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Original Research Article

Morphometric Epididymis Postnatal Development of White Rabbit Bucks Population in Algeria

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ABSTRACT:
The aim of this study is determinated the ultra structural morphometric changes of the epididymis during the postnatal development of rabbits of the white population. For this, 148 rabbits aged between 4 and 28 weeks were sacrificed, epididymides are fixed for the study of microscopic parameters. The morphometric study of epididymis shows that the principal cells of 4 to 12 weeks are characterized by weak morphofunctional characters. From 14 weeks, these cells acquire morphometric characters marked by high values of hcp and zsn and low values of the report by N / hcp. This is suggestive of a physiological differentiation indicating the acquisition of cellular polarity and the development of secretory and / or absorptive character. The high N / hcp ratio of young individuals between 4 and 12 weeks of age could be an indicator of the existence of cell divisions. The structural changes of the epididymis during the postnatal development is manifested by a progressive increase of the height of principal cells and the height of the zone supranuclear. The values of hcp and zsn are higher at the level of the épididyme proximal from 16 weeks which correspond in the entered in puberty.

Keywords: Epididymis; rabbit; principal cell; morphometric.

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INTRODUCTION

The rabbit (*Oryctolagus cuniculus*) offers many advantages in the field of reproduction. It allows the demonstration of some reproductive processes such as morphological changes of epithelial epididymal cycle (Ewuola and Equnike, 2010).

The Algerian rabbit production turned to the use of the rabbit of "white population", the productive aspect of which diversified and heterogeneous requires identification and knowledge of these biological and zootechnic capacities. The majority of the research work carried out has dealt on the one hand with the reproductive aspects of the various existing genetic types in rabbits and on the other hand, semen analysis in male rabbits.

The study of the sexual development of the animal involves the knowledge of the profiles of growth and maturation of tissues of the reproductive system bound the potential capacity of spermatic production (García-Tomás et al., 2007). The implementation of the puberty is coupled with a proliferation and a maturity of epididymis cells (Vigueras-Villasenor et al., 2013).

The epididymis plays a very important role in male fertility, thanks to the post-testicular maturation events that result from a constant interaction between the male gametes and the specific environment of the epididymal fluid, composed mainly of synthesized and secreted proteins highly regionalised by epididymal epithelium (Turner, 1991; Olson et al., 2002).

In order to characterize epididymal postnatal development of the white population rabbit, histogenesis of cellular structures are explored by histomorphometric methods.

MATERIAL AND METHODS

Biological material

This study was conducted at the Animal Physiology Laboratory of Mouloud Mammeri University of Tizi-Ouzou. A total of 148 rabbits bucks aged 4, 8, 12, 14, 16, 18, 20, 24 and 28 weeks were used in this study. These animals come from an existing local population in Tizi-Ouzou city and called "white population". They are rabbits of commercial hybrids, imported from France between 1980 and 1987.

The animals are kept in individual cages and fed ad libitum with a commercial granulated feed (11.16% of total nitrogenous matter and 10.32% of crude cellulose) and subjected to the same breeding conditions (conditions of illumination and natural temperature). The water is distributed in permanent free access by individual pipettes.

Sampling gonads and histomorphometric study

After sacrificing, the gonads are removed, defatted and the right epididymes are fixed, for histological and morphometric study, in the Holland Bouin for 5 days are dehydrated in ethanol baths at increasing degrees (50°, 70°, 80°, 90°, 100°) and then included in the paraffin with the help of a circulation and coating apparatus of the Leica type.

The paraffin packed samples are cut into 5µm thick serial histological sections using the Leica Microsystem-type microtome at the anatomical pathology laboratory of the Tizi-Ouzou University Hospital Center. The topographic stains used are Masson trichrome (Goldner variant) and Hematoxylin-Eosin (Martoja et Martoja, 1967).

