Journal of Cytology & Histology

ISSN: 2157-7099

Khamas et al., J Cytol Histol 2014, 5:6
DOI: 10.4172/2157-7099.1000287

Research Article Open Access

# Histological Description of the Testis, Epididymis and Ductus Deferens of the Northern Great Grey Kangaroo (*Macropus giganteus giganteus*)

**Wael Khamas[1]*, Mohamed Al-Tikriti[2], Mohanad Albayati[3], Suzana Tkalcic[1] and Curtis Eng[4]**

[1]*College of Veterinary Medicine, Western University of Health Sciences, 309 E Second Street, Pomona, CA 91766, USA*
[2]*College of Osteopathic Medicine of the Pacific, Western University of Health Sciences, 309 E Second Street, Pomona, CA 91766, USA*
[3]*Department of Pharmacology and Physiology, College of Veterinary Medicine, University of Baghdad, Al-America, Baghdad-Iraq*
[4]*Head Veterinarian, LA Zoo Health center, 5333 Zoo Drive, Los Angeles, CA, USA*

## Abstract

The *Tunica albuginea* surrounding the testis of the great grey kangaroo was thick and there were large number of Leydig cells present in between the seminiferous tubules. These findings were the striking features among other histological findings of an 18 year old kangaroo which was kept in captivity in Los Angeles zoo all his life.

Eighteen year old great grey kangaroo testes, epididymis and ductus deferens were collected and processed using standard histologic techniques. The animal was kept all his life in captivity in LA zoo. Two different stains were used to differentiate tissues and cells. Histological characteristics of all organs under study were found to be similar to other animal species of younger age with few striking exceptions. The *Tunica albuginea* was very thick and large number of Leydig cells was present between the seminiferous tubules. Animals in captivity with excellent care and veterinary services will continue to be fertile and actively producing sperms when compared to animals of the same age live in the wild.

**Keywords:** Kangaroo; Testis; Histology; Epididymis; Ductus deferens

**Abbreviations:** DD: Ductus Deferens; DE: Ductuli Efferentes; LA: Los Angeles; LC: Leydig Cell; RT: Rete Testis; ST: Seminiferous Tubule; TA: *Tunica albuginea*; TR: Tubuli Recti

## Introduction

Animals' reproduction in captivity is better than in the wild as it has been reported that wild animals may perform much better in captivity due to better and regular availability of food and veterinary care throughout their lives [1]. Also, some animal species show seasonal reproductive behavior which has to be taken into consideration in any study of wild animal. It has been reported that the Northern great grey kangaroo (*Macropus giganteus giganteus)* may live up to 25 years in captivity and could weigh up to 85 kg [2].

In the testis of certain marsupials, seasonal changes in volume fraction of Leydig cells (LC) followed similar changes in plasma androgen concentration. This was confirmed during winter season in male swamp wallabies where notable decreased in plasma androgen concentration was reported [3].

Williamson and co-workers [4] recorded the timing of maturation of different cell types within the testes of tammar wallaby (*Macropus eugenii*). Besides, it was reported that seasonal regression of the testis is rare in male macropod marsupials, even in species in which breeding is strictly seasonal [5-7]. In tammar wallaby, there are no seasonal testicular and/or epididymal change in size, and are potentially fertile year-round [8]. It was also established in the same species that the levels of plasma testosterone and luteinising hormone (LH) increases during breeding season but only in the presence of oestrous females [9]. Paplinska and his co-workers [3] reported that LC was larger and more numerous during summer season than winter in swamp wallabies. However, during sexual development, in gray short-tailed opossum (*Monodelphis domestica)*. Leydig cells are significantly reduced among seminiferous tubules (ST) before puberty and reappear at maturity [10]. Peritubular clusters of LC are thought to be the source of the renewed interstitial cells and this may represent a separate adult LC population in brown marsupial mouse [11].

Annual regression of the testes associated with cessation of spermiogenesis after highly restricted seasonal mating and birthing periods in ringtail possums and greater gliders were reported by several researchers [12-14]. Regressed testes were reported to be similar to immature testes of marsupials [14]. Taggart and Temple-Smith [15] described the structural changes in the epididymis of a marsupial, the brown antechinus (*Antechinus stuartii*). No study of the male genital system of the northern great grey kangaroo was found in the available literature. This report describes the histological architecture of the testis, epididymis and ductus deferens of mature Northern great grey kangaroo.

## Materials and Methods

The data used in this report was from a necropsied single captive animal in Los Angeles (LA) Zoo. Eighteen year-old male northern great grey kangaroo presented with a rapid onset of anorexia, fatigue, arthritis and poor vision. The animal weight was taken two days before death and reported to be 46.4 Kg. Due to grave prognosis, the animal was humanely euthanized by an intravenous injection of 200 mg of ketamine, 3 mg medtomidine and 12 ml euthasol and necropsied at the Veterinary Pathology Center at Western University of Health Sciences, Pomona, California.

