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Immunolocalization of laminin during postnatal development of the testis, epididymis and vas deferens of albino rat.

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Abstract

Background: Disorders of testicular function may have their origins in fetal or early life as a result of abnormal development. Laminin-1 is emerging as the key molecule in early embryonic basement membrane assembly. Accumulating evidence supported the idea that extracellular matrix (ECM) molecules and mesenchymal cells might influence Sertoli and spermatogenic cell functions.

Aim of the work: detecting the changes in the distribution and prevalence of laminin-1 assembly during postnatal development of the testis, epididymis and vas deferens in albino rats.

Materials and methods: Thirty male albino rats were used and divided into six groups (n= 5 each) according to the age (postnatal day). These were one day, 1 week, 2 weeks, 3 weeks, 4 weeks, and 8 weeks postnatal. Specimens were fixed and processed, sectioned and stained by hematoxylin and eosin and immunohistochemical stain for laminin-1. The area percent of positive laminin immunostaining was measured and results were statistically analyzed.

Results: at day one postnatal, the testis was formed of solid un-canalized cords of seminiferous tubules with abundant laminin expression in the cells of the cords. With advancement of development the cords were luminized and the laminin expression declined to involve the basement membrane and the apical portions of the Sertoli cells at the 8th week postnatal. The epididymis at postnatal day one had a small diameter and narrow lumen and laminin expression involved the cytoplasm of the epithelial lining. As development proceeded the expression became confined to the apical portion, the site of stereocilia together with its presence in the basement membranes. The same pattern of changes in laminin expression together with morphological appearance was detected in the vas deferens.

Conclusion: The present study was able to demonstrate a change in the distribution as well as the prevalence of laminin-1 immunoreactivity within the testis, epididymis and vas. During the period of postnatal development starting at postnatal day one up to 8 weeks postnatal. This would reflect an essential role for laminin in early postnatal period of development.

Key words: Laminin, testis, epididymis, vas deferens, development, immunohistochemistry.

Introduction

Basement membranes (BMs) are cell-associated sheet-like extracellular matrices covering the basal aspect of all epithelia, endothelia, surrounding muscle, fat and peripheral nerve cells. BMs are essential for tissue formation in all animals. They provide

mechanical stability and barriers between different cell types and are critically involved in cell differentiation, survival, and migration [(1)].

The first BM protein to be analyzed biochemically was collagen IV, soon followed by the discovery of the major non-collageneous BM glycoprotein, laminin [(2)]. Laminin-1 is emerging as the key molecule in early embryonic basement membrane assembly. The classic laminin-1 is a cross-shaped molecule comprising α1, β1 and γ1, chains and is the most important isoform in early development. It spontaneously self-assembles into polygonal lattices in vitro through calcium-dependent interactions between all the three short arms. Assembly *in vivo* is believed to occur by the same mechanism, but additionally requires the long arm of laminin to be tethered to receptors on the cell surface [(3)]. Early genetic evidence pointed to a hierarchy in BM formation, with polymerizing laminin-1 acting as a scaffold for the recruitment of other BM components [(4)]. Generally, laminins have contributed to several critically important activities including cell adhesion and differentiation, cell shape and movement, maintenance of tissue phenotypes, and promotion of tissue survival [(5)].

The attention drawn toward the seminiferous tubule ECM components arose partially from observations that male infertility was associated with abnormal thickening in the seminiferous tubule lamina propria or boundary tissue [(6)]. In fact, accumulating evidence supported the idea that extracellular matrix (ECM) molecules and mesenchymal cells might influence Sertoli and spermatogenic cell functions [(7)].

The process of sperm production does not occur at birth but is initiated at puberty. This makes testicular development somewhat different from that of other organs, many of which develop almost exclusively during embryonic life. Although embryonic testicular development is substantial, additional changes occur at puberty during the initiation of spermatogenesis when, for instance, the seminiferous tubule diameter increases more than twofold in the mouse testis to accommodate additional spermatogenic cell types [(8)]. After development in the testis, the spermatozoa travel through the epididymis where they mature, gaining motility and the ability to fertilize the oocyte. Each segment of the epididymis synthesizes and secretes a specific set of proteins, thus creating the unique luminal environment needed for the sperm maturation process [(9)].

Although the identity of most BM proteins might probably be known, but many of their activities still remain to be elucidated. This study focuses on the changes in the distribution and prevalence of laminin-1 assembly during postnatal development of the testis, epididymis and vas deferens in albino rats.

Materials and methods:

Thirty male albino rats were the subject of the current study; they were assigned to six groups (5 each) according to the age (postnatal

day PND). These were one day postnatal, 1 week, 2 weeks, 3 weeks, 4 weeks, and 8 weeks postnatal (age of sexual maturation) [10]. Animals were sacrificed and the testis, epididymis and vas deferens were excised and fixed in 10% formol saline. Specimens were processed to obtain paraffin blocks that were cut into five to six µm sections. These sections were stained by: hematoxylin and eosin and immunohistochemical stain for laminin-1 [11].

Laminin Ab-1 is a rabbit polyclonal antibody. The antibody was provided as avail of 0.5 ml to be used at a dilution of 1:50. Being formalin fixed, paraffin-embedded sections required pretreatment by protease XXV. This was supplied as lyophilized powder which was reconstituted to 2ml by phosphate buffer saline (pH 7.4) at 1mg/ml concentration.

Immunostaining was completed by the use of ultravision detection system. Counterstaining was done using Mayer's hematoxylin. Primary antibody, citrate buffer, ultravision detection system, protease and Mayer's hematoxylin were purchased from lab vision Labvision, Thermoscientific, USA.

Morphometric study:

The area percent of positive laminin immunostaining was measured at magnification X400 in 10 non overlapping fields in every specimen for all animals. Image analysis was done using "Leica Qwin 500C" image analyzer computer system (England) present in Histology Department, Faculty of Medicine, Cairo University.

Statistical analysis:

The results were expressed as mean ± standard deviation (SD) and were analyzed statistically using the software "Statistics for windows SPSS" version 9. This was done using one-way analysis of variance ANOVA followed by "tuckey" post hoc test. Results were considered significant when P value was <0.05 [12].

Results:

1- Haematoxylin and eosin stain results:

a- Testis:

Examination of the testis one day postnatal demonstrated the seminiferous tubules with non-canalized lumena (cords). They were characterized by a single basal layer of cells formed mainly of Sertoli cells with few gonocytes inbetween. One week postnatal it was the same as the seminiferous tubules were still non-canalized and displayed the single basal layer of cells of abundant Sertoli cells with few spermatogonia (Fig 1). Examining the testes two weeks postnatal showed that the seminiferous tubules started to be partially canalized being lined with 2 or 3 layers of spermatogenic cells with few primary spermatocytes near the centre (Fig 2). By the third postnatal week, the testis revealed seminiferous tubules lined with several layers of developing spermatogenic cells reaching only to stage of primary spermatocytes. However, some of the tubules were still not fully canalized (Fig 3). As development proceeded at the fourth week postnatal all the seminiferous tubules were lumenized and they were lined with

several layers of spermatogenic cells. Clearly developing spermatozoa could be seen extending their heads deep between the spermatocytes (Fig4). On examination of the testis at 8 weeks postnatal, it showed the appearance of adult seminiferous tubules lined with spermatogenic cells in various stages of spermatogenesis. These included basal spermatogonia, primary spermatocytes having large nuclei with dense chromatin strands and adluminal round spermatids with small nuclei. In addition sertoli cells with their characteristic pale oval nuclei could be also seen inbetween the spermatocytes. The presence of multiple spermatozoa was evident with their heads attached to the Sertoli and their tails extending into the lumen of the tubule (Fig 5).

b- Epididymis:

Examination of the epididymis one day post natal revealed its small diameter and narrow lumen being lined by a single layer of cuboidal cells. Such appearance was persistent by the end of the first postnatal week they were similarly lined by simple cuboidal cells that lacked any signs of the characteristic sterocilia (Fig 6). At two weeks postnatal the lumens of the epididymis were relatively wider with larger diameter. However, the lining epithelium appeared to be formed of densely packed cells with rounded nuclei with scarce primitive stereocilia (Fig 7). Examination of the epididymis at three weeks postnatal showed sections in it with relatively large diameter and wider lumen. The cells of the lining epithelium were densely packed some of them had rounded nuclei and others had oval ones. Some cells possessed few stereocilia. Four weeks postnatal the epididymis was lined by pseudostratified epithelium which was formed of basal cells with rounded nuclei and few other more superficial cells with oval nuclei and abundant long stereocilia. The lumen demonstrated evidence of the start of spermatogenesis by the presence of small group of spermatozoa. The wall was surrounded by 1 or 2 layers of spindle-shaped smooth muscle cells (Fig 8). As development advances the epididymis at eight weeks postnatal was characteristically of large diameter and evidently wider lumen. It was lined with pseudostratified columnar epithelium with stereocilia and surrounded by a thin layer of circularly-arranged smooth muscle cells. The lumen was filled with numerous spermatozoa (Fig 9).

C- Vas deferens:

Examination of sections of the vas deferens one day postnatal revealed that it was of small diameter and narrow lumen. The epithelial lining is formed of densely packed small non-ciliated cells with rounded nuclei. The wall was formed of irregularly arranged smooth muscle cells with rounded nuclei. One week postnatal the vas deferens was similar to that of one day old, it was formed of small diameter and narrow lumen. The epithelial lining is formed of densely packed small non-ciliated cells with rounded nuclei. However few of the lining cells had scarce, short and primitive stereocilia. The wall was also

formed of irregularly arranged smooth muscle cells with rounded nuclei (Fig 10). As development proceeded the vas at two and three postnatal weeks appeared to be of relatively larger diameter. The epithelial lining was formed of densely packed cells with rounded nuclei giving the appearance of pseudostratification having few short stereocilia. The underlying lamina propria was thin and dense and the musculosa was formed of compact smooth muscle cells with oval vesicular nuclei (Fig 11). At four postnatal weeks the vas deferens was of a large diameter and a wide lumen. It was lined by pseudostratified epithelium formed of densely packed cells some having rounded nuclei and other superficial principle ones with oval nuclei and numerous long sterocilia lying on the dense lamina propria. The musculosa is formed by relatively regular smooth muscle cells with oval vesicular nuclei (Fig 12). By the eighth postnatal week the vas deferens acquired a mature appearance becoming of a large diameter and a wide lumen. The mucosa demonstrated some folding with pseudostratified epithelial lining formed of basal cells with rounded nuclei and densely-packed superficial tall principal cells with oval nuclei and multiple long and well-developed stereocilia. The underlying lamina propria was dense and thin. Smooth muscle cells of musculosa were compact and well-developed (Fig 13).

