2012) [6] observed Sertoli cells as irregularly columnar cells that were extended from the basal lamina to the lumen of tubules in adult Bakerwali goat; Buergelt (1997) [8] described that the Sertoli cells were based at periphery of seminiferous tubules and identifiable from surrounded germinal cells by convoluted nucleus in domestic animals; Goyal (1971) [18] reported that the Sertoli cells were distinguished by light stained nuclei and were found vertical to basement membrane in buffaloes, Burgos *et al.* (1970) [9] mentioned that in most species tall columnar Sertoli cells had very irregular contour and germinal cells arranged in layers in close relationship with the columnar cells.

The average width of Sertoli cells was 7.90 ±0.61 µm which varied from 3.50 µm to 11.55 µm. Whereas, Yaseen (2009) [27] observed wider Sertoli cells (9.879±0.49 µm) in Marwari goat, Mohammed *et al.* (2011) [22] (13.58±2.11 µm) in Indigenous male goat and Singh (2013) (9.433±0.38 µm) [25] in Marwari sheep.

4. Conclusion

The seminiferous tubules were lined with the stratified epithelium of spermatogenic cells and Sertoli cells. The basement membrane of each tubule along with framework of collagen, reticular and elastic fibres, which supported the germinal epithelium. The average thickness of seminiferous tubules were 253.70±14.29 µm. The peri-tubular cells were made a layer around the seminiferous tubules. Two types of spermatogonia were recognized, these were Type A and Type B. Sertoli cells were seen as fewer tall elongated cells

sandwitched between spermatogenic cells which were arranged radially from basement membrane to the lumen of seminiferous tubules. The average width of Sertoli cells were 7.90±0.61 µm.

5. Acknowledgments
The authors are very grateful to Dean, College of Veterinary and Animal Science, Bikaner, RAJUVAS, Bikaner, Rajasthan for providing necessary facilities to carry out research work. The authors are profoundly thankful to all members of the Department of Anatomy for their help during the completion of this work.

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