Ascites and perigastric serosal edema were evident during necropsy.

***Corresponding author:** Wael Khamas, College of Veterinary Medicine, Western University of Health Sciences, 309 E Second Street, Pomona, CA 91766, USA, Tel: 909-469-5526; Fax: 909-469-5635; E-mail: wkhamas@westernu.edu

**Received** September 16, 2014; **Accepted** October 16, 2014; **Published** October 18, 2014

**Citation:** Khamas W, Al-Tikriti M, Albayati M, Tkalcic S, Eng C (2014) Histological Description of the Testis, Epididymis and Ductus Deferens of the Northern Great Grey Kangaroo (*Macropus giganteus giganteus*). J Cytol Histol 5: 287. doi:10.4172/2157-7099.1000287

The animal had moderate multifocal ulcerative gastritis, hepatitis and mild enodcarditis. Male genital system was reported to be apparently normal with the exception of a white milky secretion dripping from the urethra.

Three specimens of each testis (one close to *Tunica albuginea* -TA, one deeper to it, and one close to tubuli recti-TR), three samples from the epididymis (sections of the caput, corpus, and cauda) and one from the ductus deferens as it continue from the tail of the epididymis were collected and placed in 10% neutral buffered formalin. All specimens were processed following standard histological procedures. Five to seven micrometers thick sections were stained routinely with hematoxylin and eosin and with a special stain (Masson's trichrome to differentiate smooth muscle from connective tissue) [16].

Measurements of the height of epithelium, lumen diameter and perimeters were recorded as well as numbers of LC in three different regions of the testes as mentioned above. Only LC with regular oval or rounded nucleus was measured to avoid any discrepancies due to sectioning incidence. Both regular and irregular nuclei LC are counted as active as long as they had regular cell membranes and foamy cytoplasm. Lipid laden LC with large globules of lipids were considered inactive.

## Statistical Analysis

All calculations were performed with the mean and standard deviation of 10 readings from each slide using ImageJ (NIH) [17,18]. Photography and measurements were performed using Olympus microscope BX 41 with attached Olympus camera DP 71.

## Findings

### *Tubica albuginea* (TA)

The testis was surrounded by thick TA which sends trabeculae through the parenchyma of the testis to divide it into ill-defined compartments containing the seminiferous tubules (ST). The trabeculae carry blood vessels and nerves to supply the parenchyma.

The TA was composed of a dense regular connective tissue with a thickness of 482.9-2388.6 µm. Testicular vessels pass through the TA to supply and drain the testicular parenchyma were observed (Figure 1). The thickest region was found to be the exit area of the ductuli efferentes (DE) from the testicular parenchyma. Loose connective tissue separates the TA from the testicular parenchyma where DE emerges to join the head (caput) of the epididymis. Relatively dense connective tissue fibers interrupted with smooth muscle cells were observed around the tubuli recti (TR) inside the testicular parenchyma. Much thinner layers of loose connective tissue extend inside the testis to separate individual ST from each other. Numerous small arteries, arterioles, venules and capillaries resided within the interstitial connective tissue.

### Testicular tissue

Seminiferous tubules of the kangaroo testis are well developed (Figure 2). Outer diameters of ST close to the TA, in the deeper portion of the testis parenchyma and around the region of the TR are presented in Table 1. The inner diameters of the tubules in the same regions of the ST as well as the stratified epithelium thickness of the ST were recorded and averages of 10 readings for each parameter with standard deviation are presented in Table 1.

Leydig cells were identified by their relatively dark stained eosinophilic cytoplasm and rounded nuclei. Large number of regular and very few lipids laden LC were identified in the testis. Active cells were characterized by a foamy cytoplasm and clear circumscribed nucleus and well defined cell membrane, while lipid laden cells were considered inactive. LC nucleus diameter is 5.7- 6.0 ± 0.9 µm while entire LC diameter is 12.4 ± 2.0 µm (Figures 3 and 4). No difference in diameters is detected in the TR, deep and peripheral (close to TA) regions. Calculated LC index (cell/nucleus ratio) is 2.09 ± 0.38.

