POSTNATAL DIFFERENTIATION AND REGIONAL HISTOLOGICAL VARIATIONS IN THE DUCTUS EPIDIDYMIDIS OF RAMS*

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ABSTRACT
The caput, corpus and cauda regions of the ductus epididymidis consisted of six histological segments that showed regional variations. The postnatal differentiation of the different segments started from birth and progressed till maturity. The simple epithelium at birth gained progressive pseudostratification. Similarly, the appearance of stereocilia, different types of cells in the epithelium and the smooth muscle layers around the epididymal tubules progressed and at different periods of postnatal development, the typical adult characteristics were achieved. The diameter of the epididymal tubules also increased, as age advanced. The developmental progression was disto-proximal i.e., the postnatal differentiation started in the terminal segment and then ascended through the middle and initial segments. The progression in the increase in height of the epithelium was varied in the three regions, whereas the progression in the increase in the diameter of epididymal tubules was uniform.

Key words: Histology, Ductus Epididymidis, Ram lamb

INTRODUCTION
The epididymis is important for transportation, maturation and storage of sperms. The spermatozoa acquire the progressive forward motility as they pass through the epididymis. They accumulate in the cauda region of the epididymis, which acts as a reservoir, which is important for species such as goat and sheep that copulate frequently. During emission, the semen is conveyed by the peristaltic contractions of the muscular wall into the pelvic urethra. The epididymis consisted of three regions with six segments that showed regional variations. Most authors differed in subdividing these caput, corpus and cauda regions into different histological segments. Reports were available on the histology of the epididymis in adults of different species but very few reports were available on the histology during postnatal development. A thorough knowledge of the histological changes is very essential to gain a comprehensive knowledge on the reproductive physiology. The present study was undertaken to study the postnatal differentiation and the developmental progression from birth to sexual maturity.
MATERIALS AND METHODS

The present study was undertaken using the samples obtained from thirty-two Madras Red rams in the Chennai Corporation abattoir. The tissue pieces were obtained immediately after slaughter from the caput, corpus and cauda regions of the epididymis. The rams used were divided into four age groups viz; pre-weaning (birth to 3 months), pre-pubertal (4 to 6 months), pubertal (7 to 9 months) and post-pubertal (10 to 12 months), each of which consisted of eight animals. The determination of age was ascertained based on the eruption of teeth (Noden and de Lahunta, 1985). The tissue pieces collected were fixed in 10% neutral buffered formalin and Bouin’s solution. Sections of 5-6 µm thickness were cut and stained by the Haematoxylin and Eosin method for routine histological study, Van Gieson’s method for collagen fibres and Masson’s Trichrome method for collagen and muscle fibres (Luna, 1968). The mean epithelial height and the mean diameter of the epididymal tubules were measured using the Carl Zeiss Videoplan image processing system and KX 300 software.

RESULTS AND DISCUSSION

The ductus epididymidis comprised the epididymal tubules and the inter-tubular area, which consisted of the loose connective tissue. The circular smooth muscle fibres surrounded the epithelium and their thickness increased proximo-distally. On microscopic examination the caput consisted of segments I to III proximo-distally, the corpus consisted of the segment IV and the cauda consisted of segments V and VI proximo-distally.

Segment I was the first and major portion distal to the ductuli efferentes. The columnar principal cells and the small polygonal basal cells were the two cell types invariably present in all the segments of the epithelium. Additional cell types like the apical cells were also seen in the different segments. At the junction of Segment I with the ductuli efferentes there was an abrupt increase in the height of the pseudostratified ciliated columnar epithelium due to preponderance of the columnar principal cells. Segment II was a short portion distal to the segment I. The foldings of the pseudostratified ciliated columnar epithelium were noticed due to the variations in the height of the principal cells. Segment III was distal to segment II and was larger than it. It continued into the segment IV. The height of the pseudostratified ciliated columnar epithelium was uniform in this portion and the folding of the epithelium was less compared to segment II.

Segment IV was very long and constituted the whole of the corpus epididymidis till it joined the segment V in the cauda epididymidis. The height of the principal cells was lesser compared to that in the three segments of the caput epididymidis. Slight folding of the epithelium was noticed. The height of the stereocilia was more compared to that in the segments V and VI, but less compared to the segments I to III.

Segment V showed an increase in thickness compared to the previous segments. Segment VI occupied the terminal portion of the cauda epididymidis. It continued into the ductus deferens. This segment showed a very thick peritubular smooth muscle layer surrounding the epithelium.