The morphometric measurements of the epididymis of rabbits were taken on more transverse histological sections (rays are almost equal R1 / R2 close to 1). The counting and cellular measurement of the epididymal structures: height of the principal cells (hcp), height of the supranuclear area (zsn) and height of the nuclear (N) are performed on the photos scanned by the Nano Digital Zoomer with the software Pannoramic viewer and the Ziess Axio Vision image analysis software. The height of the infranuclear area (zin), the ratio of the supranuclear zone to the total size of the cell (hzsn / hcp) and the nucleocytoplasmic ratio (N / hcp) are calculated.

Statistical analysis

The set of measured and calculated variables was subjected to an analysis of the variance (ANOVA test) with the software Origin pro 7.5 (2007 version). The values presented are expressed as averages assigned to the Standard Mean Error. The difference is considered significant when $p \leq 0.05$.

To better characterize the morphometry of epididymal structures, Principal Component Analysis (PCA) is applied. It is a method of projection of data from a space of K dimensions of k variables to a space of P dimensions of p variables (p<k), so that the maximum of dimensions is reduced while keeping the information and is represented by the total variance of the sample.

RESULTS

The rabbit epididymis is a strongly curled canal, connected to the testicle upstream and the vas deferens downstream. Anatomically, it is subdivided into three major parts: a head that covers the testicle and constitutes the proximal epididymis, a tapered body and a thick tail that represents the distal epididymis.

Morphometric measurements of the proximal and distal epididymis at the cellular level are subjected to a variance analysis between two successive ages for the different parameters studied.

Height of principal cells (hcp)

The morphometric study of the proximal and distal epididymis reveals a progressive increase in the height of the principal cells of rabbits aged 4 to 28 weeks (Fig. 1) with a difference of 37.49µm in the proximal epididymis and a gap of 17.66µm in the distal epididymis. This increase is significant at 8, 12, 14, 16 and 24 weeks in the proximal epididymis and at 8, 14 weeks and at 24 weeks in the distal epididymis.

The comparison of the height of the principal cells between both perties of the epididymis noted that these values are higher at the level of the distal epididymis between 4 and 14 weeks and at the level of the proximal epididymis from 16 weeks.

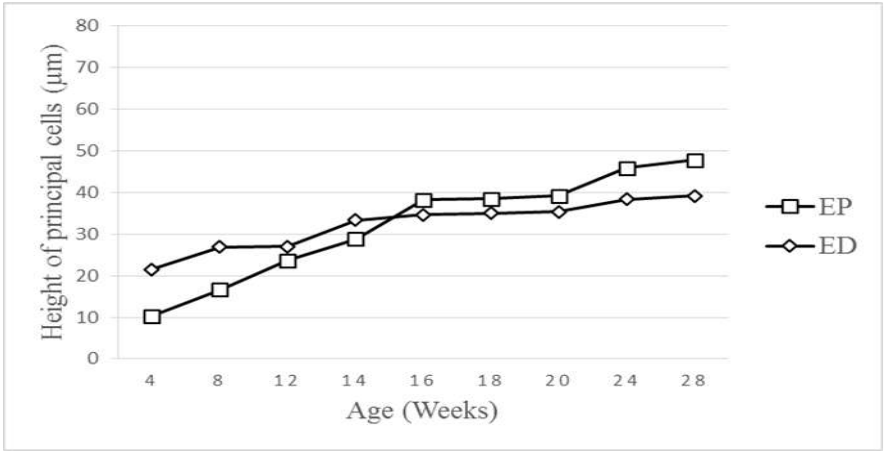

Figure 1: Evolution of the height principal cells according to the age in the proximal (EP) and distal epididymis (ED) of back rabbit white

Height of the supranuclear area

The evolution of the height of the supranuclear area in the proximal and distal epididymis shows a gradual increase from 4 to 20 weeks with a difference of 16.55µm in the proximal epididymis and 7.24µm in the distal epididymis, then gradually decreases to 28 weeks (Fig. 2). These variations are significant at 8, 12, 16, 18, 20 and 28 weeks in the proximal and at 12 weeks in the distal epididymis.

The comparison of the height of supranuclear area between both parts of the epididymis noted that these values are higher at the level of the distal epididymis between 4 and 14 weeks and at the level of the proximal epididymis from 16 weeks.

