POSTNATAL DEVELOPMENT OF GONADS IN RELATION TO THE BODY GROWTH IN THE MALE RABBIT WHITE ALGERIAN POPULATION

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Abstract

This study aimed to characterize the gonadal postnatal development in rabbits from white population found in Algeria. 122 rabbits aged between 4 and 20 weeks were sacrificed, gonads were weighed and fixed for histological and morphometric investigation. The macroscopic parameters including body weight, testicular weight and volume were measured, as well as the parameters of the microscopic testicular seminiferous tubule. The comparison between the different variables showed an increase in live weight and gonadal depending on age. The obtained data were subjected to analysis of variance using the Origin 7.5 software pro. Histological study showed the onset of 1 to 12 weeks spermatocytes, and smoothed and elongated spermatids at 16 weeks, while the first sperms were visible at 20 weeks. All histological findings recommended a rabbit reproduction implementation for the studied population from 20 weeks.

Key words: *gonads, testis, rabbit, spermatogenesis, histomorphometry*

1. INTRODUCTION

The rabbit (European rabbit) offers many advantages in reproduction field. It allows the detection of some breeding process as morphological changes of the seminiferous epithelial cycle (Ewuola et Equnike, 2010).

In Algeria, the majority of research works were dealing on one hand, with reproductive aspects in rabbits of various existing genetic types (initiated by the University of Tizi-Ouzou and livestock technical institute Baba- Ali ITELV) and on the other hand, with semen analysis in male rabbit.

The male influences reproductive performance of the female, it is known for its semen quality and sexual ardor. Indeed, several studies have revealed male effect on low performance recorded in rabbits of local populations produced in hot countries (Nabi, 2012).

The study of rabbit sexual development needs knowledge of growth profiles and tissue maturation or parts of reproductive system related to the potential capacity of sperm production (Garcia-Tomas et al 2007). Male fertility is marked by adequate gonadal differentiation, maturity of the hypothalamic-pituitary-testicular axis, a differentiation of neonatal testicular cells, testicular descent and early puberty coupled with proliferation and maturity of the testicular cells. The crucial period for the emergence of these changes varies from species to species and between individuals of the same species, it remains undetermined (Vigueras-Villasenor et al 2013).

The testis consists mainly of seminiferous tubules where takes place spermatogenesis and interstitial tissue rich in Leydig cells. These synthesize and release androgens, mainly testosterone playing a role in maintenance of spermatogenesis (Curtis et Amann, 1981; Eurell et Frappier, 2006).

In order to characterize gonadal development of white rabbit population, histogenesis, weight and testicular volume are investigated by histologic and morphometric methods.

2. MATERIALS AND METHODS

This study was performed at the laboratory of animal physiology at Mouloud Mammeri University (Tizi-Ouzou, Algeria). The rabbits used are from a state breeding unit of Djebla located in the town of Ouaguenoune, 18 km north-east of Tizi-Ouzou.

2.1. Biological material

A total of 122 male rabbits aged of 4 to 20 weeks, placed in seven batches according to age (4, 8, 12, 14, 16, 18 and 20 weeks) were used in this study. These animals come from an existing local population at the Tizi-Ouzou region and called "white people" described by Zerrouki et *al.* (2007). They are descendants of commercial hybrid rabbits imported from France between 1980 and 1987.

The animals are placed in individual cages and fed ad libitum with a commercial pelleted feed (11.16% of crude proteins and 10.32% crude fibers) and subjected to the same culture conditions (lighting conditions and natural temperature). Water is distributed in permanent free access by individual pipettes.

2.2. Measurement of live weight and gonads remove

Before any sacrifice, animals of each lot were weighed in the morning, using an electronic scale of 6 ± 0.002 Kg. After sacrifice, the gonads are collected, degreased and weighed with a 0.01g precision balance. The right testicles are fixed for histological study, while the left ones are frozen in liquid nitrogen for further use.

2.3. Histological study

Testes fixed in Holland Bouin for 5 days are dehydrated in ethanol bath at increasing degrees (50°-100°C) and then included in paraffin via by means of a moving and coating device (Leica type) from pathology laboratory of the University Hospital of Tizi-Ouzou. Samples included were cut into serial histological sections of 5 mm thickness with Leica Microsystem microtome at the Laboratory of Developmental Biology and Reproduction (BDR) INRA Jouy-en-Josas.

Topographical coloring used were Masson's trichrome (Goldner variant) (Martoja et Martoja 1967). The observation of histological sections was performed by OPTIKA microscope at different magnifications.

2.4. Histomorphometry

A morphometric study was carried out on testicular structures of rabbits aged of 4 to 20 weeks in order to determine the diameter of the seminiferous tubules (DTS), the surface of the seminiferous tubule (STS), the surface of the epithelium (MS) and surface of the light (SL) using the Axio Vision Ziess image analysis software.