11- Laminin immunostaining results:

A-Testis:

Examination of sections of the testis of one day old rats immunostained for laminin revealed the presence of positive laminin immunostaining within the cells of the seminiferous tubules. This involved most of the cytoplasm. It was also detected as thin fibers related to some of the stromal cells. By the first postnatal week, the same was detected since the cord like seminiferous tubules still showed positive laminin immunostaining involving most of the cytoplasm of their cells and in their centre. Positive immunoreactivity could also be seen in the scarce spindle shaped cells surrounding the base of these cords, in the walls of blood vessels in addition to thin fibers in the interstitium (Fig 14). Examination of the testis of the two weeks-old rats, they revealed scanty positive laminin immunostaining within some of their cells, at the basement membrane and in the myoid cells. (Fig 15). However the three weeks-old rats' testis specimens showed scanty positive laminin immunostaining within some of the cells lining the seminiferous tubules and at the basement membrane (Fig 16). At four weeks postnatal the testis demonstrated a scanty positive reaction of a longitudinal manner toward the centre of the tubules together with strong positive reaction at the basement membrane (Fig 17). By the age of 8 weeks (2 months) the testis maintained the longitudinal pattern of the positive laminin immunostaining which appeared to be related to cells resting on the basement membrane with triangular nuclei (sertoli cells). The positive immunoreactivity was evident at the basement membrane. In the large blood vessels the

reaction was related to their endothelium as well as the cells of their media (Fig 18).

B-Epididymis:

Examination of the specimens of the epididymis showed that at one postnatal day laminin immunopositivity was located in the cytoplasm of the epithelial lining together with positive reaction in the cells present within the surrounding stroma. Similar findings were observed by the end of the first week postnatal (Fig 19). Epididymis by two postnatal weeks showed strong positive laminin immunostaining mainly in the apical part of the cytoplasm of the epithelial lining with noticeable reaction at the bases. Positive reaction was also evident within the surrounding stroma (Fig 20). By the third week postnatal the epididymis showed strong positive laminin immunostaining mainly in the apical part of the cytoplasm of the epithelial lining and within the surrounding stroma. Positive reaction was also related to the endothelium of the blood vessel (Fig 21). With progression of development at four postnatal weeks laminin positive immunostaining was mild at the apical part of the cytoplasm of the epithelial lining of the epididymal ducts as well as their bases. Also it could be noticed in the stroma and related to the blood vessels. Similar results were recognized at eight weeks postnatal (Fig 22).

C-Vas deferens:

Examination of the vas deferens of a one day old rat revealed an abundant strong positive laminin immunostaining within the cytoplasm of the epithelial lining, the same abundant positive reaction was related to smooth muscles of the musculosa as well as within the connective tissue stroma. The same pattern and distribution of positive reaction was detected by the end of the first postnatal week (Fig 23). Specimens of vas deferens at both two and three postnatal weeks revealed the presence of moderate positive laminin immunostaining within the cytoplasm of the epithelial lining and related to smooth muscles of the musculosa (Fig 24). As development proceeded, laminin immunostaining was mild in the epithelial lining and musculosa in both four and eight weeks old rats vas deferens (Fig 25).

D- Morphometric results:

Mean area percent of laminin immunopositivity (Table 1) was of maximum levels at early postnatal ages (one day and one postnatal week) and gradually declined with progress of development to be at the least levels at the sexually mature age (8 postnatal weeks) in the testis (Chart 1), epididymis (Chart 2) and vas deferens (Chart 3). Thus, all the three organs showed the same pattern of decline in laminin immunoexpresion with the progress of age (Chart 4). The decrease in mean area percent of laminin immunopositivity in the testis at eight postnatal weeks was statistically significant when compared to the one day and one week aged groups ($P=0.0001$), but statistically insignificant when compared to those of two weeks, three weeks and four weeks old ones ($P= 0.078$, 0.967 and 0.997 respectively).

As regard the epididymis, the mean area percent of laminin showed a statistically significant decrease at eight postnatal weeks when compared to the younger age groups from one day until three weeks postnatal (P ranged between 0.0001 and 0.006), but statistically insignificant when compared to those of four weeks old (P=0.97).

However, comparing the results of laminin immunoreaction mean area percent in the vas deferens there was also a decline with advancement of age to reach its least level at age of eight postnatal weeks. The decrease at eight postnatal weeks was statistically significant when compared with the younger ages, one day, one week and two postnatal weeks (P=0.0001, 0.0001 and 0.011 respectively) but statistically insignificant when compared to those of three weeks and four weeks old ones (P=0.991 and 0.998 respectively).

Table1: mean area percent ± SD of positive laminin immunostaining in the testis, epididymis and vas deferens in all age groups.

Age / Organ	1day	1 week	2 weeks	3 weeks	4 weeks	8 weeks
Testis	17.454 ±2.042	16.872 ±1.534	9.956 ±2.205	5.533 ±2.178	4.86 ±0.603	3.869 ±1.446
Epididymis	19.504 ±1.534	15.239 ±2.083	13.213 ±1.081	11.871 ±1.111	7.171 ±2.784	3.482 ±1.053
Vas deferens	39.649 ±2.338	28.682 ±1.183	21.595 ±2.936	17.629 ±2.802	11.536 ±0.936	11.01 ±2.95

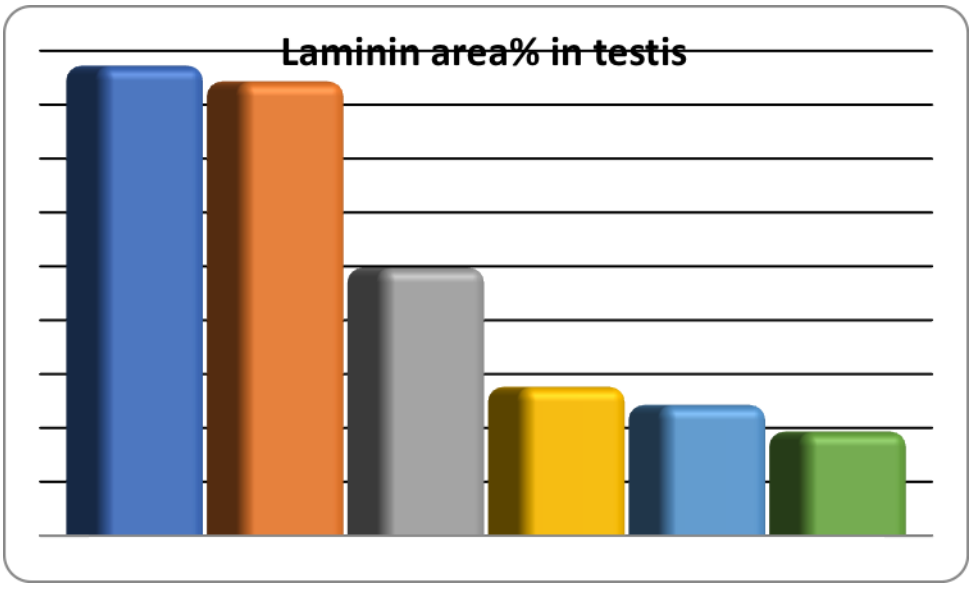

Chart 1: Mean area percent of laminin immunostaining in the testis of all the tested ages.

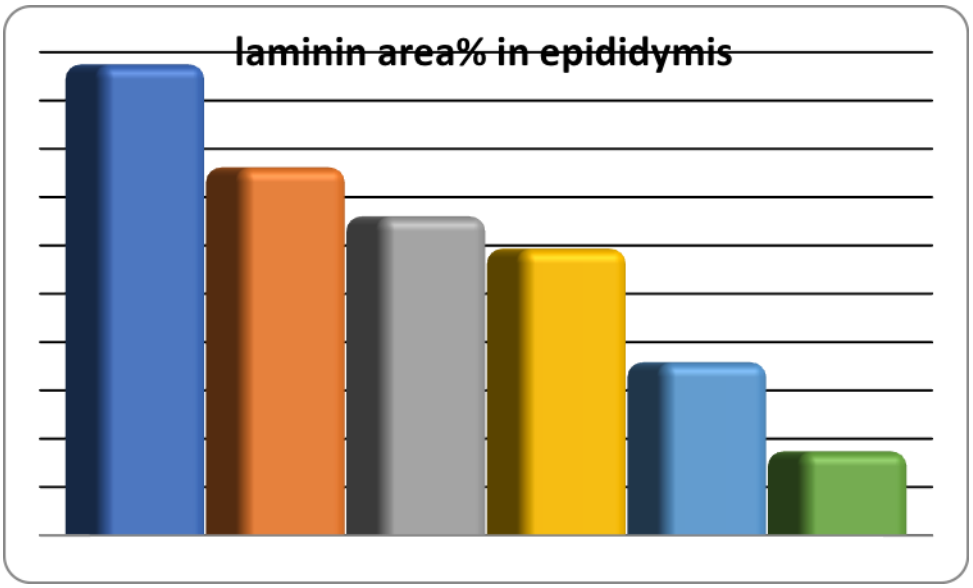

Chart 2: Mean area percent of laminin immunostaining in the epididymis of all the tested ages.