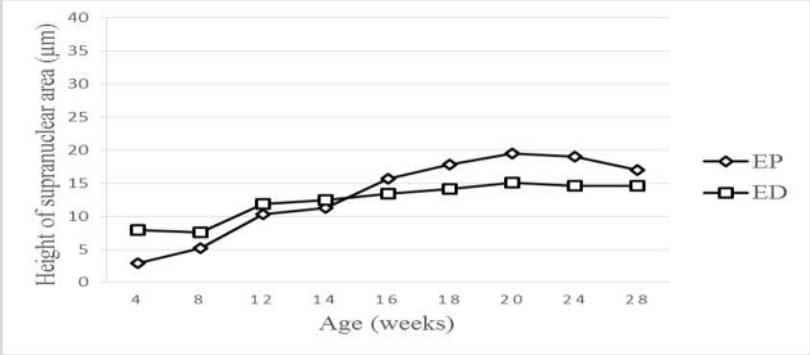

Figure 2: Evolution of the height of supranuclear area according to the age in the proximal (EP) and distal epididymis (ED) of back rabbit white

Height of the nucleus

The height of the nucleus, in the epididymis during the postnatal development (Fig. 3), notes a progressive increase from 4 to 14 weeks, with a difference of 3.92µm. Then marks a plateau until the age of 18 weeks to gradually decrease to 28 weeks of age to the value of 7.72 ± 0.18µm. But in the distal epididymis this height increase to 4 weeks at 28 weeks with a difference of 8.79µm. These variations are significant at 4, 8, 14, 20 and 28 weeks in the proximal epididymis and at 8, 12, 14, 18 and 28 weeks in the distal epididymis.

The comparison of the height of nucleus between both parts of the epididymis noted that these values are higher at the level of the distal epididymis between 4 and 12 weeks and at the level of the proximal epididymis from 20 weeks. The values of this height is similar between the proximal and distal epididymis to 14 at 18 weeks.

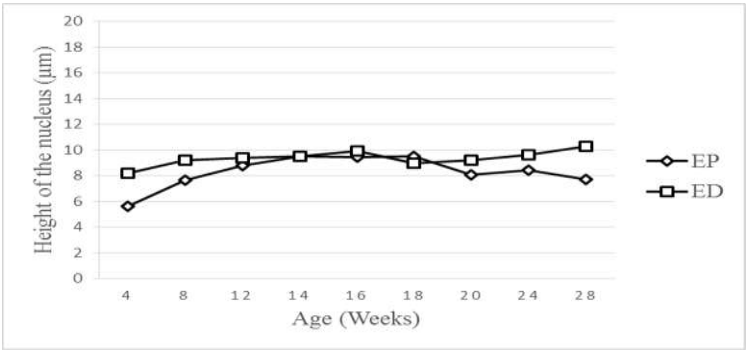

Figure 3: Evolution of the height of the nucleus cells according to the age in the proximal (EP) and distal epididymis (ED) of back rabbit white

Height of the infranuclear area

The evolution of the height of the infranuclear area in the proximal and distal epididymis reveals a progressive increase from 4 to 28 weeks of age (Fig. 4). This height increase with a difference of 21.37µm, in the proximal epididymis and 21.37 µm in the distal epididymis. These variations are significant at 8, 14, 18, 24, 28 weeks in the proximal epididymis and at 8, 14, 18, 24 and 28 weeks in the distal epididymis.

The comparison of the height of nucleus between both parts of the epididymis noted that these values are higher at the level of the distal epididymis between 4 and 12 weeks and at the level of the proximal epididymis from 20 weeks. The values of this height is similar between the proximal and distal epididymis to 14 at 18 weeks.