After scanning the cuts by a Nano Zoomeur Digital Laboratory of Developmental Biology and Reproduction (INRA, Jouy en Josas, France), the volumes of the different testicular structures: seminiferous tubules (VTS) epithelium (VEP) and light tubes (VL) were measured only on old rabbits of 12 and 20 weeks, with the image analysis software "Mercator nova explored".

2.5. Statistical analysis

All measured and calculated variables were subjected to analysis of variance (ANOVA) with the Origin Pro 7.5 software (version 2007). The values are expressed as means with standard error. The difference is considered significant when $p \leq 0,05$.
To better characterize morphometry of the seminiferous tubules, the principal component analysis (PCA) was applied. This is a method of data projection from a space with K dimensions of k variables to a space of P dimensions of p variables ($p<k$), so that reduce the maximum dimensions of the retaining maximum information and is represented by the total variance of the sample.

3. RESULTS

The results related to body weights, weight and testicular volume in rabbits as a function of age are shown in Table 1 indicating only values of variance analysis carried out between two successive ages considering various parameters studied. All comparisons revealed a highly significant difference with respect to the age of 4 weeks.

3.1. Variation of live weight and testis weight

The evolution of rabbit live weight from white population shows a progressive increase in weight and follows a sigmoidal curve. Indeed, the average value of the live weight of the population increased from 824.4 ± 71,0g to 2660.7 ± 70.6 g in older rabbits between 4 and 20 weeks with a highly significant difference of 1836g ($p < 0.0001$). This increase is highly significant ($p < 0.0001$) in 8 weeks aged rabbit of and significant ($p < 0.05$) in those aged from 12 and 20 weeks. The highest percentage increase is observed at four weeks of age (103%) while the lowest was observed at 16 weeks of age (7.7%).

The evolution curve of testicular weight rabbits is sigmoidal and progressive. Indeed, the absolute average weight of the testicle between 4 and 20 weeks vary from 0.17 ± 0.02 g to 4.49 ± 0.31 g with a highly significant difference ($p < 0.0001$) of 4.32g. A percentage of 300% was observed at 8 weeks and 119% at 16 weeks of age. These variations in testicular weight are highly significant ($P < 0.001$) at 8 and 20 weeks (respective average weight of 0.59 ± 0.08 g and 4.49 ± 0.31 g) and significant at 12 and 16 weeks (respective average weight 1.19 ± 0.17g and 2.02 ± 0.26g).

Table 01: Change in live weight, testicular weight and testicular volume rabbits depending on age. % Percentage increase between two successive ages

Ages	N	Live weight (g)			Testicular Weight (g)		
		Average	%	W	Average	%	W
4	10	824,44±70,99			0,17±0,02		
8	10	1633,67±76,31	103,1	0	0,59±0,08	300,1	0
12	19	1891,16±74,85	9,7	0,019	1,19±0,17	103,5	0,016
14	21	2091,57±80,6	10,6	Ns	1,39±0,18	62,3	Ns
16	23	2207,96±97,39	7,7	Ns	2,02±0,26	119,9	0,048
18	21	2440,81±77,06	18,4	Ns	2,71±0,28	94	Ns
20	18	2660,67±70,59	8,13	0,04	4,49±0,31	97,7	0.0001

3.2. Histological study of the testicles

At birth, the animal has a stock of some developed stem cells called spermatogonia. The spermatogenic cycle represents all divisions and cell differentiation resulting in the formation of sperm (Boussit, 1989).

The histological structure of testes revealed considerable variability with animal age (Figure 01). Indeed, in four weeks aged rabbits, the testis revels at low magnification an overall histological structure presenting different forms of seminiferous tubules without light (Figure 01 A). At high magnification, the seminiferous epithelium consists of three cell types: spermatogonia, Sertoli cells and rarely spermatocyte. Smaller spermatogonia with rounded nuclei and condensed chromatin are distributed over the entire section of the tube, the center and / or at the periphery. Sertoli cells are recognizable by their nuclei of irregular shapes. They emit expansions that extend to the center of the

seminiferous tubule. An important vascularized interstitial space is visible between the seminiferous tubules. At high magnification, Leydig cells are organized in clusters or dispersed in this space.