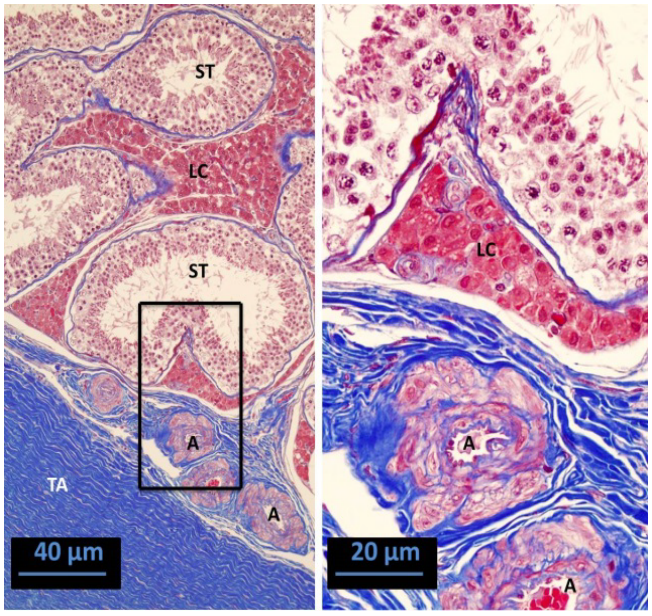


**Figure 1:** Across section in the wall of the testis of the kangaroo showing tunica albuginea (TA), tortuous testicular artery branches (A), Leydig cells (LC) and seminiferous tubules (ST). Trichrome stain, Line scale=40 µm, enlargement image line scale=20 µm.

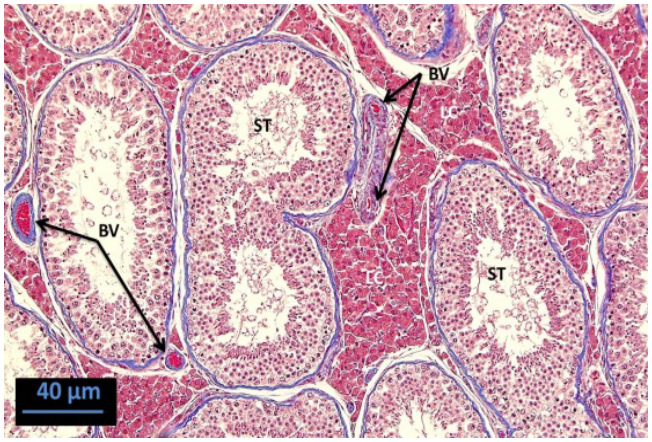


**Figure 2:** Kangaroo testis section taken from a deeper zone (middle) showing well developed ST as well as relatively large number of LC. Trichrome stain, Line scale=40 µm.

| ST | Close to TA | Deeper zone | Around TR |
|---|---|---|---|
| Outer diameter | 337.1 ± 77.3 | 462.8 ± 151.3 | 295.5 ± 54.1 |
| Inner diameter | 148.9 ± 46.4 | 181.9 ± 47.7 | 126.5 ± 16.1 |
| Epithelial height | 188.2 ± 92.2 | 239.7 ± 107.8 | 164.4 ± 57 |

**Table 1:** Measurements of kangaroo seminiferous tubules at three different regions inside the testis, ST- seminiferous tubules, TA-*Tunica albuginea*, TR-tubuli recti. All measurements are average of 10 readings in micrometers.

Number of LC per seminiferous tubule in different regions of the testes showed few differences. The smallest number of LC per tubules present in the deep region of the testes (40 ± 14 µm) followed by the region close to the TA (58 ± 23.4 µm). The largest number of LC was detected around the region of the TR/RT (73 ± 2.4 µm) (Figures 3 and 4). LC close to the blood vessels tended to have smaller and irregular nuclei with variation in position and shape. Spermatogenic cells and yield of the ST in stage VII of the kangaroo testis were counted and presented in Table 2, (Figure 5).