These findings are in accordance with the description of Nicander (1958) and Crabo (1965) in bull, Wrobel and Fallenbacher (1974a) in adult boar, and Goyal and Dhingra (1975) and Singh and Roy (1993) in the Indian buffalo. On the contrary, Abdou et al. (1985) in buffalo, Goyal and Williams (1991) in goat and Ariyaratna et al. (1996) in the pre-pubertal swamp buffalo reported eight, five, and four segments respectively. These authors also differed in the localization of these segments within the three regions of epididymis.
Regional histological variations were observed from caput to cauda in the various segments. In all age groups, the height of the principal columnar cells in the pseudostratified ciliated columnar epithelium gradually decreased from the beginning of the caput to the end of the cauda. Wrobel and Fallenbacher (1974b), Goyal and Williams (1991), Dellmann and Eurell (1998) and Sanchez et al. (1998) reported similar findings in the adult boar, goat, domestic animals and in the cat respectively. However, Delhon and Lawzewitsch (1994) described that in llama, the second and third segments showed the maximum height.

The height of the stereocilia on the apical surfaces of the pseudostratified ciliated columnar epithelium gradually decreased from the beginning of the caput to the end of the cauda in all age groups. These findings are in agreement with the reports of Dellmann and Eurell (1998) in domestic animals, Sanchez et al. (1998) in cat and Islam et al. (2002) in Black Bengal goat. On the contrary, Goyal and Dhingra (1975) described that the second and fourth regions were devoid of stereocilia in buffalo.

The tubular and luminal diameters of the epididymal tubules gradually increased from the beginning of the caput to the end of the cauda in all age groups. Wrobel and Fallenbacher (1974b) reported that the sixth (last) segment in the cauda had the largest diameter in the adult boar. Goyal and Dhingra (1975) reported that the tubular diameter of the tubules showed a gradual increase from regions I to III but region VI showed a marked increase in diameter as compared to the other regions in the buffalo. These findings are in accordance with the description of Pal and Bharadvaj (1986) in buffalo, Goyal and Williams (1991) in goat, Naidu (1991) in ram lamb and Sanchez et al. (1998) in cat. Hafez and Hafez (2000) also reported similar findings and described that there was a progressive decrease in the height of the epithelium and stereocilia, but a widening of the lumen occurred throughout the three regions in the farm animals.

At birth, while the caput and corpus regions showed a simple cuboidal epithelium, the cauda region showed a low columnar epithelium. The epithelium in all these regions later differentiated into pseudostratified columnar with stereocilia. These findings are in agreement with the reports of Yao and Eaton (1954) and Harshan et al. (1978) in goat, and Pal and Bharadvaj (1986) in buffalo. Wrobel and Fallenbacher (1974b) reported that in pig, at postnatal day 10, the whole length of the epididymal duct was lined with a single layer of epithelial cells. Goyal and Dhingra (1975) reported that in buffalo, a simple columnar epithelium with a few basal cells was seen from three weeks of age up to sixteen weeks of age in the regions I to III. However, in the regions IV and V, it was pseudostratified columnar from three weeks of age up to thirty-two weeks, but it was pseudostratified low columnar in region VI, at all stages of development. Nilnophakoon (1978) reported that in the Swedish Landrace ram, the whole epididymal duct had a simple low columnar epithelium with a marked increase in diameter as compared to the other regions in the buffalo. Pal and Bharadvaj (1986) reported that the simple columnar epithelial lining of the duct in the newborn buffalo calf changed to pseudostratified columnar with stereocilia in older buffalo calves.

The height of the epithelium in the epididymal tubules increased within each region, as age advanced. In the caput region (segment III), while there was no significant difference in the increase from birth to 3 months, a highly significant difference (P<0.01) was observed among the above age groups and the subsequent age groups of 6, 9 and 12 months. In the corpus region (segment IV), a highly significant difference (P<0.01) was observed from birth to 3, 6 and 9 months. However, there was no significant difference between 9 and 12 months. In the cauda region (segment VI), a highly significant difference (P<0.01) was observed only between birth and 3 months. In the subsequent age groups of 6, 9 and 12 months there was no significant difference. The developmental progression was slightly...
different in the three regions. Though there was an increase in height, as age advanced, in each region, this increase was not proportionate proximo-distally within each age group, compared to the other age groups. As a result, the height of the epithelium at birth, which was the lowest in the caput and the highest in the cauda, changed to the highest in the caput and the lowest in the cauda at 12 months of age.