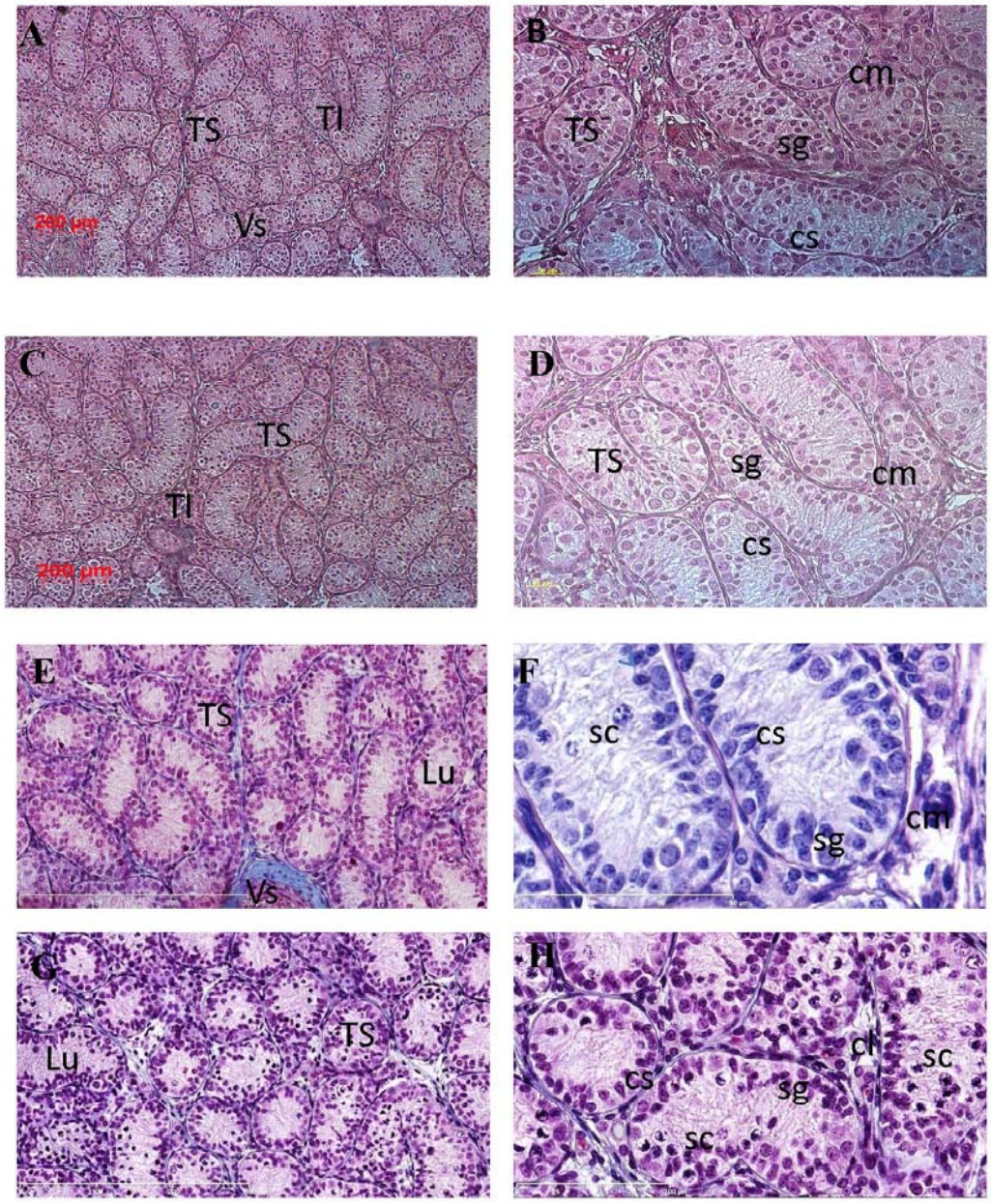

Figure 01 : Histological structure of rabbit testes in white population depending on age.

A et B Testis structure at 4 weeks of age ; **C et D** Testis structure at 8 weeks of age;

E et F Testis structure at 12 weeks of age ; **G et H** Testis structure at 14 weeks of age.

TS : seminiferous tubules; Lu : Lumen e ; Sg : Spermatogony ; CS : Sertoli cells ; SL : Leydig cells ; SC : Spermatocyte ; cm : Myoïde cells ; Vx : blood vessel

Fusiform cells called peritubular cells or myoid cells surrounding the seminiferous tubules (Figure 01 B).

The general appearance of histological sections of the testes of 8 weeks old rabbits shows seminiferous whose central portion is showed hollowed small gaps; they lead to the establishment of the light of these tubes. At high magnification, the seminiferous epithelium contains spermatogonia occupying the periphery and rarely spermatocytes in the wall of the tube. The interstitial space narrows under the effect of seminiferous tubules development and progressively takes triangular shape (Figure 01 C and D).