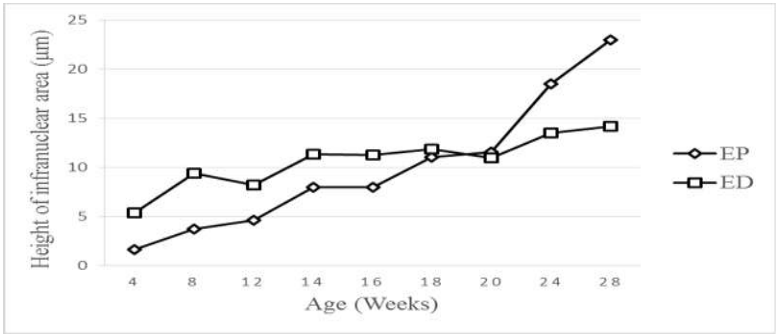

Figure 4: Evolution of the height of the nucleus cells according to the age in the proximal (EP) and distal epididymis (ED) of back rabbit white

Supranuclear area to the height of the cell ratio (zsn/hcp)

The ratio of the supranuclear area to the height of the cell fluctuates with values between 0.29µm at 4 weeks of age and 0.54µm at 20 weeks in the proximal epididymis and between 0.29µm and 0.44µm in the distal epididymis (Table 1). These variations are significant at the different age.

Nucleocytoplasmic ratio (N/hcp)

However, the nucleocytoplasmic ratio decreases progressively from 4 to 28 weeks from the value of 0.55 ± 0.01 µm to 0.16 ± 0.01 µm in the proximal epididymis and weeks from 0.40 ± 0.01µm to 0.27 ± 0.01 µm in the distal epididymis (Table 1). These variations are significat in the different age.

Table 1: Evolution of the ratio of Supranuclear area to the height of the cells and Nucleocytoplasmic ratio in function of age

Age	zsn/hcp (µm)		N/hcp(µm)		zsn/hcp (µm)		N/hcp(µm)	
	Moyenne	p	Moyenne	P	Moyenne	p	Moyenne	P
4	0.29±0.01		0.55±0.01		0.36±0.01		0.4±0.01	
8	0.31±0.01	ns	0.47±0.01	0	0.29±0.01	0	0.36±0.01	0.0013
12	0.45±0.04	0	0.02±0.01	0	0.44±0.01	0	0.26±0.01	0
14	0.37±0.01	0,011	0.37±0.02	0	0.37±0.01	0.0001	0.29±0.01	0.0034
16	0.42±0.02	0	0.25±0.01	0	0.29±0.01	0	0.39±0.01	0
18	0.46±0.01	0	0.25±0.01	ns	0.41±0.01	0	0.26±0.01	0
20	0.50±0.01	0,018	0.21±0.01	0	0.42±0.01	ns	0.27±0.01	Ns
24	0.41±0.01	0	0.19±0.01	0.015	0.39±0.01	0.016	0.26±0.01	Ns
28	0.36±0.01	0	0.16±0.01	0.002	0.37±0.01	ns	0.27±0.01	Ns

Principal Component Analysis (PCA) of the various variables of the proximal epididymis

The extraction of the principal components (Table 2) shows that the principal cell height (hcp), supranuclear area height (zsn) and the nucleocytoplasmic ratio (N / hcp) variables strongly contribute to the constitution of the factor1 (0, 95, 0.92, 0.82). The infranuclear area (zin) and the ratio of the supranuclear area to principal cells height (zsn / hcp) strongly contribute to the constitution of factor 2 (-0.72, -0.73) (Fig. 5).

The projection of the observations on the two factors brings more than 77% (55 + 22) of the total inertia (Fig. 6); it shows that the principal cells of animals aged 4 weeks are characterized by weak morphofunctional characters that is to

say: height principal cells (hcp), supranuclear area (zsn), and a hight proportion of the ratio (N /hcp). We find a point cloud between individuals aged 4 and 12 weeks; these have weak morphofunctional characters. From the 14th week, the principal cells acquire physiological characters marked by high values of (hcp), (zsn) and low values of N / hcp. This is suggestive of the acquisition of cellular polarity and the development of secretory and/or absorptive character. The high N/hcp ratio for young individuals at 4 to 12 weeks of age could be an indicator of cell division and / or lack of secretory activity.