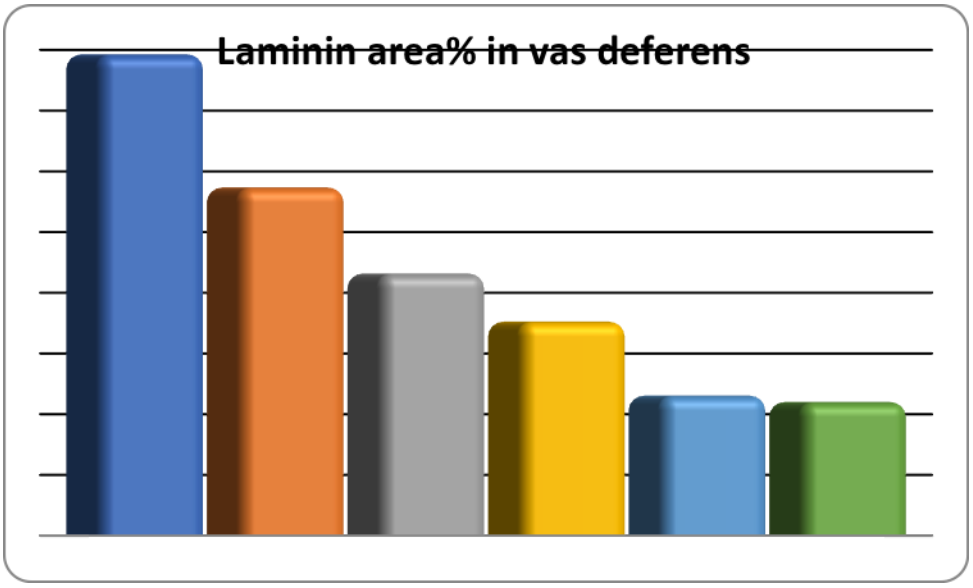

Chart 3: Mean area percent of laminin immunostaining in the vas deferens of all the tested ages.

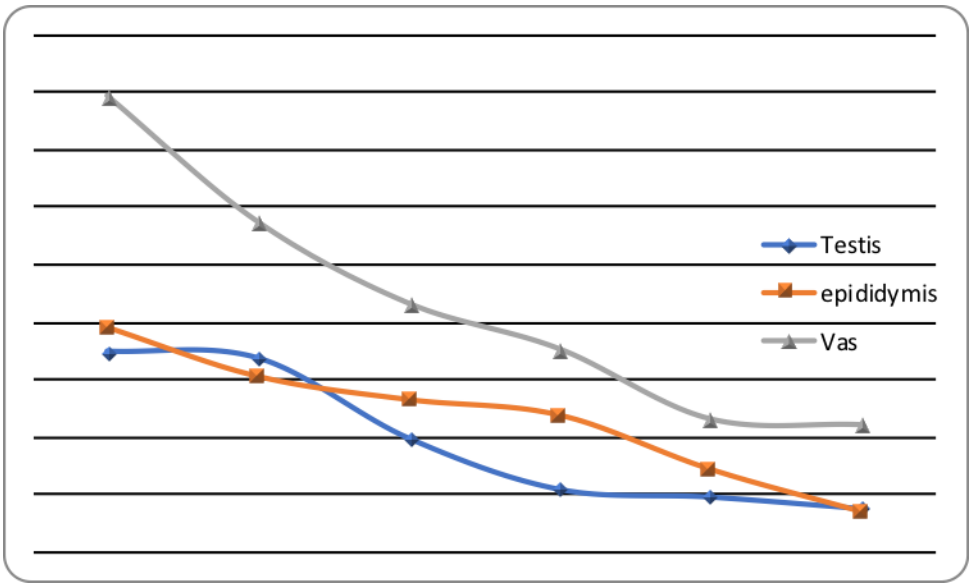

Chart 4: Linear correlation between the mean area percent of laminin immunopositivity in the testis, epididymis and vas deferens of all the tested ages.

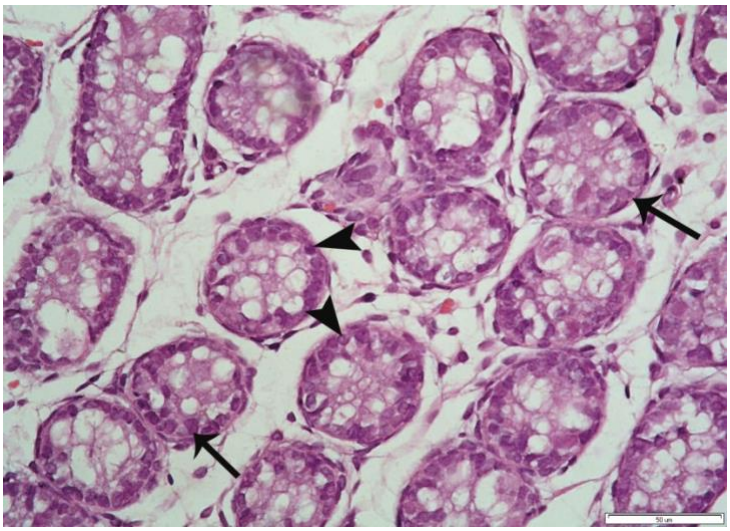

Fig.1: A photomicrograph of the testis of a 1 week-old rat showing the seminiferous tubules with non-canalized lumen and a single basal layer of cells formed of many Sertoli cells (arrow) and few gonocytes (arrowhead). (H&E X400)

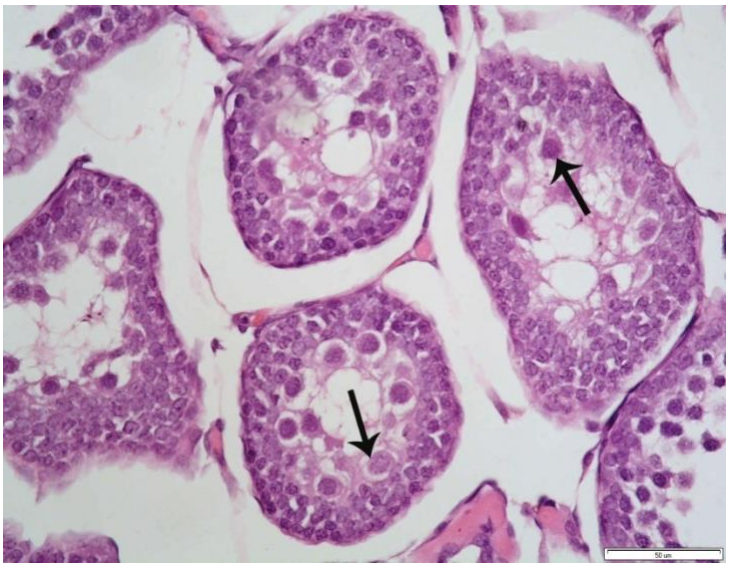

Fig.2: A photomicrograph of the testis of 2 weeks-old rat showing the seminiferous tubules with partially canalized lumen lined with 2 or 3 layers of spermatogenic cells with scarce primary spermatocytes (arrow) near the centre. (H&E X400)

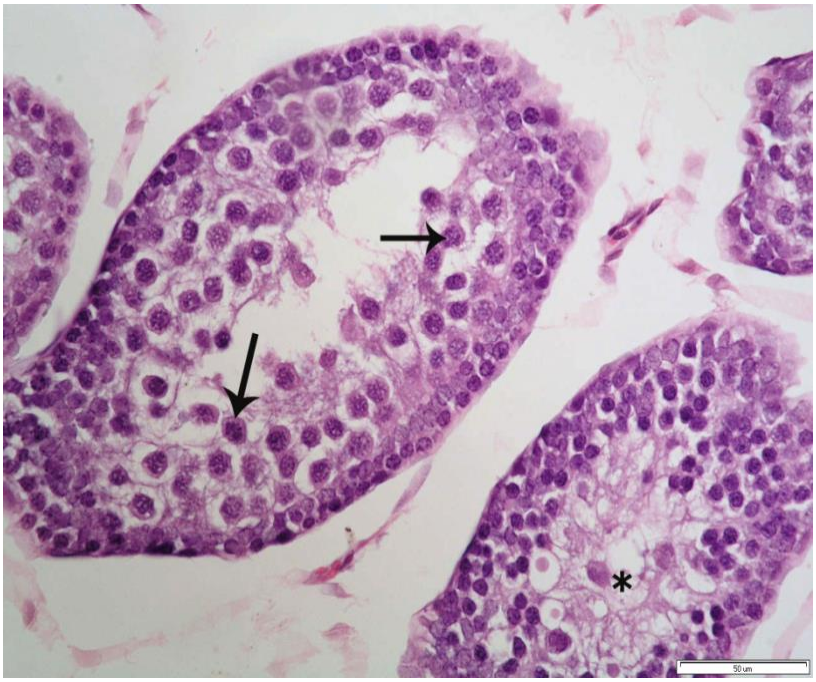

Fig.3: A photomicrograph of the testis of a 3 weeks-old rat showing the seminiferous tubules lined with several layers of developing spermatogenic cells reaching only to stage of primary spermatocytes (arrow). Note that some tubules are still not fully canalized (asterix). (H&E X400)

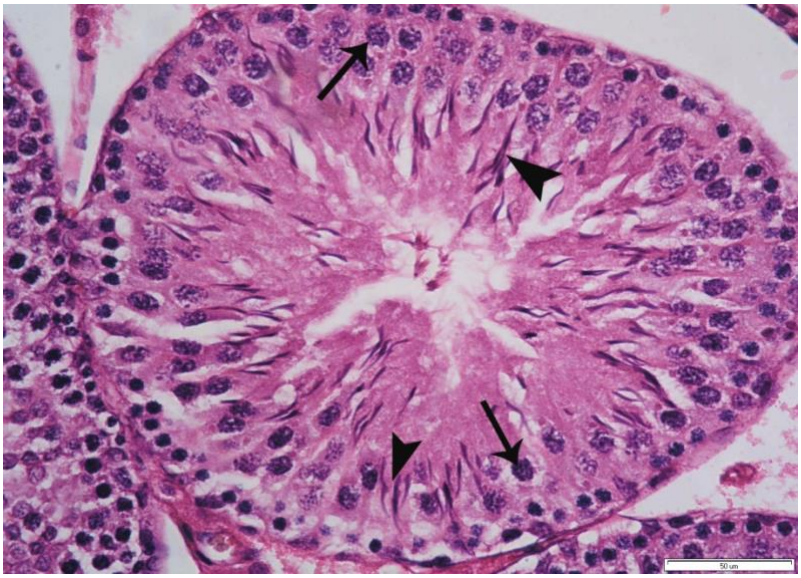

Fig. 4: A photomicrograph of the testis of a 4 weeks-old rat showing a seminiferous tubule lined with several layers of spermatogenic cells. Note the primary spermatocytes (arrow) with dense chromatin strands. Developing spermatozoa (arrowhead) can be seen extending their heads deep between the spermatocytes. (H&E X400)

