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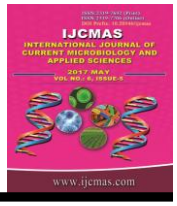

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Age Related Changes in the Histoarchitecture of Seminiferous Epithelium in Mice

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A B S T R A C T

In the present study, the histoarchitecture of seminiferous epithelium of testis in pre-pubertal and post-pubertal mice were observed. Mice belonging to the age three days post-partum to eight weeks were used. The histological observation of the testes up to six days post- partum in the present study showed that the semeniferous epithelium was made up of two distinct cell types' viz., gonocytes and sertoli cells. At day eight postpartum, both Type A and Type B spermatogonia were observed on the basement membrane of seminiferous tubules. After ten days post-partum, primary spermatocytes were observed in two to four layers next to spermatogonial layer. Both the age groups showed the presence of Leydig cells and Sertoli cells. In addition to these cellular populations, testes of post-pubertal age groups showed secondary spermatocytes, round and elongated spermatids.

Introduction

Testis is the primary organ of male reproductive system and is a bipartite glandular organ, with both exocrine and endocrine components (Siu and Cheng, 2004 and Moustafa *et al.*, 2015). To carry out these dual roles, testicular parenchyma is composed of two compartments, a seminiferous tubular compartment and an interstitial compartment. The tubular compartment consists of an outer layer of peritubular connective tissue and an inner layer of seminiferous epithelium resting upon acellular basement membrane. The seminiferous epithelium consists of two types of cells, germ cells and sertoli cells (Ravindranath *et al.*, 2003).

The histomorphological features of the testis, at various stages of growth and development,

have been described in several domestic animal and avian species (Carmon and Green, 1952; Orsi *et al.,* 1987; Sanchez *et al.,* 1993; Wrobel, 2000; França and Godinho, 2003; Zayed and Moustafa, 1996; Kannan *et al.*, 2015), whereas, the present study emphasised on the histomorphological changes in cellularity of seminiferous epithelium of mice.

Spermatogenesis is a continuous process in adult life due to the presence of unipotent adult stem cells, defined as the spermatogonial stem cells (SSCs) located along the basement membrane of the seminiferous tubules. SSCs were derived from gonocytes, which in turn, arose from primordial germ cells (PGCs). During embryogenesis, PGCs migrated from the yolk sac to the genital ridge. The arrival of PGCs stimulated the formation of the primitive sex cords. Once the seminiferous cords were fully formed, the PGCs were considered gonocytes (Senger, 2005). The first biologically active SSCs appeared 3 to 4 days postpartum in the male mouse (McLean *et al.,* 2003). Spermatogenesis occurs in 35 days in mice (Treuting and Dintzis, 2012), 5-7 days after birth in rodents and 10-13 years after birth in humans (Dym *et al.,* 2009).

The knowledge about histoarchitecture of seminiferous epithelium helps in understanding the spermatogenesis in mice. Hence, the present study was carried out in age relate changes in seminiferous epithelium of pre-pubertal and post-pubertal mice.

Materials and Methods

Testis samples were collected from eight pre-pubertal (0-4 weeks) and eight post-pubertal (4-8 weeks) mice. The mice were purchased from the Laboratory Animal Medicine unit, Madhavaram Milk Colony, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 051. At the time of collection, the animals were apparently healthy and maintained in controlled environment.

Animals were euthanized by using chloroform instead of CO2 asphyxiation and testes were removed as per Geetha Ramesh *et al*., (2016). Tissue pieces were collected from testes of pre-pubertal and post-pubertal age groups and were rinsed in normal saline and fixed in 10 per cent neutral buffered formalin and Bouin's fluid. The fixed tissues were dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax (Kannan *et al.,* 2015). Tissue sections were cut at 3-5 micron thickness in rotary microtome and used for the routine Haematoxylin-eosin staining method (Bancroft and Stevens, 2014).

Results and Discussion

In the present study, in both pre-pubertal and post-pubertal age groups, the parenchyma of the testes was composed of numerous densely packed semeniferous tubules with interstitial cells in between (Fig.1) which is in accordance with the findings of Treuting and Dintzis (2012).

Pre-Pubertal

In the pre-pubertal mice, the seminiferous epithelium was composed of gonocytes, spermatogonia, primary spermatocytes and sertoli cells. The histological observation of the testes up to six days of post partum in the present study showed that the semeniferous epithelium was made up of two distinct cell types viz., gonocytes and sertoli cells. The gonocytes were evident as large, round cells in the centre of the tubule. The cytoplasm contained a spherical nucleus dispersed with homogenous chromatin and prominent nucleolus (Fig. 2). These findings are in accordance with Bellve *et al*., (1977) in pre-

pubertal mice and McLean *et al*., (2003) in neonatal mice.

The distribution of gonocytes at the centre of the tubule indicated that the mitosis has not been initiated in gonocytes until birth (Orth *et al.*, 2000; McLean *et al.*, 2003).