Table 2: Factorial weights without rotation and extraction of the principal components (marked weights > 0.70)

	Facteur 1	**Facteur 2**
hcp	**-0,954917**	0,210225
zsn	**-0,920881**	-0,276762
N	-0,323545	-0,383016
zin	-0,664469	**0,724082**
zsn/hcp	-0,564607	**-0,735022**
N/hcp	**0,826223**	-0,135448
Var. Exp	3,307513	1,350392
Prp.Tot	0,551252	0,225065
hcp : Height of the principal cells, **zsn :** Height of supranuclear area, **N :** Height of nucleus, **zin :** Height of the infranuclear ara, **hzsn/hcp :** ratio of the supranuclear area to principal cells height et **N/hcp** : nucleocytoplasmic ratio.		

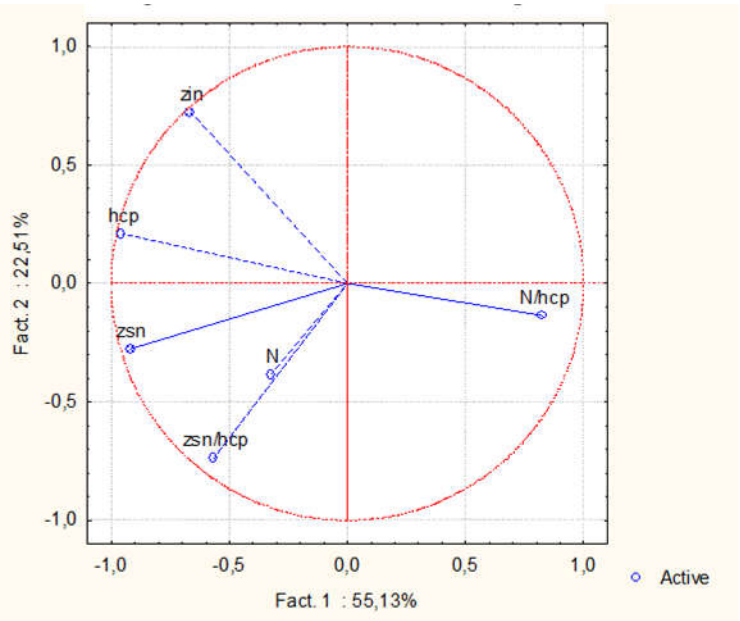

Figure 5: Projection of the various variables on the factorial plan

Principal Component Analysis (PCA) of the various variables of the distal epididymis

The principal components extraction (Table 3) shows that the principal cell height (hcp), supranuclear zone height (zsn) and nucleocytoplasmic ratio (N/hcp) variables contribute significantly to the formation of the factor1 (-0.93, -0.80, 0.79) and are very close to the correlation circle (Fig. 06). The ratio of the supranuclear zone to the total size of the cell (zsn/hcp) strongly contributes to the constitution of the factor 2 (-0.90).

Table 3: Factorial weights without rotation and extraction of the principal components (marked weights > 0.70)

	Facteur 1	**Facteur 2**
hcp	**-0,939395**	0,261172
zsn	**-0,800210**	-0,437200
N	-0,190490	0,370145
zin	-0,672465	0,669791
zsn/hcp	-0,361098	**-0,904054**
N/hcp	**0,795230**	0,113125
Var. Exp	2,774077	1,675094
Prp.Tot	0,462346	0,279182
hcp : Height of the principal cells, **zsn :** Height of supranuclear area, **N :** Height of nucleus, **zin :** Height of the infranuclear ara, **hzsn/hcp :** ratio of the supranuclear area to principal cells height et **N/hcp** : nucleocytoplasmic ratio.		

The projection of the observations on the two factors brings more than 73% (46 + 27) of the total inertia (Fig. 6); it shows that the principal cells of animals aged 4 weeks are characterized by weak morphofunctional characters, ie: cell height (hcp), supranuclear area (zsn) and a high proportion of the ratio N/hcp. We observe an evolution in the acquisition of morphophysiological characters during the age and which will reach its maximum at the age of 28 weeks. These changes are indicators of a gradual evolution with age.