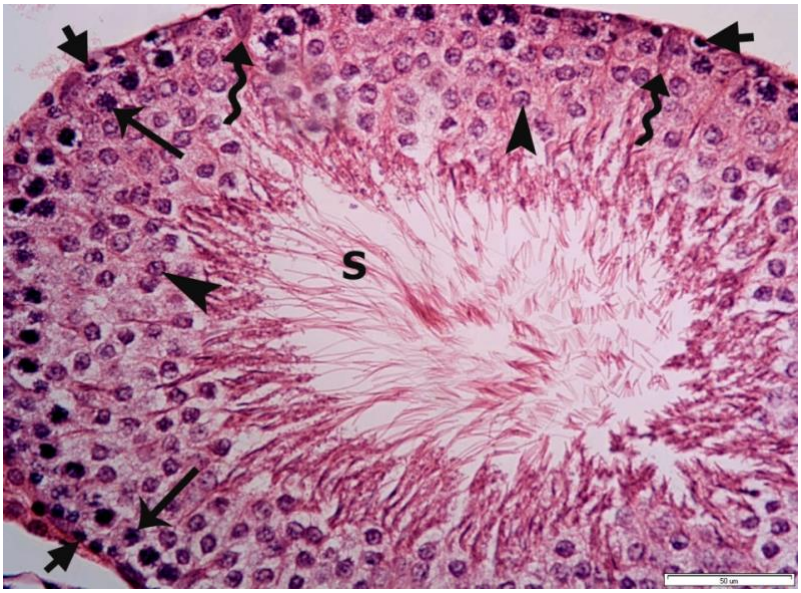

Fig. 5: A photomicrograph of the testis of an 8 weeks-old rat showing a seminiferous tubule lined with spermatogenic cells in various stages of spermatogenesis, basal spermatogonia (short arrow), primary spermatocytes (long arrow) having large nuclei with dense chromatin strands and adluminal spermatids (arrowhead) with small nuclei. Sertoli cells (wavy arrow) with pale oval nuclei can be seen inbetween the spermatocytes with multiple spermatozoa attached to them by their heads and their tails (S) extending in the lumen of the tubule. (H&E X400)

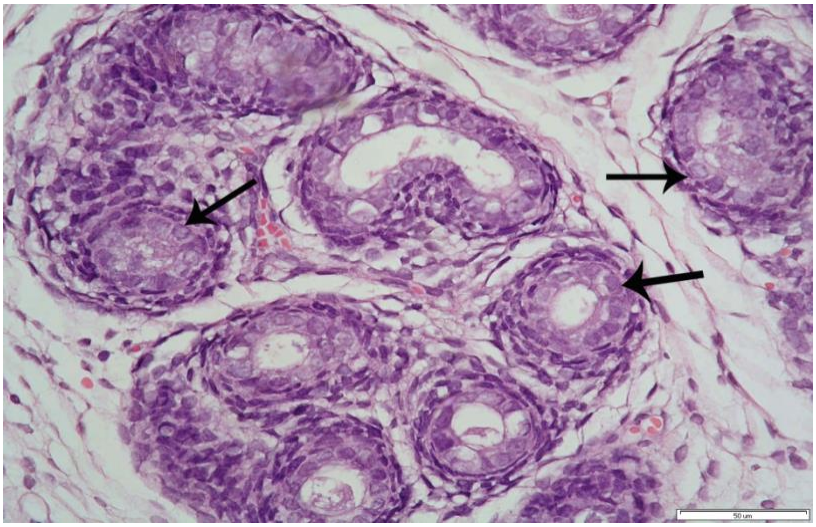

Fig. 6: A photomicrograph of the epididymis of a 1 week-old rat showing its small diameter and narrow lumen. The lining epithelium is formed of a single cuboidal cells layer (arrow). Note the absence of stereocilia. (H&E X400)

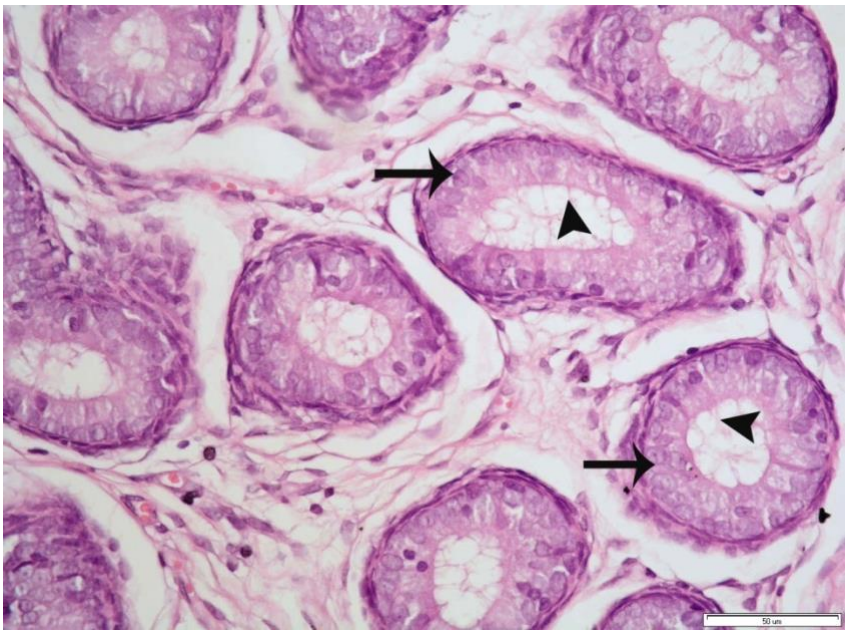

Fig. 7: A photomicrograph of the epididymis of a 2 weeks-old rat showing its relatively larger diameter and lumen, and the lining epithelium (arrow) being formed of densely packed cells with rounded nuclei with scarce primitive stereocilia (arrowhead). (H&E X400)

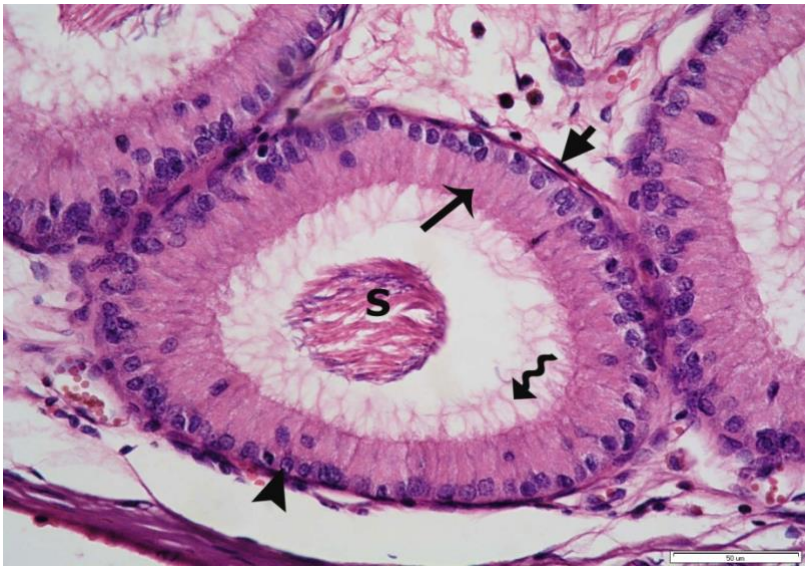

Fig. 8: A photomicrograph of the epididymis of a 4 weeks-old rat showing its lining epithelium formed of basal cells of rounded nuclei (arrowhead) and few other more superficial cells with oval nuclei (long arrow) and many long stereocilia (wavy arrow). A small group of spermatozoa (S) can be seen in the lumen. The wall is surrounded by 1 or 2 layers of spindle shaped circular smooth muscle cells (short arrow). (H&E X400)

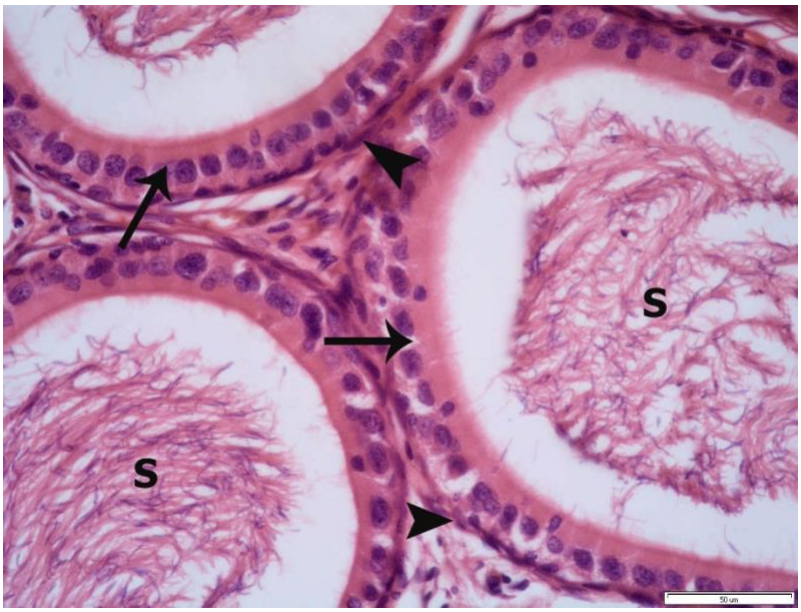

Fig. 9: A photomicrograph of the epididymis of an 8 weeks-old rat showing its wide lumen lined with pseudostratified columnar epithelium (arrow) with steriocilia. The wall is surrounded by circularly-arranged smooth muscle cells (arrowhead). The lumen is filled with numerous spermatozoa (S).

