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ORIGINAL ARTICLE

The Age at Puberty of the Nigerian Local Dogs in Ibadan. Southwest, Nigeria

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SUMMARY

The age at puberty of the Nigerian Local Dogs (NLD) in Ibadan was studied by monitoring the changes in their weekly live body weight, semen parameters, testicular, epididymal biometry and testosterone profile. The puppies were selected randomly from different healthy litters at different regions of Ibadan metropolis. The puppies were randomly assigned into four age groups consisting of 3 animals in each group, namely, group-A (12 weeks), group-B (16 weeks), group-C (20 weeks) and group-D (24 weeks). Weekly live body weights of the puppies were obtained from 7 weeks to 24 weeks of age. Blood samples were collected at 12, 16, 20 and 24 weeks into EDTA bottles and the plasma decanted were assayed for testosterone. The puppies were castrated at 12, 16, 20 and 24 weeks. Semen was collected from the caudal epididymis and evaluated. The result showed a positive linear relationship ($P < 0.05$) between the age and body weight of the dogs with R (correlation coefficient) = 0.980. The right testes had higher values of the testicular and epididymal parameters compared to the left testes. There was a linear relationship among weight of dog, testicular weight and corresponding percentage. There were fluctuations in the levels of testosterone during this study and a significant difference in the results of testosterone before and after castration ($P < 0.05$). It was therefore concluded that Nigerian local dogs do not have spermatozoa present in their semen and have very low serum testosterone level before 24 weeks of age, therefore puberty in the Nigerian local dog does not occur before 24 weeks of age and results of this study can be used as a baseline data in the further study of the classification and characterization of the NLD.

Key words: Age, epididymis, Nigerian Local Dog, testes, testosterone.

INTRODUCTION

The unclassified and unselected Nigerian local dogs are the indigenous breeds of dogs found in Nigeria. They are popularly referred to as "mongrels" by indigenes. They are dolichocephalic (long-headed) dogs and are highly domesticated, with their feeding pattern being largely omnivorous due to the high level of domestication (Igado, 2011). They are medium sized with short and fine coat. Their colours range from brown; black; bicolour (brown and white; black and white); with white feet, chest and

tail tip. The adult Nigerian local dog weighs between 15 and 25kg. Most of these dogs are used for hunting by the native hunters because they can run faster and are very intelligent. They understand the hunters' language and readily communicate with them and are also trained to hunt wild games. They also serve as guard dogs and pets in various metropolitan cities of the country (Olayemi *et al.,* 2009), and are also kept by people who cannot afford the exotic breeds or even their crosses. Households of medium socio-economic status, with adequate fences are more likely to own dogs. In the city, owners provide better husbandry, health care and feeding of their dogs (Ortega-Pacheco *et al.*, 2006). The Nigerian local dogs have been used for many biomedical studies and also as models for the study of human diseases (Ajala *et al.,* 2012).

For most Nigerians, dogs are often not allowed inside the house and are primarily kept as guard animals. Thus, guarding and protecting as well as commercial breeding seem to be the most common function of dogs in urban and rural areas. In some parts of Nigeria, the local dog is regarded as a delicacy and in one way helps to make up for their protein requirement (Dipeolu, 2010).

In Nigeria, indigenous breeds are well adapted to the tropical condition (Ajala *et al.,* 2009). Owing to the fact that the dog is already adapted to this environment, it is therefore able to stand many harsh conditions that other breeds of dogs are unlikely to stand. There is an upsurge in the acquisition of this local breed of dogs, which is probably due to their being cheaper to care for than the exotic breeds. Also, it seems that they are preferred because of their resistance to diseases caused by blood parasites, such as canine babesiosis and trypanosomosis (Olayemi *et al.,* 2009). There are about 4.5 million indigenous dogs in Nigeria. (Arowolo, 1999; Olayemi *et al.,* 2009).