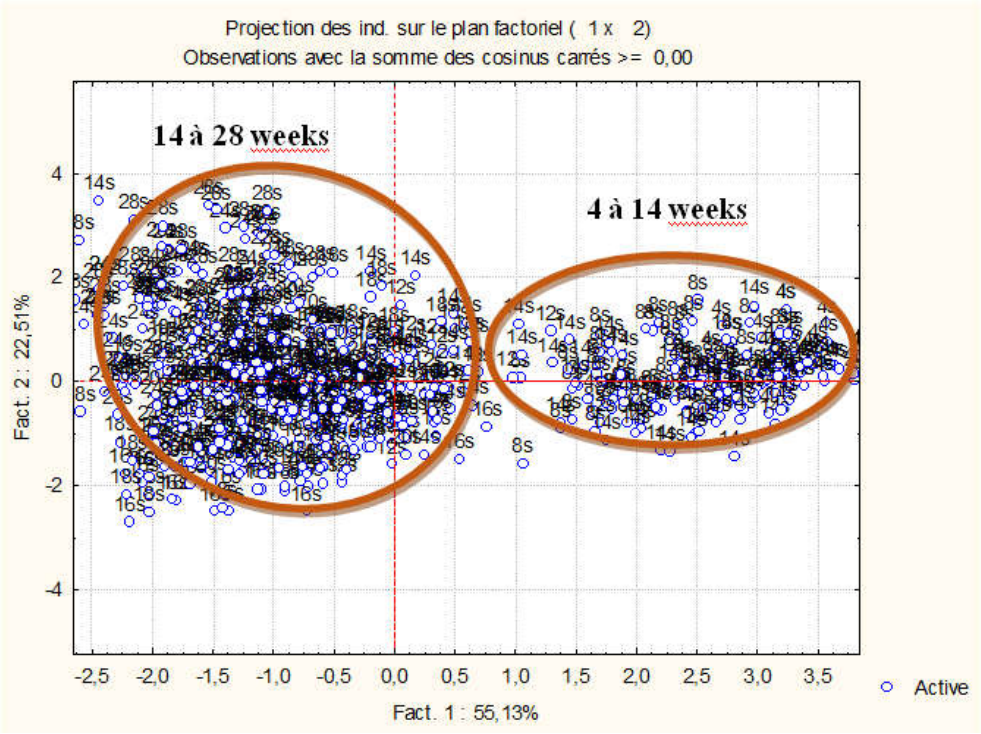

Figure 6: Projection of the individuals on the factorial plan

DISCUSSION

The results obtained during this study show that during the postnatal development comment of the epididymis proximal and the distal the height of the various parameters studied increase with the age. However, the values of the height of the principal cells, the supranuclear area and the infranuclear area are higher at the level of the distal epididymis until 14 weeks for both first one parameters and until 20 week for the third parameter. While they are higher in the proximal epididymis from 16 weeks for hcp and zsn and from 24 weeks for the zin.

We observe a gradual evolution of the morphofunctional characteristics of the principal cells between the 4th and the 28th week. This is manifested by a progressive increase of the hcp and the snz between the different ages, which is accompanied by the acquisition of the apocrine secretion confirming a parallel evolution with the testicular development already described by Lakabi et al. (2016).

We also observed a parallel evolution of hcp and snz between the proximal and distal epididymis with more marked values in the proximal epididymis. Indeed, the comparison of the height of these cells between the two regions of the epididymis shows that the height of the principal cells is greater at the level of the distal epididymis during the infant phase and pre-puberty. Contrarily, during the puberty and adult phases it is the principal cells of the proximal epididymis that are higher. The height of the supra-nuclear zone (snz) of the principal cells is higher in the distal epididymis between 4 and 14 weeks (in the infant and pre-pubertal phases) and in the distal epididymis between 20 and 28 weeks (in the puberty and adult phases).

According to Lakabi et al. (2016), spermatocytes I appear to 12 weeks in seminiferous tubules of the rabbits of this population, what corresponds to the starting up of the spermatogenesis and the entered in phase pre puberty. From this age the epididymis must be ready to receive sperm cells testicular and to lead their maturations thanks to secretions apocrine by the principal cells what can explain that from 16 weeks the height of these cells and that of the supranuclear area became higher and what presents structural characteristics of a grown-up cell.