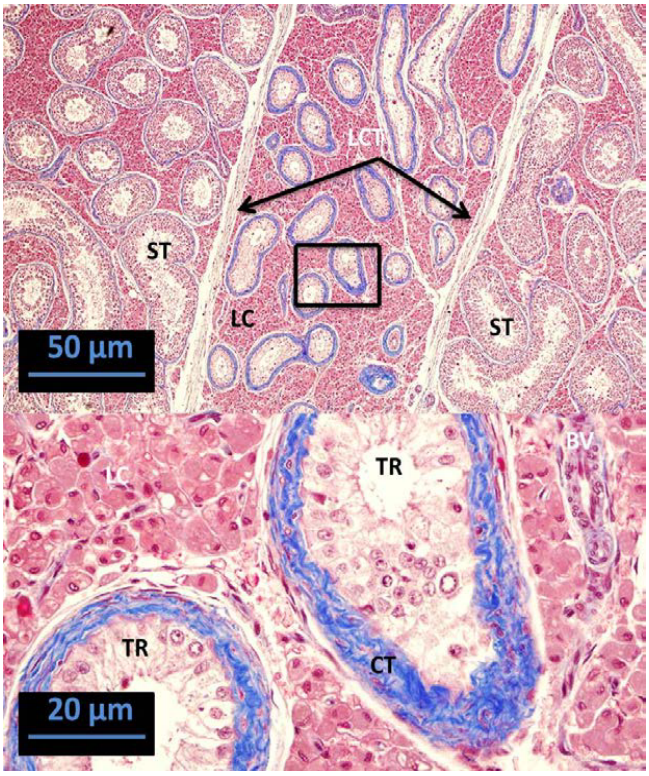


**Figure 3:** A cross section of the kangaroo testis through the middle portion where the seminiferous tubules (ST) end into the tubuli recti (TR) separated by loose connective tissue (LCT). Notice the abundance of the Leydig cells (LC). Trichrome stain, line scale=50 µm , while the enlarged image line scale=20 µm.

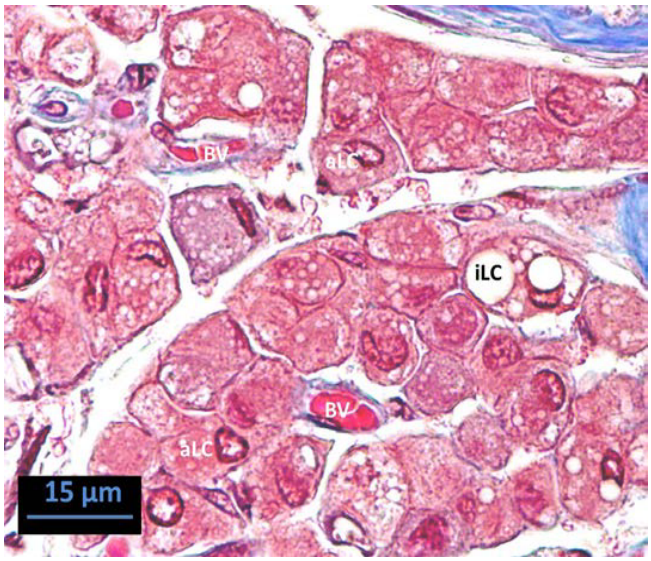


**Figure 4:** Kangaroo LC from the region around the TR. Most of the cells are active cells (aLC) with foamy cytoplasm while one inactive (iLC) lipid laden cell which has two large lipid droplets, scattered blood vessels (BV) among LC. Trichrome stain, line scale=15 µm.

| Spermatogenic cells | Ratio |
|---|---|
| Spermatogonia to Preleptotene | 1: 39.76 ± 1.96 |
| Preleptotene to Leptotene | 1: 12.72 ± 0.71 |
| Leptotene to Pachytene | 1: 17.41 ± 1.28 |
| Pachytene to step 7 spermatids | 1: 27.39 ± 0.71 |
| Spermatid step 7 to Spermatid step 16 | 1: 11.50 ± 0.84 |
| **Crude numbers** | |
| Spermatogonia | 3.58 ± 0.21 |
| Preleptotene | 131.17 ± 3.76 |
| Leptotene | 49.63 ± 2.50 |
| Pachytene | 70.91 ± 2.97 |
| Step 7 spermatids | 78.35 ± 2.21 |
| Spermatid step 16 | 79.68 ± 4.50 |

**Table 2:** Kangaroo testis, number of spermatogenic cells and yield in stage VII of seminiferous tubule "n=6 stage VII seminiferous tubule".

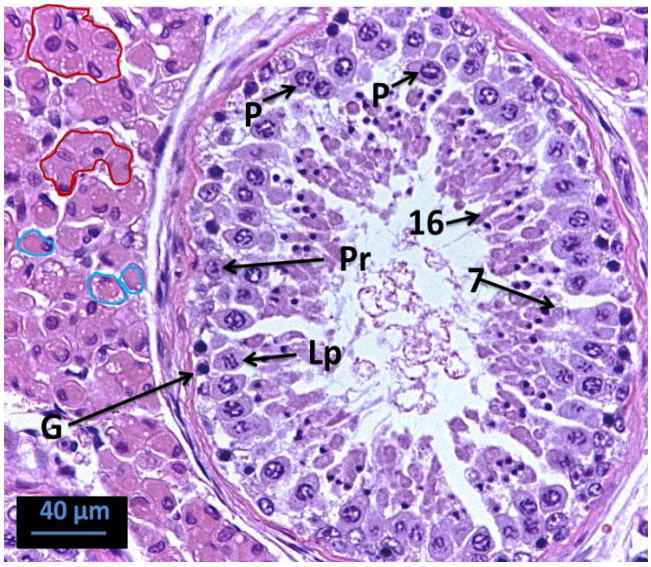


**Figure 5:** spermatogenic cells and yield in Stage VII of seminiferous tubules mature Kangaroo testis, spermatogonia (G), preleptotene (Pr), leptotene (L), pachytene (P), spermatid stage VII (7), stage XVI (16), red outline (Leydig cell clusters), blue outline (individual cells). Hematoxylin and eosin stain. Line scale=40 µm.