Observation of testicular sections of 12 weeks old rabbits revealed variable diameter of seminiferous with an apparent light and a wall formed of an epithelium comprising cells of the germ line: spermatogonies with smoothed and fused nucleus ; some spermatocytes I with enlarged nuclei and decondensed chromatin colored as coarse clusters and Sertoli cells with triangular kernel. These tubes were surrounded by a connective tissue rich in vascularized intertubular rich in peritubular cells with flattened nuclei and Leydig cell with smoothed nuclei (Figure 01 E and F).

At the age of 14 weeks, we can see two stages of spermatogenesis in a thick epithelium: spermatogonia, of small size and with light nucleus, were located close to the basal lamina; spermatocytes I larger with enlarged nuclei and Sertoli cells to oval or triangular core occupying the entire wall. A reduction of the interstitial layer was observed in favor of the seminiferous tubules which increase in volume; Leydig cells, rounded core were grouped into clusters (Figure 01 G and H).

Testicular structure of rabbits aged of 16 and 18 weeks reveals a thick spermatogenic epithelium with presence of light; spermatogenesis stops until the appearance of the first rounded and smaller spermatids with dense and elongated nucleus. A reduction in the volume of the interstitial tissue in favor of seminiferous tubes was also observed (Figure 02 A,B, C and D).

At the age of 20 weeks, the testicular histological study showed a thick epithelium containing whole cell mosaic spermatogenesis from stage spermatogonia to stage sperm. Interstitial tissue looked the same of that observed at 16 and 18 weeks (Figure 02 E and F).

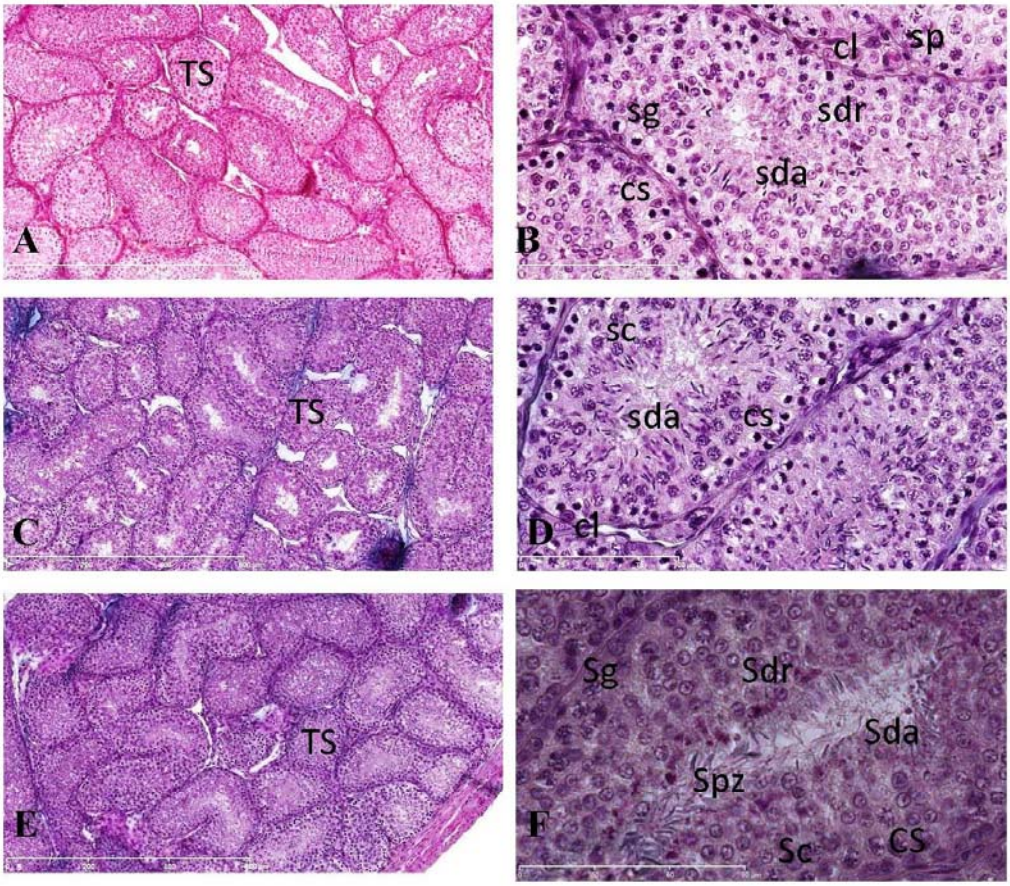

Figure 02 : Histological structure of rabbit testicules in white population depending on age.

A et B Testis structure at 16 weeks of age; **C et D** Testis structure at 18 weeks of age; **E et F** Testis structure at 20 weeks of age.