(H&E X400)

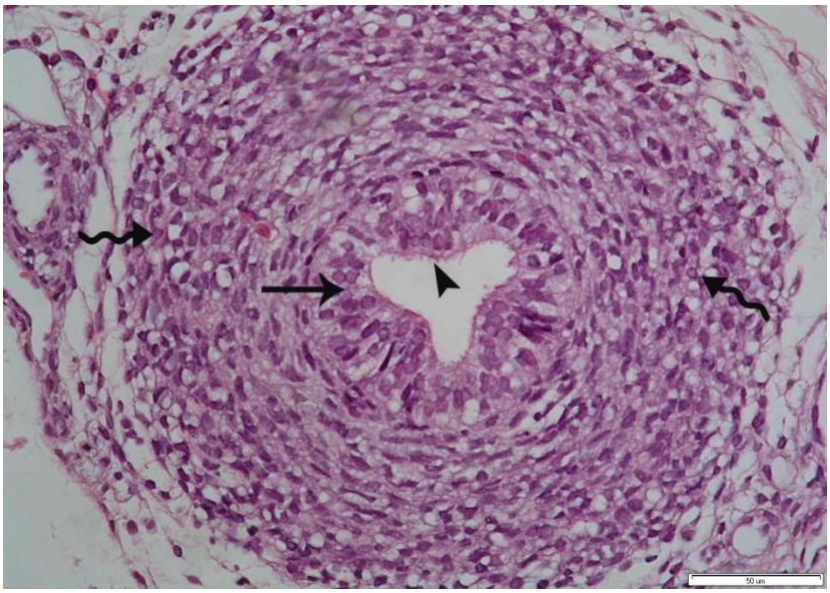

Fig. 10: A photomicrograph of the vas deferens of a 1 week-old rat showing is small diameter and narrow lumen. The epithelial lining is formed of densely packed small cells with rounded nuclei (straight arrow) and the wall contains irregularly arranged muscle cells with rounded nuclei (wavy arrow). Note the appearance of very scarce, short and primitive cilia (arrowhead). (H&E X400)

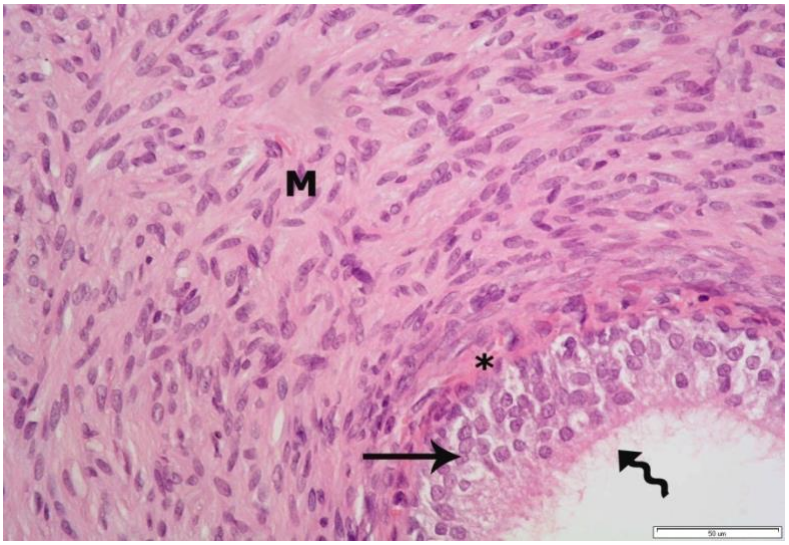

Fig. 11: A photomicrograph of the vas of a 3 weeks old rat showing the relatively larger diameter and lumen. The epithelial lining (straight arrow) is in the form of pseudostratified densely packed cells with rounded nuclei and short cilia (wavy arrow). The underlying lamina propria (asterix) is thin and dense. The muscle cells of the musculosa (M) are compact with oval vesicular nuclei. (H&E X400)

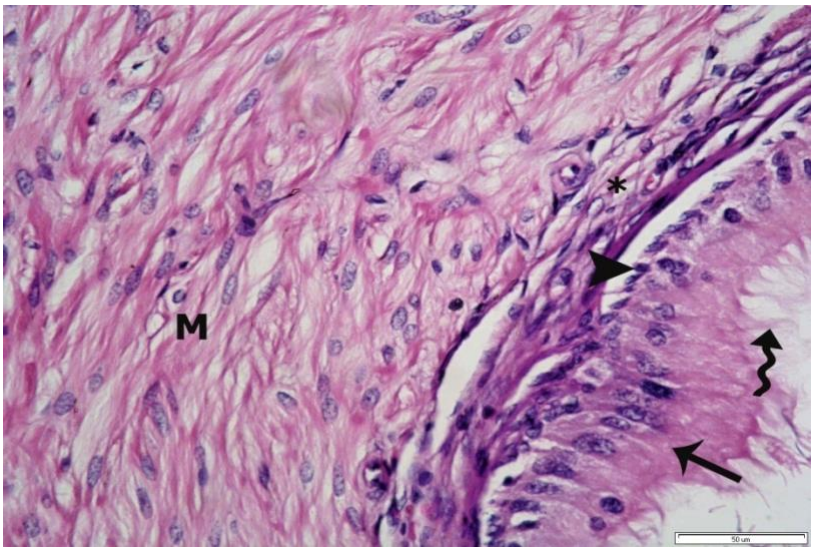

Fig.12: A photomicrograph of the vas of a 4 weeks-old rat showing the pseudostratified epithelial lining formed of basal cells with rounded nuclei (arrowhead) and superficial principal cells with oval nuclei (straight arrow) and numerous long stereocilia (wavy arrow), lying on dense lamina propria (asterix). The musculosa (M) is formed by relatively regular smooth muscle cells with oval vesicular nuclei.

(H&E X400)

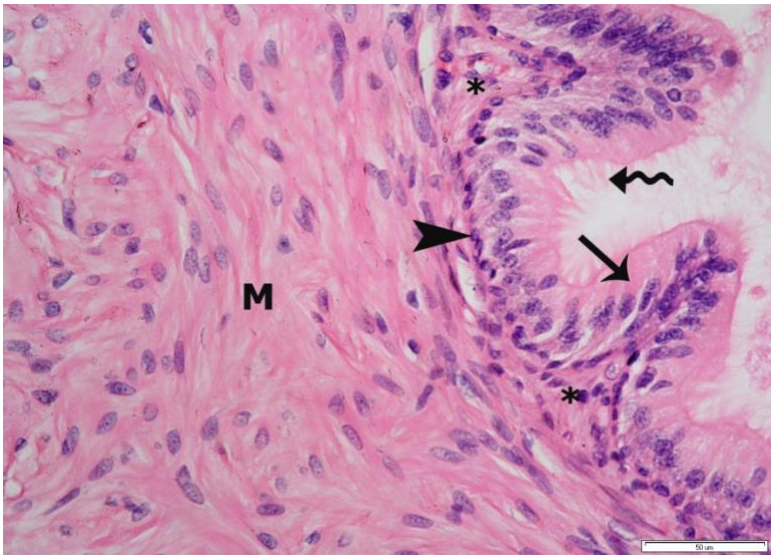

Fig.13: A photomicrograph of the vas of an 8 weeks-old rat showing its lining pseudostratified epithelium formed of basal cells with rounded nuclei (arrowhead) and densely-packed superficial tall principal cells (straight arrow) with oval nuclei and multiple long and well-developed stereocilia (wavy arrow), with underlying thin lamina propria (asterix). The compact well-developed musculosa (M) can be seen.

(H&E X400)

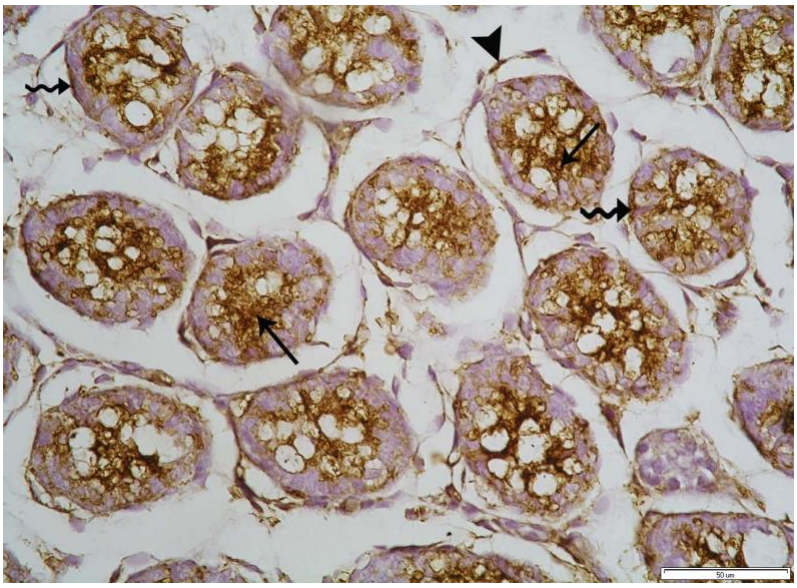

Fig 14: A photomicrograph in a section of the testis of a 1 week old rat showing the non-canalized small seminiferous cords with strong positive laminin immunostaining involving most of the cytoplasm of their cells and in their centre (straight arrow). Positive immunoreactivity could also be seen in the scarce spindle shaped cells surrounding the base of these cords (wavy arrow), in the wall of a blood vessel and as thin fibers (arrowhead) in the interstitium. (Laminin immnostaining X 400)