## Tubuli Recti (TR)

The straight tubules or TR are lined with simple columnar to high cuboidal epithelium with basal nuclei. The total wall thickness was 40 ± 7 µm with epithelium and 18 ± 12 µm wall thickness without epithelium. TR are surrounded by relatively limited amount of dense connective tissues of collagen fibers surround the TR with a few (1-3) consistently present myoepithelial cells (Figure 3). Cells of testicular origin (spermatocytes) at different stages of development were observed inside the lumen of the TR. These tubules are surrounded by a relatively large number of LC (Figure 3). Small blood vessels, arterioles, venules and capillaries are scattered among small number of fibrocytes.

### Rete Testes (RT)

Rete testes are small tubules within collagenous connective tissue lined with simple cuboidal epithelium connecting the TR with the ductuli efferentes (Table 3).

### Ductuli Efferentes (DE)

Ductuli efferentes (Efferent ductules) have much thinner wall thickness compared to TR. Simple cuboidal (high to low) ciliated and non-ciliated epithelium (18.2 ± 2.8 µm) are observed in the DE that connect the RT with the head of the epididymis (Figure 6).

### Epididymis

The epididymis is lined by pseudostratified columnar epithelium with stereo cilia. Large number of sperms within the lumen present in the entire length of the epididymis. Basal, principal (nuclei are at different levels), and intermediate (darkly stained) cells are observed with variable numbers depending on the epididymis specific segments. Epididymal tubules are surrounded by large number of arterioles and capillaries. The caput (head) had relatively thin wall with one to two smooth muscle cell layers and a little more collagenous connective tissue.

Lumen diameter of the epididymis was recorded for different segments, caput, corpus, and cauda epididymis. Epithelium height in different segments is found to be highest in the corpus and lowest in cauda (Table 4). Smooth muscle cells were rarely present in the caput and corpus (one to two cell layers), while smooth muscle thickness is found to be 34.9 ± 9.1 µm in the cauda epididymis.

| | |
|---|---|
| Sertoli cells/seminiferous tubule | 5.01 ± 0.18 |
| Leydig cells/ seminiferous tubule | 71.97 ± 3.88 |
| Leydig cells in cluster per seminiferous tubule | 86.39 ± 5.91 |
| Leydig cells clusters per seminiferous tubule | 25.56 ± 1.05 |

**Table 3:** Calculated indices of Sertoli and Leydig cells in the kangaroo testis.

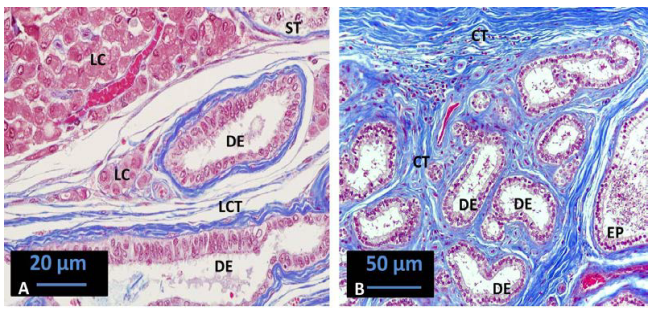


**Figure 6:** (A) Outer part of the kangaroo testis close to the tunica albuginea showing loose connective tissue (LCT), Efferente ductules (DE), Leydig cells (LC), notice the change of the epithelium from high to low cuboidal. Trichrome stain, line scale=20 µm. (B) Ductuli efferentes (DE) embedded inside connective tissue (CT) of the tunica albuginea before they open into the epididymis (EP). Trichrome stain, Line scale=50 µm.

| Epididymis | Caput | Corpus | Cauda |
|---|---|---|---|
| **Lumen diameter** | | | |
| Minimum | 160.29 ± 39.27 | 159.6 ± 25.3 | 167. ± 19.7 |
| Maximum | 295.45 ±115.90 | 348.7± 93.8 | 286.5 ± 1 00.2 |
| Epithelium height | 30.2 ± 6.5 | 36.1 ± 5.9 | 20.3 ± 5 |
| Number of smooth muscle cell layers | 1-None | 1-2 | 4-9 |

**Table 4:** Measurements of the epididymis including minimum and maximum diameters of the three segments, epithelium height and smooth muscle cell layers. Measurements are in micrometers.