TS : seminiferous tubules; Lu : Lumen ; Sg : Spermatogonye ; CS : Cellule Sertoli cells ; SL : : Leydig cells ; SC : Spermatocyte ; cm : Myoïde cells ; Vx : blood vessel

3.3. Morphometric study of the testicles

Addtionally to histological study, we performed morphometric measurements on the pictures taken on histological sections observed bylight microscope. Several parameters were measured including seminiferous tubule diameters and surfaces, light

Table 02: Variation of seminiferous tubules diameter and surface, light surface; surface of the seminiferous epithelium and the surface of the interstitial tissue in male rabbits of the Caucasian population, according to the age.

Ages (weeks)	Diameter of TS (µm) Average	W	Area light (µm²) Average	w	Area of seminiferous tubule (µm²) Average	w	Area of the seminiferous epithelium (µm²) Average	w	Area interstitial tissu (µm²) Average	W
4	48,92±0,86		0±0		2049,72±56,62		2049,72±56,62		4309,3±391,5	
8	67,45±1,44	0,0000	0±0	Ns	3973,97±164,41	0,0000	3973,97±164,41	0,0000	2146,9±195,31	0,0000
12	81,91±1,48	0,0000	858±61,82	0	5985,48±202,71	0,0000	5127,48±227,56	0,0001	1978,3±381,13	Ns
14	102,12±3,64	0,0000	829,67±76,86	Ns	8744,33±417,03	0,0000	7914,67±402,31	0,0000	2137,9±240,04	Ns
16	135,37±3,41	0,0000	288,91±45,58	0,0000	16220±755,14	0,0000	15931,09±753,07	0,0000	1297,33±226,97	0,014
18	138,8±2,8	Ns	253,21±81,09	Ns	16120±614,26	ns	15866,79±610,31	Ns	881,87±98,43	Ns
20	138,33± 1,54	0,027	36,97±16,89	0,01	14458,06±537,75	0,046	14421,1±536,49	Ns	414,35±25,12	0,0000

Surface and interstitial tissue. The surface of the seminiferous epithelium was calculated from the previous surfaces measured (Table 02). Variances analysis values were carried out between two successive ages of the various parameters studied since all comparisons revealed a highly significant difference with respect to the age of 4 weeks.

Morphometric study demonstrated a gradual increase highly significant ($p < 0.001$) in the diameter of the seminiferous tubules of rabbits aged of 4 to 16 weeks with a difference about 86.38 microns (48.92± 0.86µm vs 135.37± 3.41µm), followed by a plateau of 16 to 20 weeks of age.

The evolution of seminiferous tubules surface showed a highly significant gradual increase ($p < 0.0001$) for 4 to 16 weeks and then gradually decreases until 20 weeks but with insignificant difference. Indeed, the surface of the seminiferous tubules increased from 2049.72 ± 56.62µm² to 16220 ± 755.14µm² with a gap of 14170,28µm² then decreases to the value of 14458.06 ± 537.75µm² (Table 02)

The surface of the epithelium revealed a highly significant gradual increase ($p < 0.0001$) for 4 to 16 weeks and then decreased gradually until the age of 20 weeks. In fact, it increased to 15931.09 ± 753,07µm² to 2049.72 ± 56,62µm2 with a gap of 13881,37µm² and then decreases to the value of 14421.1 ± 536.49 µm² (Table 02).

The evolution of the surface of the interstitial tissue and the surface of the light is inversely proportional to that surface of the seminiferous tubules and the surface of the epithelium. However, the surface of the interstitial tissue decreased to 4309.3 ± 25.12µm² to 414.35 ± 391.5µm²; these variations are highly significant ($p < 0.001$) at the age of 8 and 20 weeks and not significant at 16 weeks (Table 02).

The light of the seminiferous tubules occurs only from 12 weeks with a surface of 858 ± 61.82µm² which gradually decreased until the value of 36.97 ± 16.89µm² in old rabbits of 20 weeks with a difference about 821.64µm2. These variations are highly significant at 16 weeks and less significant at 20 weeks (Table 02).

Measuring the volume of different structures of the testes of rabbits aged of 12 weeks and 20 showed an average volume of the seminiferous tubule and higher epithelium in 20 weeks old rabbits. Conversely, the volume of the interstitial tissue and light tubes are higher in 12 weeks aged rabbits (Figure 03).