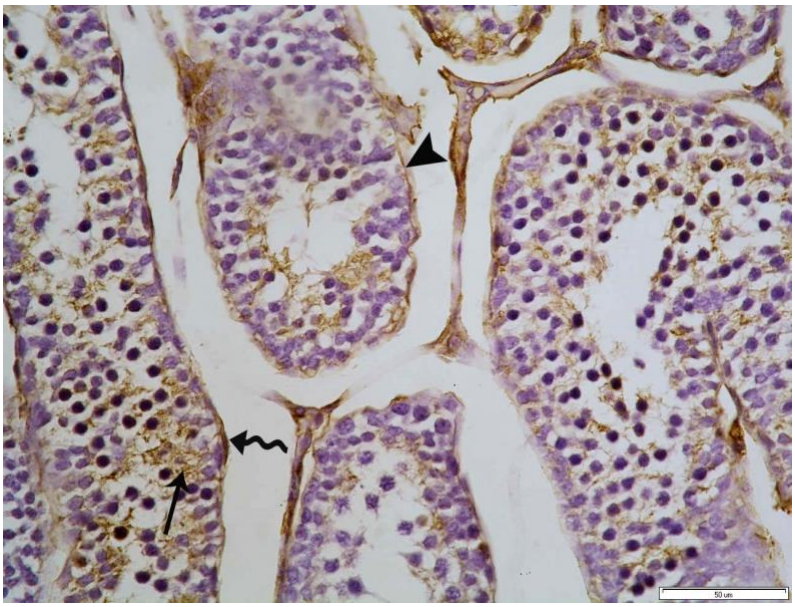

Fig 15: A photomicrograph in a section of the testis of a 2 weeks old rat showing the seminiferous tubules with scanty positive laminin immunostaining within some of its cells (straight arrow), at the basement membrane (arrow head) and in the myoid cells (wavy arrow). (Laminin immnostaining X 400)

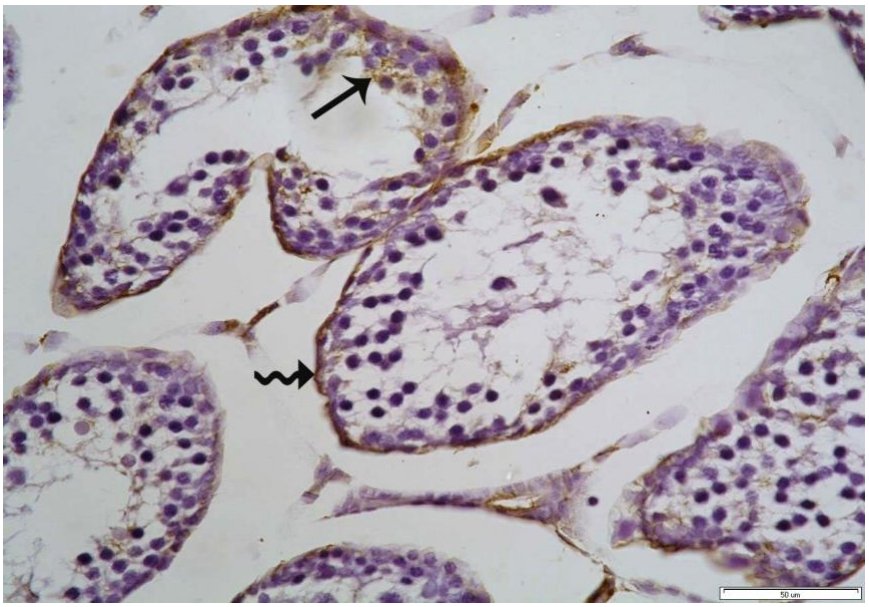

Fig 16: A photomicrograph in a section of the testis of a 3 weeks old rat showing the seminiferous tubules with very scanty laminin immunopositivity within some of the cells (straight arrow) and surrounding the base of the tubules (wavy arrow). (Laminin immnostaining X 400)

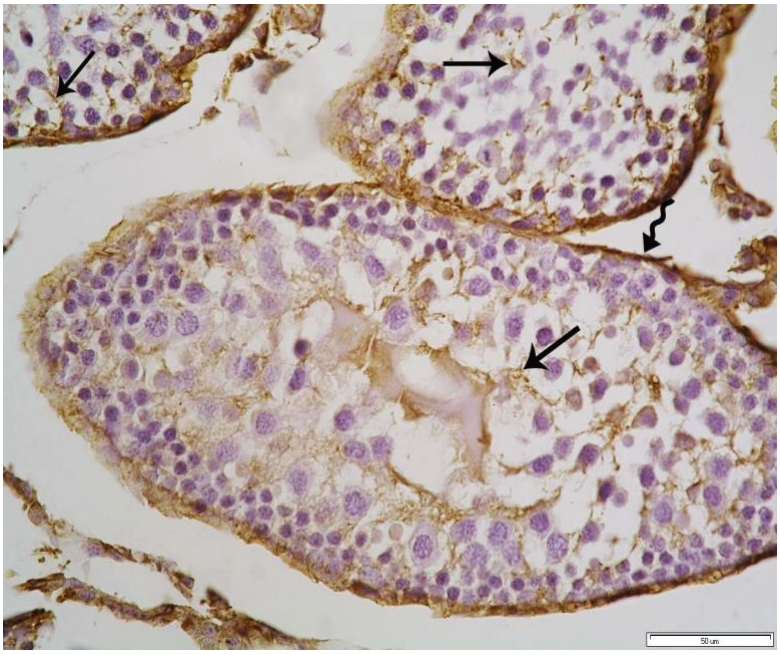

Fig 17: A photomicrograph in a section of the testis of 4 weeks old rat showing the seminiferous tubules with minimal positive laminin immunestaining within some of the cells (straight arrow) and strong reaction at the basement membrane (wavy arrow). (Laminin immnostaining X 400)

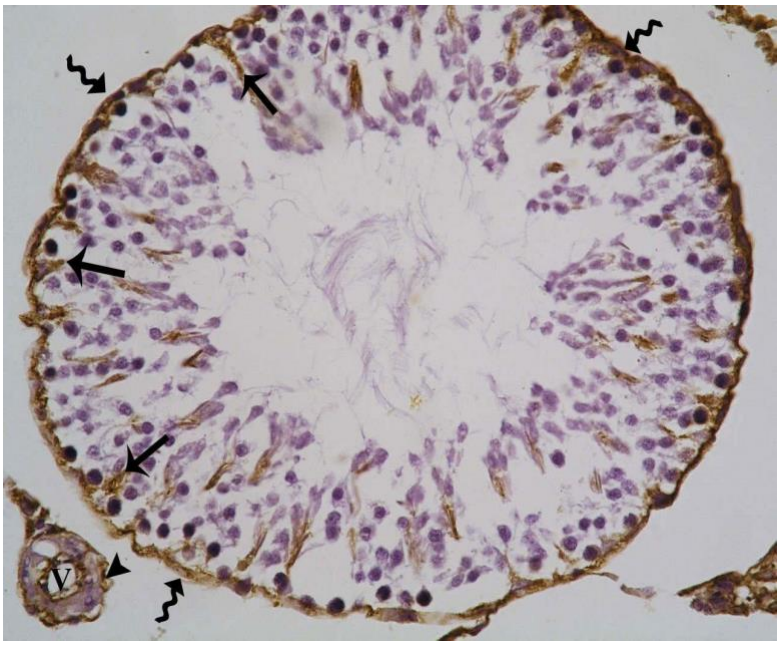

Fig 18: A photomicrograph in a section of the testis of an 8 weeks old rat showing the seminiferous tubules with strong positive laminin immunostaining surrounding the base of the tubules (wavy arrow) and in as streaks within the cytoplasm of sertoli cells (straight arrow). Note positive immunoreactivity related to the endothelium of blood vessels (V) and in association with cells of its media (arrow head).

(Laminin immnostaining X 400)

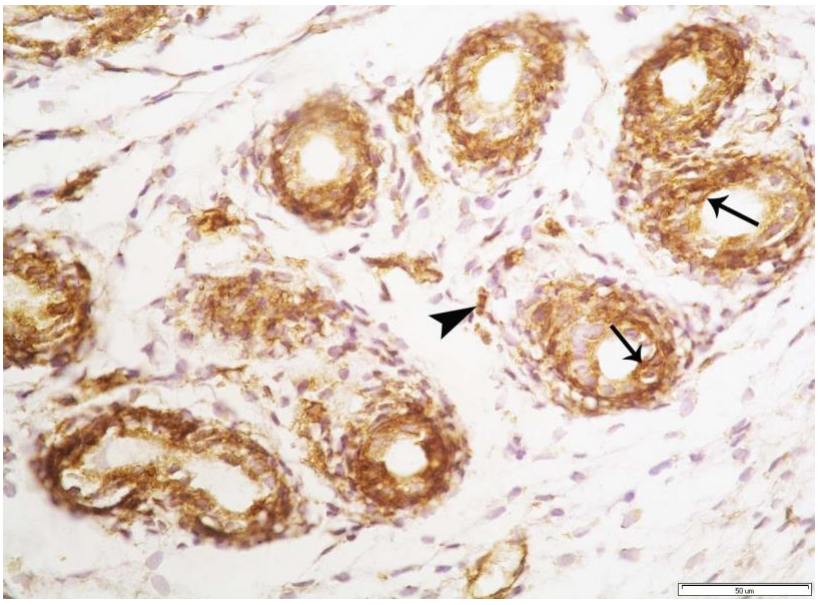

Fig 19: A photomicrograph of a section in the epididymis of a one day old rat showing strong positive laminin immunostaining involving the cytoplasm of the epithelial lining (arrow) and related to cells within the surrounding stroma (arrowhead). (Laminin immnostaining X 400)