The management of the Nigerian local dogs in this region can be classified as intensive, semi-intensive and extensive systems of management. Dogs under the intensive management system are well fed and housed with adequate veterinary care. Dogs under this management system are quite healthy. In the semi-intensive system of management, the duty of catering for the feeding and housing of the dog is carried out by both the dog and owner. Dogs under this management occasionally roam about in search of food and are more exposed to diseases and harsh conditions. In the extensive system of management, dogs here are termed "stray dogs" and are usually not owned by anybody and they roam about in search of food and shelter. The dogs usually have no name, no identification and are relatively independent of people. Such dogs are usually unhealthy and constitute a nuisance to the environment (Boitani and Ciucci, 1995).

Male dogs are generally believed to reach puberty at 6-12 months of age with smaller breeds showing evidence of precocity (Harrop, 1960). Collies and Beagles produced their first ejaculate at 365 days (Ford, 1969) and 235 days at the point of increase in peripheral plasma testosterone concentration respectively (Ford, 1969). Whereas, the Nigerian local dogs are yet to be characterized and classified since established data on male vital reproductive statistics is not as robust as other exotic breeds. The pubertal age, testosterone profile and other testicular parameters in the male is very important in understanding the reproductive biology of the dog. Therefore the current study was conducted to determine the pubertal age, testosterone profile and other testicular parameters in the male NLD in Ibadan, Southwest of Nigeria.

MATERIALS AND METHODS

Source of experimental animals

Twelve 6-week old male Nigerian local puppies were used for this study. The

puppies were selected randomly from different healthy litters at different regions of the Ibadan metropolis in Oyo state, Nigeria. They were kept for two weeks to acclimatize. During this period, the puppies were housed and fed on mashed rice and cereals for about two weeks and gradually introduced to more solid meals. They were vaccinated against rabies, canine distemper, hepatitis, leptospirosis, parainfluenza and parvovirus diseases. The puppies were dewormed and veterinary attention was given when necessary.

Live body weight

Live body weight was obtained on a weekly basis with the use of a bathroom/digital scale (IDDEXX Scale) and the weight of each dog was recorded

Sample collection

Blood collection

Blood was collected from the cephalic vein using a 21 gauge needle into labeled sample bottles containing Ethylene Diamine Tetra Acetic acid (EDTA). The blood in the EDTA bottle was centrifuged and the plasma decanted into Eppendhorf tubes and assayed for testosterone.

Blood samples were collected at 12, 16, 20 and 24 weeks of age.

Collection of testicles

The animal was restrained by tape-muzzle around the mouth. 1ml of lignocaine (a local anaesthetic) was infilterated into the scrotal skin and around the testes. A midline/prescrotal incision was made through the scrotal skin and fascia. The testicles were milked out of the incision site and exposed by incising the tunica vaginalis. The spermatic cords were exposed, ligated and incised. The skin was then closed with catgut according to Oyeyemi *et al.* (2002). The testicles were weighed; semen evaluation and histology of the collected testicles were carried out.

Semen collection

The right and left epididymis were carefully trimmed off the body of the testes and semen samples were obtained from the cauda epididymis through an incision made with a scalpel blade into the lumen of the cauda epididymis as described by Oyeyemi *et al.* (2002). The semen was then milked out through the incision made on the epididymis into a clean tissue slide. 2-3 drops of 2.9% sodium citrate kept at 37^0C was used to flush the incision. A half of the collected semen sample was stained with Wells and Awa (Wells and Awa, 1970) which is a stain for morphological studies and Eosin-Nigrosin for live-dead ratio. The second half was mixed with 0.5ml 2.9% sodium citrate to study spermatozoa motility.

Semen analysis

After the semen samples had been obtained, the following parameters were considered- the colour, sperm motility, sperm livability, morphology and sperm cell concentration or density.

Sperm motility

Motility means the percentage of sperm cells in a unidirectional progressive movement over a field on a slide observed under a microscope (Zemjanis, 1970). It was evaluated in a small drop of semen into which one drop of warm sodium citrate was added. The mixture was covered with a glass coverslip and viewed under the microscope at a magnification of about 40X. Only sperm cells moving in a straight forward unidirectional motion were included in the motility count while sperm cells moving in circular, backward direction or showing pendulation movement were excluded.