The tubular diameter of the epididymal tubules also increased, as age advanced. In the caput region (segment III), while there was no significant difference in the increase of the external diameter from birth to 3 months. A highly significant difference (P<0.01) was observed among these age groups and the subsequent age groups of 6, 9 and 12 months. In the corpus region (segment IV), the developmental progression was similar to that in the caput region (segment III). However, in the cauda region (segment VI), a highly significant difference (P<0.01) was observed between all age groups from birth to 12 months. The developmental progression was uniform in the three regions. An increase in the tubular diameter was observed both within each region and within each age group, proximo-distally, as age advanced (Table 1).

The thickness of the smooth muscle around the epididymal tubules gradually increased from the beginning of the caput to the end of the cauda. Similar were the observations of Singh and Dhingra (1971) and Goyal and Dhingra (1975) in buffalo, Harshan et al. (1978) and Sharma et al. (1986) in goat. However, they stated that the number of smooth muscle fibres around the ductus epididymidis increased significantly towards the cauda region. Goyal and Dhingra (1975) further stated that the muscular wall showed a gradual increase in thickness from regions I to V but there was a sharp increase in thickness in region VI in the buffalo. Pal and Bharadwaj (1986) opined that the peri-ductular tissue in the buffalo, which contained smooth muscles along with the connective tissue was the thickest at the distal portion in the pubertal group. Goyal and Williams (1991) and Dellmann and Eurell (1998) reported that the number of circular smooth muscle fibres increased significantly towards the cauda in the goat and in domestic animals respectively. In total contrast to all these observations, Romanello et al. (1985) opined that in donkey, none of the regional differences that occurred in other species could be found.

The postnatal differentiation of the different segments of the epididymis that started from birth progressed till sexual maturity. A definite differentiation of the various histological criteria was observed and the structure typical of the adult was established. The simple cuboidal to low columnar epithelium at birth, gained progressive pseudostratification in further stages of postnatal development (Fig. 1). Similarly, the stereocilia and the different types of cells appeared in the epithelium, as age advanced (Fig. 2 and 3). The increase in the number of smooth muscle layers around the epididymal tubules progressed, and at different periods of development, the typical adult characteristics were achieved (Fig. 4).

However, within each age group, the progression in increase in height of the epithelium and increase in diameter of epididymal tubules started from the distal end and proceeded towards the proximal end. The epithelium at birth, was simple low columnar in the cauda, while in the corpus and caput it was simple cuboidal. Similarly, the small polygonal basal cells in the epithelium appeared first in the cauda region compared to the corpus and caput regions. From birth, the appearance of the number of layers of smooth muscle surrounding the epididymal tubules was also earlier in the cauda region compared to the corpus and caput regions.

These development criteria and the pattern of development indicated that the developmental progression was disto-proximal i.e., the postnatal differentiation in the epididymis of ram started in the terminal segment and then ascended through the middle and initial segments. These findings
concorded with the opinions of Wrobel and Fallenbacher (1974b) in pig and Nilnophakoon (1978) in the Swedish Landrace ram. In the present study, though the developmental progression was disto-proximal, the progression in the increase in height of the epithelium was varied in the three regions, whereas the progression in the increase in the external diameter was uniform.

REFERENCES


**Fig. 1:** Photomicrograph of the caput epididymidis (segment I) at birth showing the simple cuboidal epithelium

Ep - Epithelium  Ms - Peri-tubular smooth muscle  I - Inter-tubular area  Lu - Lumen

Van-Gieson's x 200
Fig. 2: Photomicrograph of the caput epididymidis (segment III) in three month-old ram showing the stereocilia and different cell types

Ep - Epithelium   Ms - Peri-tubular smooth muscle   CT - Connective tissue   Lu - Lumen   Ci - Stereocilia
Ba - Basal cell   Col - Columnar cell   Ap - Apical cell
Van-Gieson’s x 200

Fig. 3: Photomicrograph of the corpus epididymidis (segment IV) in nine month-old ram showing the stereocilia

Ep - Epithelium   Ms - Peri-tubular smooth muscle   CT - Connective tissue   Sp - Clumps of spermatozoa   Ci - Stereocilia
Masson’s Trichrome x 100

Fig. 4: Photomicrograph of the cauda epididymidis (segment VI) in ten month-old ram showing the very thick peri-tubular smooth muscle

Ep - Epithelium   Ms - Peri-tubular smooth muscle   CT - Connective tissue   Lu - Lumen   Ci - Stereocilia
BV - Blood vessel   Sp - Spermatozoa
Masson’s Trichrome x 100