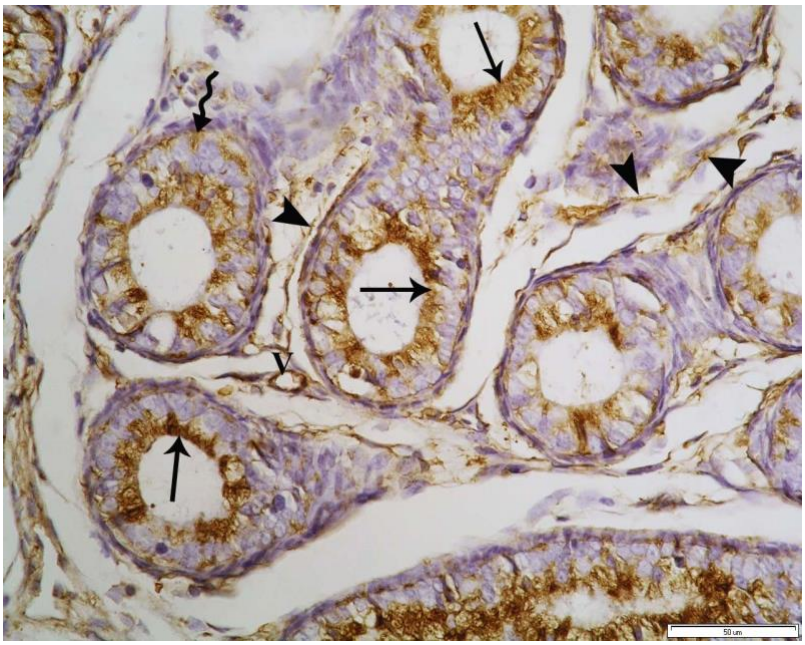

Fig 20: A photomicrograph of a section in the epididymis of a 2 weeks old rat showing strong positive laminin immunostaining mainly in the apical part of the cytoplasm of the epithelial lining (straight arrow) and some reaction at the bases (wavy arrow). Note positive reaction within the surrounding stroma (arrowhead). (Laminin immnostaining X 400)

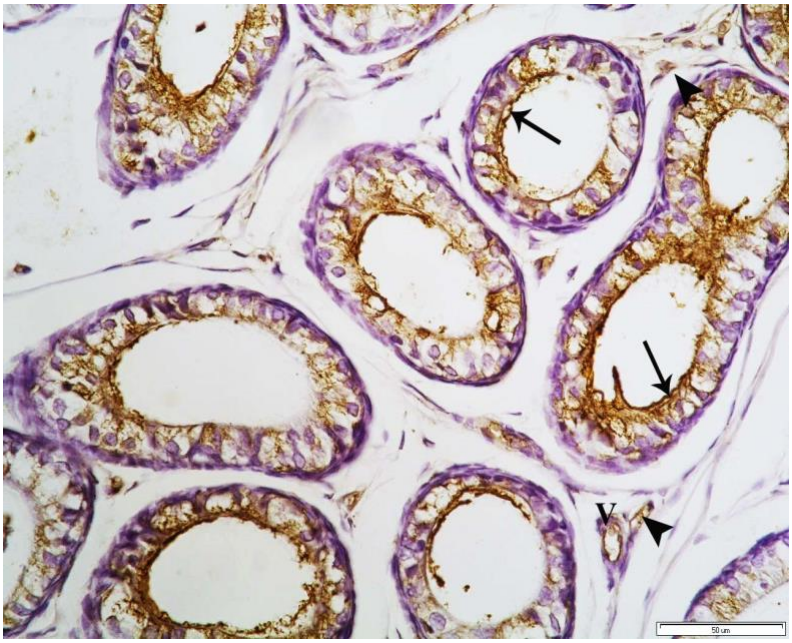

Fig 21: A photomicrograph of a section in the epididymis of a 3 weeks old rat showing strong positive laminin immunostaining mainly in the apical part of the cytoplasm of the epithelial lining (arrow) and within the surrounding stroma (arrowhead). Note positive reaction related to the endothelium of the blood vessel (V). (Laminin immnostaining X 400)

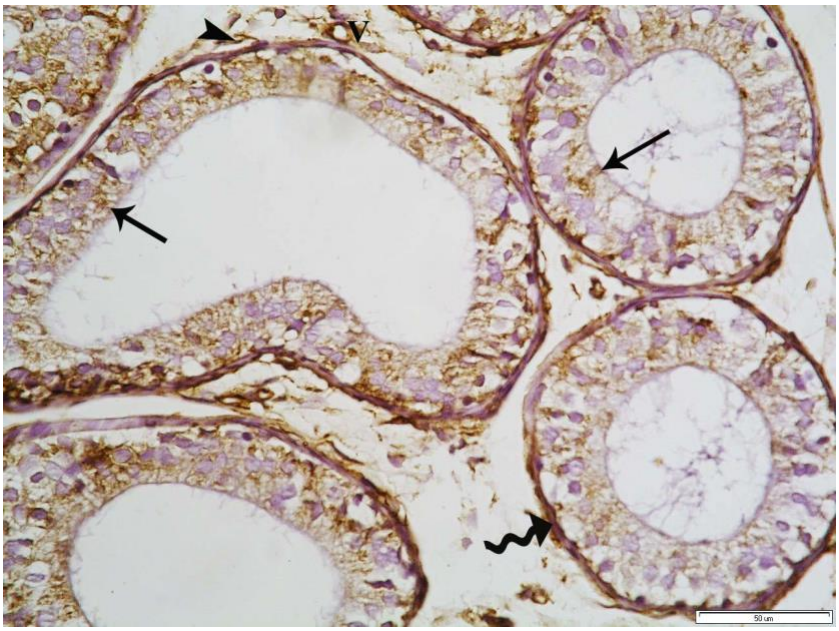

Fig 22: A photomicrograph of a section in the epididymis of a 4 weeks old rat showing mild positive laminin immunostaining in the apical part of the cytoplasm of the epithelial lining (straight arrow), at the base (wavy arrow) and in the stroma (arrowhead). Note positive reaction in the wall of the blood vessel (V).
(Laminin immnostaining X 400)

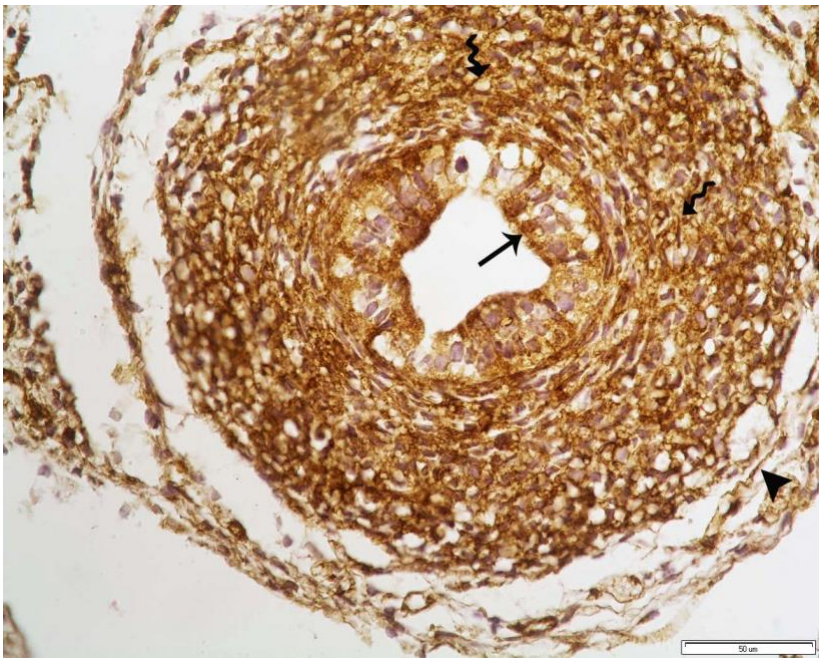

Fig 23: A photomicrograph of a section in the vas deferens of a one day old rat showing the strong positive laminin immunostaining within the cytoplasm of the epithelial lining (arrow), related to smooth muscles of the musculosa (wavy arrow) and within the connective tissue stroma (arrow head). (Laminin immnostaining X 400)

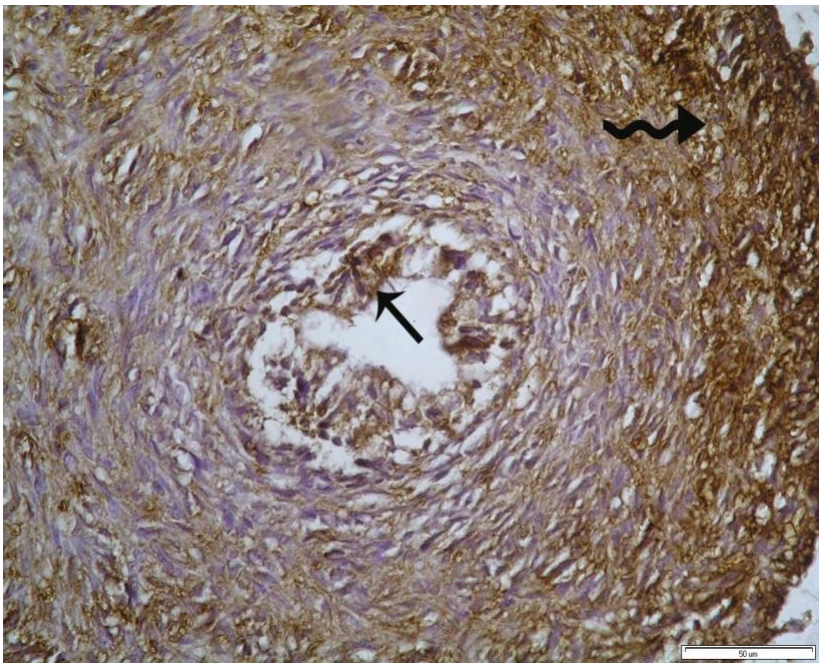

Fig 24: A photomicrograph of a section in the vas of a 2 weeks old rat showing the moderately positive laminin immunostaining within the cytoplasm of the epithelial lining (arrow) and related to smooth muscles of the musculosa (wavy arrow). (Laminin immnostaining X 400)

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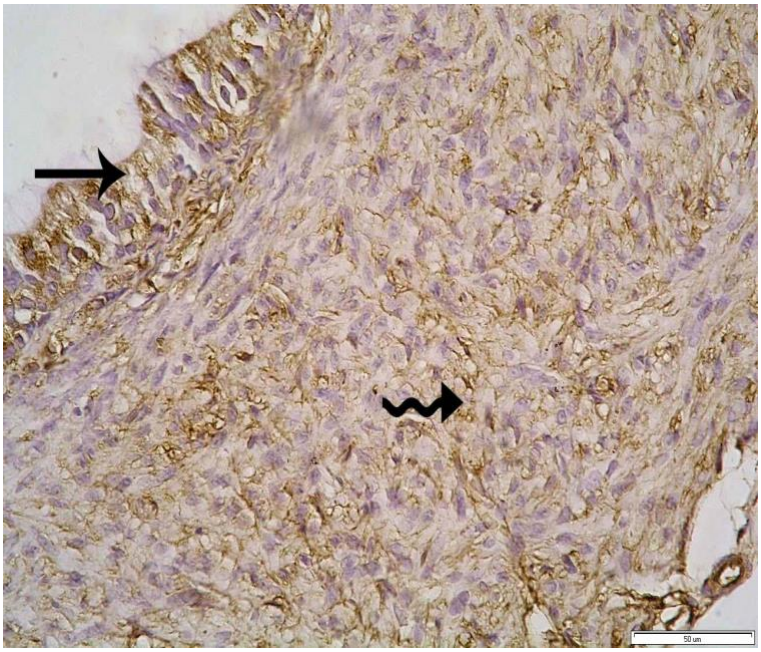

Fig 25: A photomicrograph of a section in the vas of a 4 weeks old rat showing mild positive laminin immunostaining in the epithelial lining (straight arrow) and musculosa (wavy arrow). (Laminin immnostaining X 400)

Discussion

The present study was able to demonstrate the change in the distribution as well as the prevalence of the expression of laminin-1 within the testis, epididymis and vas deferens during the period of postnatal development starting at postnatal one day up to 8 weeks, the age of sexual maturity.