### Ductus Deferens (DD)

Ductus deferens is lined with ciliated and non-ciliated pseudostratified columnar epithelium (epithelial height 32.70 ± 8.15 µm). The epithelium rested on well- defined basement membrane. Lightly and darkly stained nuclei at different levels within the epithelium are constantly present. The ductus deferens lumen average diameter was (39.11 ± 4.52 to 66.1 ± 18.03 µm) filled with sperms. Thickness of the tunica muscularis is 326.6 ± 24.3 µm, while epithelium height is 39.1 ± 5.2 µm. Large number of small blood vessels (arterioles, venules, capillaries) are present around the DD.

## Discussion

In general, the testis, epididymis and ductus deferens of the Northern great grey kangaroo were found morphologically to be similar to other mammalian species in their general organization with few exceptions. These differences included the relatively large number of apparently active LC at the age of 18 years and very well developed genital tubules filled with sperms. Besides, larger numbers of LC were present around and in between the TR even larger than in other regions within the kangaroo testes.

*Tunica albuginea* was found to be the thickest close to where the DE leaves the testis to join the caput of the epididymis. Thinner layer of connective tissue was found in the deep region to allow for the blood vessels, nerves and lymphatic to distribute through the trabeculae. The main observation was the least amount of connective tissue around the TR/RT in kangaroo testis and their lumina were filled with sperms.

Seasonal regression of the testes is rare in male macropod marsupials as reported by several investigators [5-7]. In both tammar wallaby and Bennett's wallaby there were no seasonal changes in testicular or epididymal size and males were potentially fertile all year-round [10]. Therefore, the postmortem occurrence of white milky secretion from the urethra of this kangaroo is consistent with the active seminal fluid secretion since no other pathological changes were detected within the male genital system.

Johnson et al. [19] exposed adult Syrian hamster to different photoperiods to reach the conclusion that the photoperiod did not influence the number of LC per testis in this species. Paplinska et al. [3] reported changes within the interstitium of the testes of adult male swamp wallabies during different seasons of the year. According to the available literature, we hypothesized that the kangaroo is not a seasonal animal and may show only a slight fluctuation in LC activity during various seasons. This animal was housed in the LA Zoo, California with moderate climate where the weather was pleasant most of the year. The light/dark cycle for the day of death of this kangaroo in November, 2011 was reported to be approximately 11 h /13 h. [20]. The temperature was 55-80.1°F, mean sea level pressure 30.05 in., and mean dew point 39.6°F. These photoperiods and temperature ranges reported for LA, California in this report may have influenced the kangaroo number and activity of LC combined with well-developed ST which resulted in epididymal tubules filled with sperms [21].

Two populations of LC were reported in few fetal and adult animal species like dasyurid and brown marsupial mouse [11,22]; while in *Monodelphis domestica*, one population was reported by Xie et al. [10]. It has been proven that total number of LC usually decrease with advanced age in man [23,24]. On the other hand, Grzywacz et al. [25]

demonstrated that the inability of exogenously administered LH to increase testosterone production by testes and LC of aged rats suggested that LC steroidogenic deficits in the aged brown Norway rat are unlikely to be the result of age-related changes in LH.

Differentiation between active (foamy cytoplasm) LC and non active with high amount of regular circular shape lipid droplets inside the cytoplasm was taken into consideration when LC were calculated. It was reported that lipid content is inversely proportional to the testosterone hormone levels in the testes [26]. Majority of the kangaroo LC appeared active according to the criteria used in this study.

Surmacki et al. [27] described changes in LC nuclear shape between low and high fertility periods in Chinchilla. They stated that nuclei of LC were smaller in size during the season of low fertility and located closer to the cell membrane. These differences were not considered in this case report due to very low number of inactive cells observed in all examined sections of the kangaroo testis.

Accumulation of lipid in the non-breeding season in the testes of the species that have seasonally regressing testes was described in crab eater and bank vole [26,28]. Foamy cytoplasm in kangaroo LC was considered changes due to activity and a few were heavily lipid laden cells were considered inactive and excluded from counting. Although, it would be preferable to correlate these numbers with plasma level of androgen of the same animal but unfortunately it was not done in this case report.

In tammar wallabies, Butler et al. [29] reported that LC filled most of the interstitium with little space between ST. They also described a progressive increase in overall LC size indicated by increase nuclear/cytoplasm ratio. These findings are similar to the present description of the kangaroo testes (nuclear/cytoplasmic ratio is 2.09 ± 0.38). The only difference in our case was the number of LC distribution within the three different regions of the testes. Kangaroo male sexual activities depend on the presence of active LC available at certain age. Though, the animal may behave differently when taken out of its natural environment and kept in captivity compared to those animals live in the wild. It has been demonstrated that certain animal species have different behaviors in captivity since sexual characteristics and capabilities all depend on the environmental temperature, photoperiod, availability of good quality balanced food, health status of the animal as well as the presence of a female in estrus [1,9].