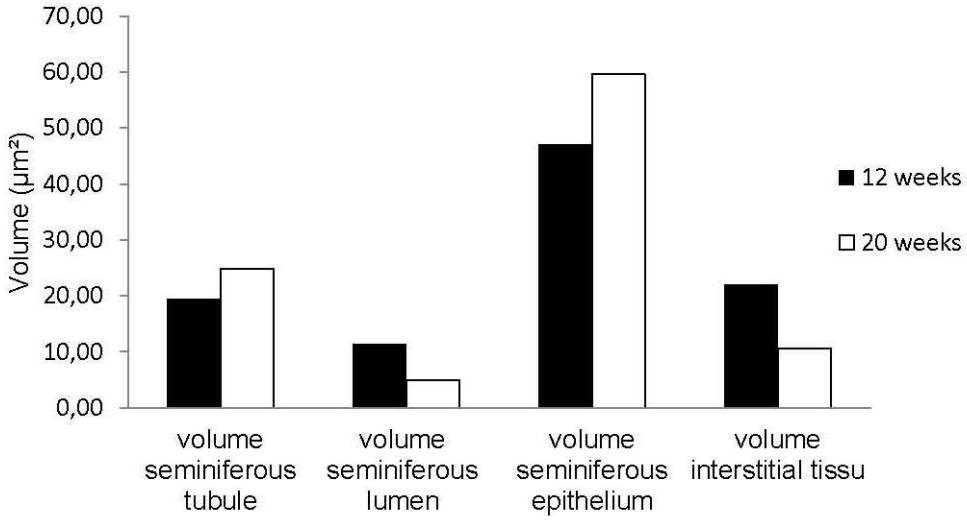

Figure 03: Evolution of the volume of the different structures of the seminiferous tubule of rabbits from white population aged of 12 and 20

The extraction of the principal components (Table 03) showed that the variable diameter of the seminiferous tubules (DST), seminiferous tube surface (STS) and area of seminiferous epithelium (SE) contribute significantly to the constitution of Factor1 (0.94, 0.98 and 0.99) and are very close from correlation circle (Figure 04). The surface of the light (SLu) and the report of the epithelium surface / seminiferous tube surface (SE / STS) are strongly involved in the formation of the factor 2 (-0.92, -0.88).

Table 03: Factorial Weight without rotation and extraction of principal components (marked weight> 0.70)

	Factor 1	**Factor 2**
DST	**0,955**	-0,208
SLu	-0,096	**-0,924**
STS	**0,987**	-0,104
SE	**0,993**	-0,036
SE/STS	0,276	**0,888**
Var. Exp	2,940	1,699
Prp.Tot	0,588	0,340

Variables projection on factoriel plan

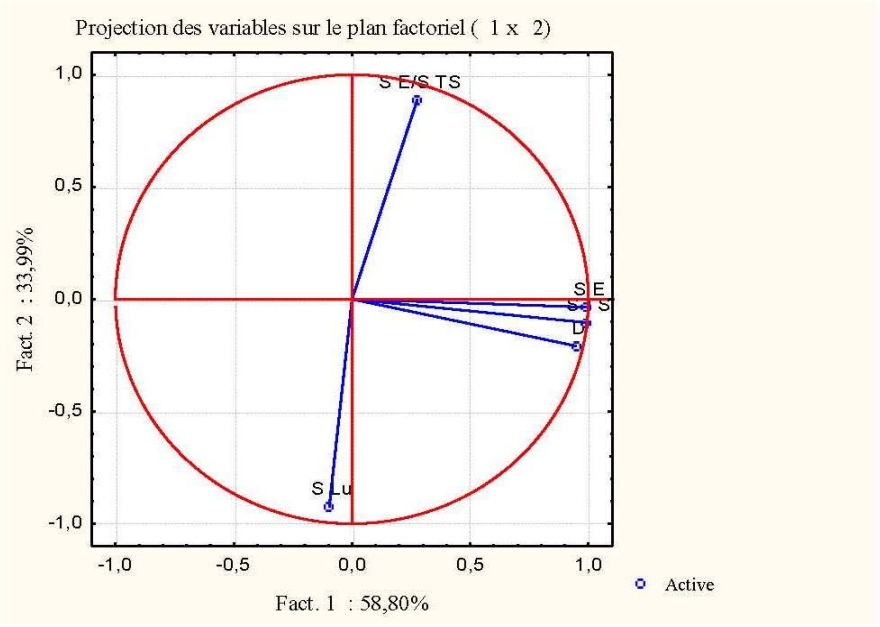

Figure 04: Evolution of the volume of the seminiferous tubule structures of the rabbits from white population aged from 4 to 20 weeks

The projection of comments on the two factors brings more than 92.78% of the total inertia (Figure 05); it showed that most individuals aged from 18 to 20 weeks have seminiferous tubules developed and characterized by a diameter of the seminiferous tubules, a surface of the epithelium and surface of the seminiferous tubule high unlike individuals of 4 and 8 weeks, the dimensions of the seminiferous tubules are still reduced. Most individuals aged of 12 weeks and 14 are characterized by a broad light of the seminiferous tubules.