As regard the testis at the postnatal day one they were formed of solid un-canalized cords of seminiferous tubules which were similarly described in a recent study that referred to them as sex cords [13]. These cords were mostly formed of Sertoli cells. This persisted up to the postnatal week one both concerning the appearance and the predominance of Sertoli. These results were in agreement with what was previously reported for other species as the marmoset [14], wild boar[15] ,Assam goats [13] and Alpine goat [16].

By the second and third postnatal weeks the seminiferous tubules were partially canalized with spermatogenic cells reaching up to the stage of primary spematocytes. However, spermatozoa were not detectable at this age. With advancement of testicular development the tubules were fully canalized at the fourth postnatal week with the heads of developing spermatozoa deepened between the multiple layers of spermatogenic cells reflecting evidence of starting spermiation. Reaching the age of eight postnatal weeks, adult seminiferous tubules with several layers of spermatogenic cells in various stages of development and numerous spermatozoa inside their lumen were clearly evident denoting the sexually maturity. In accordance with these observations, similar postnatal age-related developmental changes were recently reported [17]. They similarly described the testicular seminiferous tubules development in the rat to contain only gonocytes and somatic cells at postnatal days 0-5, by days 6-7, spermatogonia appeared, by day 13-23, spermatocytes were present, and round spermatids were observed by day 24-25, at postnatal day 30, elongating spermatids were seen, and by day 36, elongated spermatozoa were found.

Moreover, following the postnatal development in the epididymis over the same period remarkably highlighted that at birth the epididymis was canalized unlike the seminiferous tubules. What happened over this studied durations, is an increase in diameter and lumen of the epididymal ducts with advancement of age associated with change in the height and crowdedness of the epithelial cell lining. The epithelium being simple cuboidal at postnatal day one and week one with no signs of sterocilia, but primitive sterocilia were detected by the second and third week postnatal. By the fourth to the eighth week they acquired an adult appearance. It appears that development of the epididymis is a step ahead of the testicular development most probably in order to be prepared for the accommodation and transport of the sperms once they are produced. Such conclusion could gain further support from the recent a recent work.

This study found that epididymal cells differentiation was characterized by the expression of cell- and region-specific sugar chains that appeared early during postnatal development, by the age of 2 to 3 postnatal weeks, before the arrival of testicular spermatozoa in the epididymis [18].

On the other hand, as early as postnatal day one, laminin immunostaining was detected as cytoplasmic reaction involving most of the cells of the seminiferous cords together with fibrillar reaction within the stroma around the tubules. With advancement of age, the positive laminin immunostaining moved more toward the center of the seminiferous tubules becoming less abundant within the cytoplasm. At the same time it became also localized more toward the basement membranes of the seminiferous tubules as well as that of the blood vessels. By four postnatal weeks, positive reaction was minimal in the cytoplasm of the cells lining the seminiferous tubules acquiring a longitudinal manner related to the nuclei of the Sertoli cells. This was markedly evident after eight weeks; the positive immunoreactivity involved the basement membranes together with the lamina related to the smooth muscles of the media of vessels within the interstitial spaces.

The visually apparent pattern of laminin-1 expression was supported by the morphometric results that demonstrated significant decline in the prevalence during development reaching the least levels at 8 postnatal weeks that is the sexually mature age. Such pattern suggests a role for laminin-1 during the development of the testis as testicular rat development continues following birth.

These findings are consistent with numerous approaches that investigated the localization of laminin in the testis of several mammalian species including mouse, rat [19], dog [20] in addition to human [21,22] and poultry and rabbit [23]. Considering results of the previous studies in association to our current work such localization of laminin in the testis of various mammals and birds might suggest a crucial role of laminin in the male reproduction. In this concept, laminin was proposed to provide a scaffold to which the seminiferous epithelium adhere and/or serve as a barrier between the androgen-producing interstitial Leydig cells and the intratubular Sertoli and germ cells[23].

However, laminin was not exclusively localized to the basal lamina throughout the observed period of testicular development, whereas it started by being cytoplasmic within the cells of the seminiferous cords then receding by approaching the sexual maturity to be associated with the sertoli cells together with the BM. Such laminin positive immunostaining was reported by in another study which observed weak laminin staining seen in the cytoplasm of the duck's Sertoli cells [23]. Expression of laminin within Sertoli cells was similarly identified during rat testicular development [24]. The involvement of Sertoli cells with laminin production was reported in earlier studies which found that laminin was released from cultured rat

Sertoli cells [25]and also synthesized and secreted by both Sertoli and peritubular cells in human [26].

The decline in laminin expression with the advance of age could be attributed to the decrease in number of the supporting cells toward puberty. Such assumption could be supported by an earlier work which declared that the number of support cells was invariably greater than the number of gonadocytes during the early postnatal period of development of testis in pigs [27]. Similarly, such prevalence of Sertoli cells in early testicular development was reported in a more recent study that identified greater number of support cells in the impuberal phase that is the period from birth to the third month of life in the goat [16].

Through combining this observation together with the previously mentioned role of Sertoli in laminin production, it might be appropriate to assume a need for abundant laminin early in development. That would be accomplished by the numerous Sertoli cell numbers at these stages after which it declines to be confined to the apical parts of the Sertoli cells in addition to the BM. Such orientation in non BM structures was reported early in rats [28] this was in the form of apical ectoplasmic specializations in adult rat testis [29].

In addition, at all stages, the endothelial lining of the blood vessels together with the smooth muscles of the media of the medium sized vessels were positive for laminin immunostaining. This was in accordance with a study in which the testicular vasculature also exhibited intense laminin immunostaining, especially in the muscular layer of the medium sized vessels and in the basal lamina of the endothelium. It also reported the same for the epididymal vasculatures of all birds and rabbit [23]. Also the current work detected positive laminin expression as thin fibers in the interstitum between the seminiferous tubules and related to smooth muscle cells around the epididymal ducts. This is also in agreement with the same work that found short laminin positive filament-like structures around the epididymal epithelium of rabbit as well as in the basal lamina of the periductal myoid cells [23].

Laminin 1 expression in the epithelial lining of epididymis was diffuse at day postnatal one and as development continues laminin immune positivity acquired an apical position in the epithelial lining cells. This was clear and prominent at one, two and three postnatal weeks. However few punctuate areas of laminin positive immunostaining were detectable at the basement membrane. By fourth and eighth postnatal week the reaction was more confined to the basement membrane with scanty positive reaction involving the apices of the epithelial lining cells. Such weak to moderate cytoplasmic apical expression of laminin reaction was similar to what was observed in the apical surface, of the so called ciliated cells of the proximal and distal efferent ductules in chicken, duck, and pigeon[23]. Such presence of laminin was proposed by the author to help organize or stabilize the specialized cytoskeleton of the cilia. Laminin at these apical

surfaces may also participate in the anchorage of mucins to the surface.

In the present study, the same pattern of laminin expression was detected in the vas deferens, being abundant in the cytoplasm of the epithelial lining and massive reaction related to the smooth muscles of the musculosa at one day and one postnatal week. As development progresses, laminin expression receded to involve the basement membrane with scanty reaction in the smooth muscle fibers of the musculosa at the sexually mature age. This was in agreement with an earlier work in which the laminin1 immunoreactivity was seen in the ductus deferens along the epithelial basement membrane, in the lamina propria and ensheathing the smooth muscle cells of the muscular layer[28].

To our knowledge, the change in prevalence of laminin expression, localization and quantification during postnatal development of male genital tract was not assessed. This current study was able to detect changes in the prevalence of laminin as in early postnatal period the expression was high and with advancement of age toward puberty the expression declined to be localized to lesser sites. The morphometric results reflecting such changes were statistically significant and showed similar pattern in the testis, epididymis as well as in the vas deferens. This observation would reflect that in the early postnatal period of development of these male genital organs abundance of laminin is required to guide the completion of the spermatogenesis process however as adult form is reached its presence would be limited to where future need might be. This assumption could be supported by earlier studies in other organs where they stated that the patterns of laminin α chain expression within different tissues exhibited both temporal and spatial regulation as development progressed [30]. Also, in the lung, all five laminin α chains were reported to be present during early embryonic development, which primarily regressed in adult lung to only laminin α3, α4, and α5 chains[31]. This opinion supports the concept that while all types are present early during development not all types continue throughout the adult life.

In conclusion, the ECM protein laminin appears to be essential in the early postnatal developmental period of testicular and extra-testicular tubules development in the rat, considerable quantification and localization changes with the progress of age. Further elucidation of the functions and importance of laminin in these organs and other body organs, both in animals and human, would be advisable focuses of future studies.

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