The other explanation for the increase in relative numbers of LC is due to hyperplasia. It was reported that increased LH levels promote LC hyperplasia [30]. The observed LC hyperplasia in Sprague dawley rats was suggested as a compensatory effort to maintain the normal androgen status of the aged rat, which is rather successful at six months but unsuccessful at 24 months [31]. In this case report, it is very much needed to correlate antemortem LH level in the blood and number of LC to be able to prove it, which was not done in this case report.

Secretion fluid of LC are known to contribute to the maintenance and development of the epididymis. This effect was very clear in the kangaroo in this study with well-developed epithelium and relatively large lumen especially at the cauda epididymis. One study using morphometric measurement listed similar results in respect to lumen diameter and epithelium height of the epididymis in the greater cane rat bred and kept in captivity [32].

Relative numbers of LC that increase in certain cases may be attributed to shrinkage or involution of the ST or other cellular matrix within the testes. However, in this case the ST were well developed with all cell layers present in their walls. In addition, spermatids of variable quantities were seen toward the lumen of the tubules. Therefore, the increase in the number of LC could be considered normal in captivity at this age and under such photoperiod of LA, California. Thus further investigation is needed to shed some light on this animal whenever a new specimen is going to be available.

It has been reported that the volume of LC and ST was significantly lower in winter than summer despite maturing spermatozoa were found in the testes all throughout the year in male swamp wallabies [3]. Thus, further studies are very much needed to clarify this issue during different seasons. These kinds of studies should be performed on LC size and try to correlate with the androgen concentration level as done on human blood [33].

We hypothesized that the proper and regular care, the availability of balanced good quality food and veterinary services all year-round in LA Zoo resulted in increase of LC in spite of the fact that this animal is geriatric considering the reported longer life span of the kangaroo in captivity [2]. Zirkin [34] described stereogenically hypo-functioning of LC in aging rats. While in this case report, there were no clear changes in the number of LC or ST or replacement of them with fibroblasts which indicate they were still active to sustain the shape and function of these tubules.

Adebayo and Olurode [32] studied the epididymis of the greater cane cat and reported a wide range of measurements for the lumen diameters as well as the epithelium heights. They divided the epididymis into several zones as well as straight and convoluted portions. The same kinds of variations were detected in the kangaroo epididymis but three different segments were studied instead of different zones for both lumen diameter and the epithelium heights.

## Conclusion

In summary, animals in captivity may live longer and can continue to be fertile for much longer period of their lives if they are given essential veterinary care as well as a balanced diet. Other factors were also discussed like weather and temperature where the animal was housed all his life (Los Angeles zoo). The great kangaroo high numbers of leydig cells among different segments of the testis are indicative of the above mentioned reasons in spite of the relatively old age of the animal.

### References

1. Paplinska, JZ, Moyle RLC, Wreford NG, Temple-Smith PDM, Renfree MB (2008) Effect of captivity on the animal in presence or absence of female in estrous. J Anat 2008 212:257-74.

2. Poole WE (1982) Macropus giganteus. Mammalian Species. Amer Soc Mammal 187: 1-8.

3. Paplinska JZ, Moyle RL, Wreford NG, Temple-Smith PD, Renfree MB (2007) Reproduction in male swamp wallabies (Wallabia bicolor): puberty and the effects of season. J Anat 211: 518-533.

4. Williamson P, Fletcher TP, Renfree MB (1990) Testicular development and maturation of the hypothalamic-pituitary-testicular axis in the male tammar, Macropus eugenii. J Reprod Fertil 88: 549-557.

5. Poole WE, Pilton PE. Reproduction in the grey kangaroo, Macropus canguru, in captivity (1964) CSIRO Wildlife Res 9: 218–234.

6. Poole WE (1973) A study of breeding in grey kangaroos, Macropus giganteus Shaw and M. fuliginosus (Desmarest), in central New South Wales. Aust J Zool 21: 183.

7. Poole WE, Catling PC (1974) Reproduction in the two species of grey kangaroos, Macropus giganteus Shaw and M. fuliginosus (Desmarest). I. Sexual maturity and oestrus. Aust J Zool 22: 277-302.

8. Inns RW (1982) Seasonal changes in the accessory reproductive system and plasma testosterone levels of the male tammar wallaby, Macropus eugenii, in the wild. J Reprod Fertil 66: 675-680.

9. Catling PC, Sutherland RL (1980) Effect of gonadectomy, season and the presence of female tammar wallabies (Macropus eugenii) on concentrations of testosterone, luteinizing hormone and follicle-stimulating hormone in the plasma of male tammar wallabies. J Endocrinol 86: 25-33.