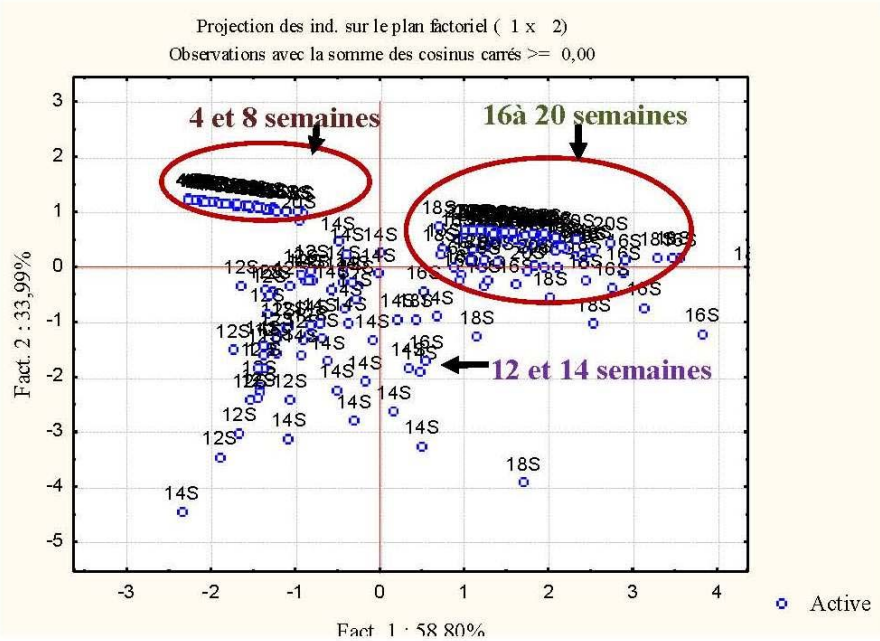

Figure 05: Evolution of the volume of different structures of the seminiferous tubule rabbits of the white population aged 4-20 weeks.

4. DISCUSSION

4.1. Evolution of growth in rabbits male from white population

The curve shape of changes in body weights of rabbits from white population is characteristic sigmoidal type with a percentage of maximum increase to 8 weeks (103.1%). These results are in accordance with those described by different populations of the same species (Garcia-Tomas *et al.,* 2009a; Vigueras-Villasenor *et al.,* 2013).

Weight gain of an animal is the result of weight development of each body component (Micol *et al.,* 1993). According to Piles *et al.* (2003), this growth is an extremely variable character depending on genetic factors, food and / or environmental. The variable growth rate observed between different batches could be related to several factors, including the level of food intake and environmental factors.

Blasco (1993) emphasize that the growth rate reaches a maximum at the age of 5 to 7 or 8 weeks, and then it slows down gradually, especially after 11 weeks, presenting a look sawtooth. The growth rate then tends to zero from the age of six months. According Garcia-Tomas et *al.* (2007), a maximum growth rate is achieved after 8 weeks of life followed by a gradual decline until 33 weeks, with different rates depending on the line.

4.2. Testicular macroscopic variables

In rabbit male, there are few studies demonstrating the relationship between the macroscopic and microscopic variables related to sexual maturation and the development of spermatogenesis. The testicular macroscopic variables such as weight, volume or scrotal circumference were considered as markers of sexual maturity in various mammals (Mandal *et al.,* 2004).

Regarding to the development of weight of the testis our results agree with those obtained by Ambriz *et al.* (2003), Garcia Tomas *et al.* (2007; 2009 a,b) ; however, the values obtained in our study remained lower than that observed by these authors. These differences could be explained by genetic and / or environmental factors.

Garcia Tomas *et al.* (2007) observed a maximum growth rate of testicular of rabbits Prat and Caldes ageed of 14 weeks, indicating that the spermatogenic activity could be engaged. However, Garcia Tomas *et al.* (2009 a,b) considered that weights testicular of rabbits are poor indicators of testicular maturity.

4.3. Testicular histology and morphometry: microscopic variables

Microscopic variables such as the appearance of elongated spermatids and spermatozoa in the seminiferous tubules, the diameter, number and size of the interstitial and germ cells have been used as indicators of maturity (Tegegne *et al.,* 1991). These parameters are consistent with moderately macroscopic variables giving additional information about the functional maturity of the testes (Chemes, 2001).

The study of the postnatal development of testicular white rabbits revealed the appearance of light and first spermatocytes at 4 weeks, the first rounded and elongated spermatids at 16 weeks and the first sperm at 20 weeks. These results do not match those obtained by Garcia Thomas *et al.* (2007, 2009a,b). According Combarnous and Valland-Nail (1997), the rabbit is a species with significant postnatal testicular differentiation, with degeneration of the interstitial tissue around 27 post partum days (ppd) and a significant prepubertal growth from 50 ppd.