At day six post-partum, the gonocytes had migrated from the centre to the basement membrane of the seminiferous tubule and differentiated to form primitive type A spermatogonia. No gonocytes were visible in the centre of the tubule after day six post-partum (McLean *et al.*, 2003). This indicated the onset of first wave of spermatogenesis in mice.

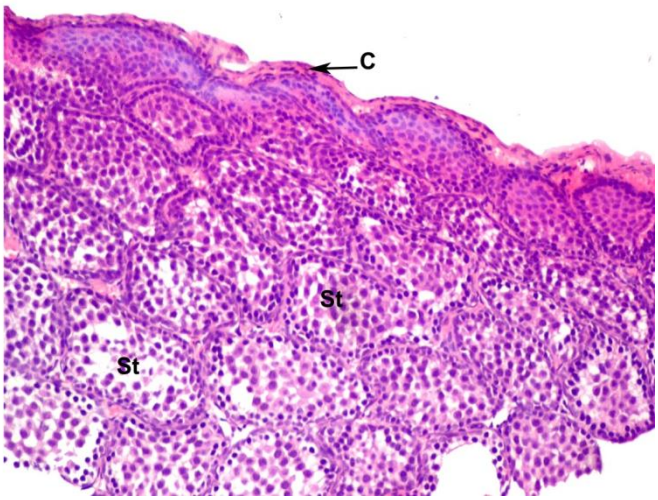
At day eight post-partum, two types of spermatogonia viz., Type A and Type B spermatogonia were observed resting upon the basement membrane. Type A spermatogonia were pale, had a reduced nuclear and cytoplasmic ratio. Nuclear chromatin was homogenous with a nucleoli. Type B spermatogonia were darker and smaller than Type A and showed an increased amount of heterochromatin (Fig.3).

At day ten post-partum, the seminiferous tubules of the testes contained primary spermatocytes. Spermatogonial cells formed a single layer and were observed to rest upon the basement membrane. Two types of spermatogonia were identified based on the appearance of the nuclear chromatin. Type A spermatogonia was pale and showed oval, intensely basophilic nucleus. Type B spermatogonia were darker and showed oval nucleus with condensed clumps of chromatin (Fig.4).

Above the spermatogonial cell layer, two to four layers of primary spermatocytes at various stages of mitotic division were observed. The primary spermatocytes were larger than the spermatogonial cell. The nucleus was round, centrally placed and basophilic in nature. It showed active chromatin.

Figure.1 Photomicrograph showing cross section of testis from pre-pubertal mouse

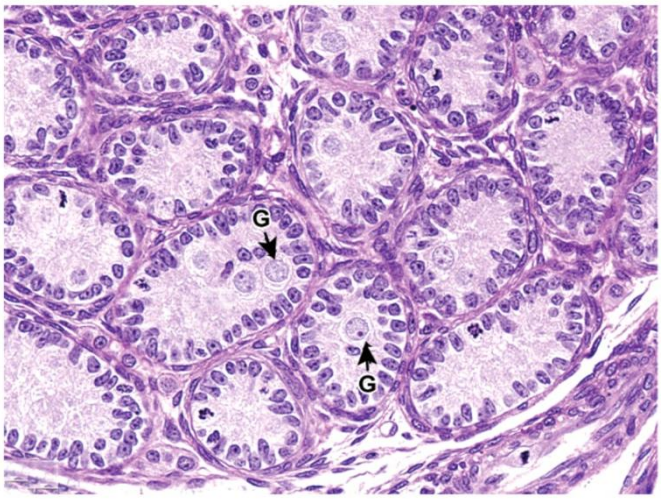
H&E X 100



C- Capsule St- Seminiferous tubules

Figure.2 Photomicrograph of testis of a three day-old mice showing the cross section of seminiferous tubules with Gonocytes

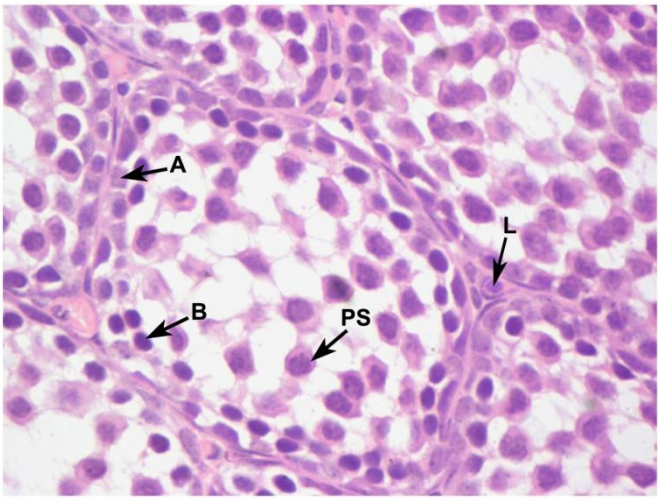
H&E x 200



G- Gonocytes

Figure.3 Photomicrograph of testis of a ten day-old mice showing the Cellular components of seminiferous epithelium