10. Xie Q, Mackay S, Ullmann SL, Gilmore DP, Payne AP, et al. (1998) Postnatal development of Leydig cells in the opossum (Monodelphis domestica): an immunocytochemical and endocrinological study. Biol Reprod 58: 664-669.

11. Taggart DA, Johnson J, Temple-Smith PD (1993) Testicular and epididymal development in the brown marsupial mouse, Antechinus stuartii (Dasyuridae, Marsupialia). Anat Embryol (Berl) 188: 87-100.

12. Hughes RL, Thomson JA, Owen WH (1965) Reproduction in natural populations of the Australian ringtail possum, Pseudocheirus peregrines (Marsupialia: Phalangeridae), in Victoria. Aust J Zool 13: 383–406.

13. Smith RFC (1969) Studies on the marsupial glider, Schoinobates volans (Kerr). I. Reproduction. Aust J Zool 17: 625-636.

14. Baldwin J, Temple-Smith PDM, Tidemann C (1974) Changes in testis specific lactate dehydrogenase isoenzymes during the seasonal spermatogenic cycle of the marsupial Schoinobates volans (Petauridae) Biol Reprod 11: 377-384.

15. Taggart DA, Temple-Smith PD (1992) Late postnatal development and differentiation of the ductus epididymidis in a dasyurid marsupial (Antechinus stuartii). Anat Embryol (Berl) 186: 259-270.

16. Bancroft J, Gamble M (2008) Theory and practice of histological techniques. (5th edn), Edinburgh, Churchill Livingstone, USA.

17. http://imagej.en.softonic.com/

18. Collins TJ (2007) ImageJ for microscopy. Biotechniques 43: 25-30.

19. Johnson L, Matt KS, Bartke A, Nguyen HB, Le HT (1987) Effect of photoperiod on the size of the Leydig cell population and the rate of recruitment of Leydig cells in adult Syrian hamsters. Biol Reprod 37: 727-738.

20. http://www.timeanddate.com/worldclock/astronomy.h tml?n=137&month=11&year=2011&obj=sun&afl=-11&day=1.

21. http://www.almanac.com/weather/history/CA/Los%20Angeles/2011-11-29

22. Chen H, Ge RS, Zirkin BR (2009) Leydig cells: From stem cells to aging. Mol Cell Endocrinol 306: 9-16.

23. Neaves WB, Johnson L, Petty CS (1985) Age-related change in numbers of other interstitial cells in testes of adult men: evidence bearing on the fate of Leydig cells lost with increasing age. Biol Reprod 33: 259-269.

24. Neaves WB, Johnson L, Petty CS (1987) Seminiferous tubules and daily sperm production in older adult men with varied numbers of Leydig cells. Biol Reprod 36: 301-308.

25. Grzywacz FW, Chen H, Allegretti J, Zirkin BR (1998) Does age-associated reduced Leydig cell testosterone production in Brown Norway rats result from under-stimulation by luteinizing hormone? J Androl 19: 625-630.

26. Sinha AA, Erickson AW, Seal US (1977) Fine structure of Leydig cells in crabeater, leopard and Ross seals. J Reprod Fertil 49: 51-54.

27. Surmacki P, Sulik M, Seremak B (2011) Morphometric studies of Leydig cells in chinchillas (Chinchilla lanigera) during high and low fertility seasons. Folia Biol (Krakow) 59: 195-201.

28. Tähkä KM (1988) Effect of differential photoperiod treatment on Leydig cell ultrastructure in the bank vole (Clethrionomys glareolus, S.). Gen Comp Endocrinol 71: 318-330.

29. Butler CM, Shaw G, Clark J, Renfree MB (2008) The functional development of Leydig cells in a marsupial. J Anat 212: 55-66.

30. Akingbemi BT, Ge R, Klinefelter GR, Zirkin BR, Hardy MP (2004) Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. Proc Natl Acad Sci U S A 101: 775-780.

31. Mendis-Handagama SM1, Gelber SJ (1995) Signs of aging are apparent in the testis interstitium of Sprague Dawley rats at 6 months of age. Tissue Cell 27: 689-699.

32. Adebayo AO, Olurode SA (2010) The morphology and morphometry of the epididymis in the greater cane rat (Thryonomys swinderianus Temmincks). Folia Morphol (Warsz) 69: 246-252.

33. Heller CG, Leach DR (1971) Quantification of Leydig cells and measurement of Leydig-cell size following administration of human chorionic gonadotrophin to normal men. J Reprod Fertil 25: 185-192.

34. Zirkin BR, Chen H, Luo L (1997) Leydig cell steroidogenesis in aging rats. Exp Gerontol 32: 529-537.