According Ricken and Viebahn (2002), the differentiation into spermatogonia is between 49 and 56 ppd, early spermatocytes appear at 8 weeks (56 ppd) and early spermatids are observed at 12 weeks (84 ppd), coinciding with the formation of the tubular light; while the first sperm appear between the 13th and 14th weeks (91-98 ppd). However, Vigueras-Villasenor *et al.* (2013) observed in chinchilla rabbits a reactivation of spermatogenesis at 60 ppd and the first spermatocytes at 80 ppd.

García-Tomas *et al.* (2009 a,b) recorded in Prat and Caldes, rabbits lines the appearance of the tubular lumen at 8 weeks (58 ppd), first spermatids ovoid at 14 weeks (98 ppd) and first sperm at the age of 16

weeks (112 ppd). Ricken and Viebahn (2002), reported that first spermatocytes become visible at the age of eight weeks, and the first spermatids occurs at 12 weeks coinciding with forming the tubular light; while the first sperm manifest at 13th and 14th weeks.

The peritubular myoid cells play an important role in spermatogenesis; they promote progression of spermatozoa by secreting factors that induce proliferation and survival and are also involved in the secretion of activating factors by Sertoli cells (Welsh *et al.,* 2009); Therefore, their training and maturity are necessary for the success of spermatogenesis. Leeson and Forman (1981) observed at 5 ppd in New Zealand rabbits 2-4 oval cell layers oriented circumferentially around the seminiferous tubules; these cells are the peritubular cells and then become condensed between 14 and 28 ppd. At 30 ppd, these cells acquire the morphological characteristics of adult peritubular cells.

Many studies showed a maximum increase of the tube diameter to the top of spermatogenesis (Chemes, 2001). Significant changes in seminiferous tube diameter were scored by Garcia Tomas *et al.* (2007) between 8-14 weeks with multiplication three or four times higher.

Laborde *et al.* (1996) reported that the height of the seminiferous epithelium decreased from the 1st to the 4th week and remains constant up to the 7th week, then increased again in relation with the start of spermatogenesis.[42,26] This is probably associated with cell death increasing with age. The same results were observed in chinchilla rabbits by Yasser *et al.* (2012).

Sexual maturity was defined as the age at which a male is used for the first time for reproduction and gives results that are considered satisfactory in industry (Brito *et al.,* 2004). In current work, male fertility was not evaluated to avoid the possible effect of early semen collection on the development of the testes and male sexual behavior. Áccording tou our results, it could be suggested to not use males for breeding an intensive pace before the age of 20 weeks; at this stage, according to Garcia-Tomas *et al.* (2007), testicular size was only 70% of its adult value.

In agreement with the anatomical development, Combarnous and Volland-Nail (1997) found that postnatal rabbit testes (20ppd) did not express a short transit (1,2kb) LH and FSH receptors, while long transits of the two receivers (between 2.2 and 3 kb) were set up at the approach of puberty.

According to Garcia-Tomas *et al.* (2010), the concentration of testosterone is undetectable at 4 weeks. A notable rise occurred between 4 and 8 weeks and then remains constant up to 14 weeks in Caldes race, while it decreases in the Prat race to finally stabilize at 16 weeks.

Testicular androgens, particularly testosterone plays an important role in the development of reproductive organs and differentiation of germ cells. The plasma level of testosterone in the New Zealandais rabbit increases from 40 to 60 ppd being consistent with accelerated growth of the testes; it stabilizes up to 90 ppd then decreases (Garcia-Tomas *et al.,* 2010). Moreover, Samia *et al.* (2005) showed that testosterone is needed to initiate spermatogenesis at puberty and maintenance of this process in adults. The combined action of FSH and testosterone-induced LH ensures proper conduct of spermatogenesis. LH stimulates Leydig cells to secrete androgens while FSH stimulates the Sertoli cells to secrete the ABP. Androgens and ABP together stimulate the development of germ cells (El-Gaafary, 1994).

In addition, there is a positive correlation between body weight, testicular weight and the plasma concentration of testosterone in growing rabbits (Berger *et al.,* 1982).

CONCLUSION

This preliminary study on the development of white rabbit testes in Algeria allowed us to see the emergence of light from the seminiferous tubules at the 12th week and spermatocytes I at the 12th and 14th week, these are probable the indicators of starting meiotic division in spermatogenesis. At the 16th week, smoothed and elongated spermatids are visible reflecting the onset of spermatogenesis, however, sperm are observed at the 20 th week. These kinetic data of postnatal development of rabbit testicles from the white population can contribute to a better knowledge about puberty age and the control of reproductive performance and production of this population in Algeria.

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