At the adult the height of the principal cells is higher at the level of the head (proximal) than of the tail (distal) of the epididymis. This variation also concerns the length of the microvillosity which hide their apical (Jones et al., 1979).

Histological characteristics allow for the easy identification of the anterior and posterior extremities of the mammalian epididymis. The thickness of the epididymal epithelium varies with the thickest portion in the proximal caput and the thinnest in the caudal region. Conversely, the luminal diameter and the thickness of the peritubular smooth muscle increases from the proximal to the distal regions (Lasserre et al., 2001; Toshimori, 2003).

Ultrastructurally, the supranuclear region of the principal cell type contains large stacks of Golgi saccules, mitochondria, multivesicular bodies and apical dilated membranous elements, while the infranuclear region is densely packed with rough endoplasmic reticulum (Robaire et al., 2000, Dacheux et al., 2005).

The majority of the studies carried out on the postnatal development of the epididymis were done on the mouse, the rat and the man, although other species was studied such as the bull, the dog, the rabbit, the marmoset and the boar (Rodriguez et al., 2002). Nevertheless, the most detailed description of postnatal epididymal development is that of rats (Robaire et al., 2006).

The studies carried out by Hermo et al. (1992) provide the basis for postnatal development of the epididymis in rats. This development can be divided into three major stages: the undifferentiated period, the differentiation period and the expansion period.

The differentiation and regionalization of the epididymis are related to the different stages of testicular maturation, the steroidogenic activity of Leydig cells, the androgen dependence of the epididymis itself and the lumicrin factors

(Robaire et al., 2006; Cornwall, 2009). Then this phase starts after the differentiation of cells in halo and finished at puberty (Arrotéia et al., 2012).

The lumicrin factors produced by the testes play an important role during epididymal postnatal development (Hinton et al., 2011). Indeed, these factors (such as androgens, growth factors and enzymes) regulate the secretory activity of epithelial cells of the epididymis and spermatozoa and participate directly in the process of epididymal maturation (Robaire et al., 2006).

The principal cells appear along the epididymal canal, but show structural differences in each region. Their structure and functions vary between different segments (Robaire and Hemo 1988) and it accounts for between 65% to 80% of the total epithelial cell population of the epididymis (Hermo and Robaire, 2002).

According to Olukole and Obayemi (2010), the positive correlation between the diameters of the head tubes and the tail of the epididymis means that an increase in one of these parameters would lead to an increase in the other, while the low correlation between epididymal tube diameter and epithelial height means that with an increase in epithelial height the increase in tubular diameter is low.

The negative correlation between the epithelial height and the diameter of the light signifies that with a decrease in the height of the epithelium, the light increases significantly.This correlation between epithelial height and lumen diameter can be attributed to function rather than structure as the tail of the epididymis where the lumen is wider since it stores the spermatozoa.

As a result, sperm concentrations are higher in the tail of the mammalian epididymis compared to the head and body (Dyce et al., 2002). According to Setchell (1989), the transit time of sperm along the epididymis is about 9 to 10 days, and 8 to 10 days according to Bedford (1967). Testosterone has a direct action in maintaining the morphology of the principal epididymal cells and in the inhibition of their apoptosis (Fan and Robaire, 1998).

CONCLUSION

In conclusion, this preliminary study on the morphometry of the postnatal development of the white rabbit epididymis in Algeria revealed that the principal cells in the proximal epididymis of rabbits aged 4 to 12 weeks are characterized by weak characters morphofonctionnel. From 14 weeks, these cells acquire physiological characteristics suggestive of the acquisition of cellular polarity and the development of secretory and / or absorptive character. An evolution of the acquisition of the morphophysiological characters of the principal cells according to the age is also observed at the level of the distal epididymis. These characters are low at 4 weeks and reach a maximum value at the age of 28 weeks. These changes are indicators of a gradual evolution with age.

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