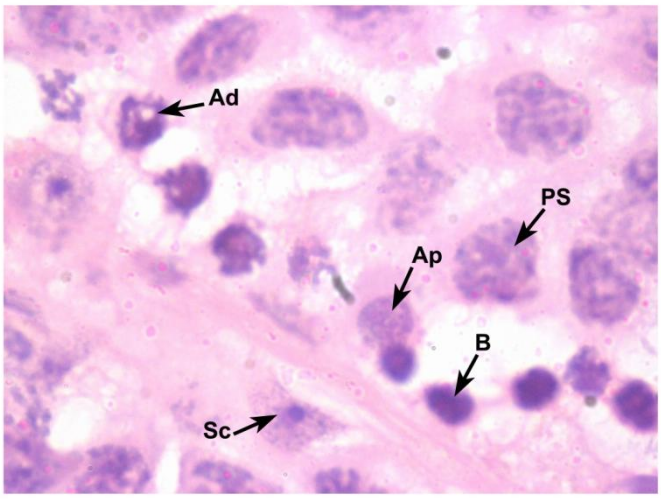
H&E X 400



A-Type A spermatogonia,
B- Type B Spermatogonia,
L- Leydig Cell,
Ps- Primary spermatocytes

Figure.4 Photomicrograph of testis of a ten day-old mice showing the cellular Components of seminiferous epithelium

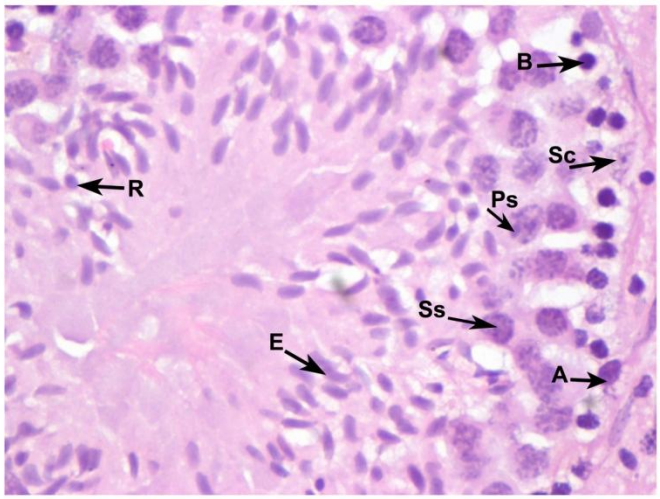
H&E x 1000



Ad- Type-A dark spermatogonia, Ap- Type A pale spermatogonia,
B- Type B spermatogonia,
PS-Primary Spermatocyte, Sc- Sertoli cell

Figure.5 Photomicrograph of testis of post-pubertal mice showing the cellular Components of seminiferous epithelium

H&E x 400



A-Type a Spermatogonia, B- Type B spermatogonia, Sc- Sertoli cell,
Ps- Primary Spermatocyte, Ss- Secondary spermatocytes,
R- Round Spermatid, E- Elongated spermatid

There were no secondary spermatocytes and spermatids observed in pre-pubertal mice. This is in contrast to the findings of Singh *et al*., (2015) in Wistar Rats who observed actively dividing spermatogonia, spermatocytes, round spermatids, Leydig cells and Sertoli cells from pre-pubertal age groups.

Post-Pubertal

In post-pubertal mice, the seminiferous epithelium was composed of spermatogonial cells, primary and secondary spermatocytes and spermatids. The supporting or sertoli cells were situated in between them as per de Rooij and Grootegoed (1998). Based on the appearance of nuclear chromatin, three types of spermatogonia were identified in the present study. Type a dark (Ad) spermatogonia showed oval, intensely basophilic nucleus. Type A pale (Ap) spermatogonia showed lightly stained oval nucleus whereas Type B spermatogonia showed oval nucleus with condensed clumps of chromatin (Figure 5).

The primary spermatocytes were larger than spermatogonial cells. It had a large nucleus with active chromatin which indicated the mitotic division. Singh *et al*., (2015) observed that the primary spermatocyte undergo first meiotic division to form the spermatids. The spermatids undergo a series of morphological and structural changes to become spermatozoa, such as formation of acrosome and tail, chromosome condensation and the removal of excessive cytoplasm at the time of spermiation.

In the present study, a concomitant increase in number of Type A and Type B spermatogonia, spermatocytes, spermatids, sertoli and leydig cells were observed in post-pubertal mice when compared to pre-pubertal age group. A similar observation has been made by Singh *et al*., (2015) in post-pubertal Wistar rats.

The secondary spermatocytes were smaller than primary spermatocytes and the nucleus contained euchromatin. Above this layer, secondary spermatocytes, numerous round and elongated spermatids were observed in the adluminal region. The sertoli cells were larger and the nuclear cytoplasmic ratio was observed more. The nucleus was oval, located in the broader portion of the cell with small nucleoli. Numerous spermatids were seen attached to the sertoli cells (Figure 5). This finding is in accordance with the observations of Eurell and Frappier (2006) in mammalian testes.

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