Sperm liveability

This was done by staining one drop of semen with one drop of warm Eosin & Nigrosin stain on a warm slide. A thin smear was then made from the mixture of semen

and stain. The smear was air dried and observed under the microscope. The ratio of the invitro dead sperm cells was observed and it is based on the principle of Eosin penetrating and staining dead autolysed cells whereas viable sperm cells repel the stain (Zemjanis, 1977)

Morphological studies

This is done to determine the presence and incidence of morphologically defective spermatozoa. Sperm cells were observed for morphological defects using Wells & Awa stained smears. A drop of semen was placed on a warm slide with a drop of Wells & Awa stain. A smear was made, air dried and observed under the microscope as described by Noakes *et al.* (2001).

Quantitative determination of testosterone in plasma

Testosterone was quantitated by the Eazyme ImmunoAssay (EIA) method as reported by Bosch, (1978).

Data analysis

Data were analyzed using one way Analysis Of Variance (ANOVA) with age as the main effect, where mean, standard error of mean and coefficient of variation and regression were calculated and compared by using Duncan Multiple Range Test (DMRT) at 5% probability level on IBM SPSS 20.0 Statistical package. The results were expressed as Mean ± SEM.

RESULTS

Semen Analysis

There were no (matured) spermatozoa observed from the semen collected from the dogs throughout the 24 weeks in this study.

Age and weight

The mean weights (kg) of the dogs from age 7 to 24 weeks are as shown in Table I.

The highest mean weight 6.04 ± 0.38 (kg) was recorded at 24 weeks while the least mean weight 1.57 ± 0.01(kg) was recorded at 7 weeks.

TABLE I: Mean weight of dogs from age 7 to 24 weeks

Age (weeks)	Mean weight (kg) Mean+/SEM
7	1.57 ± 0.01
8	2.58 ± 0.15
9	2.63 ± 0.13
10	3.17 ± 0.19
11	3.21 ± 0.17
12	3.58 ± 0.21
13	3.88 ± 0.15
14	4.33 ± 0.22
15	4.54 ± 0.33
16	4.50 ± 0.33
17	4.79 ± 0.31
18	5.29 ± 0.35
19	5.33 ± 0.28
20	5.33 ± 0.29
21	5.88 ± 0.35
22	5.96 ± 0.38
23	5.92 ± 0.38
24	6.04 ± 0.38

The relationship between age and weight was shown in the regression analysis with the magnitude of association R^2 (coefficient of determination) = 0.961 i.e. 96.1% of the variability of weight that can be explained by age. Explaining over 96% represents a good fit. Considering that other factors had been taken into consideration, such as feeding. There was positive relationship between the age and weight which shows that as the age increases, the weight tends to increase.

R (correlation coefficient) = 0.980 means age can predict weight with linear association between age and weight.

$y = a + bx$

y = independent variable (age)

x = dependent variable (weight)

ab = constant value

y (age) = 539.620 + 246.637(weight).

TABLE II: Mean testicular and epididymal parameters

Parameters (g)	Group			
	A (12weeks) Mean+/SEM	B (16weeks) Mean+/SEM	C (20weeks) Mean+/SEM	D (24weeks) Mean+/SEM
WRTE	0.61 ± 0.05^{a}	0.77 ± 0.06^{a}	1.06 ± 0.06^{b}	1.96 ± 0.31^{c}
WRT	0.35 ± 0.04^{a}	0.53 ± 0.04^{ab}	0.65 ± 0.14^{b}	1.22 ± 0.04^{c}
WRE	0.25 ± 0.03^{a}	0.25 ± 0.02^{a}	0.41 ± 0.09^{b}	0.70 ± 0.28^{c}
WLTE	0.67 ± 0.08^{a}	0.76 ± 0.04^{a}	1.22 ± 0.13^{b}	2.06 ± 0.35^{c}
WLT	0.34 ± 0.05^{a}	0.49 ± 0.05^{a}	0.83 ± 0.17^{b}	1.23 ± 0.01^{c}
WLE	0.33 ± 0.04^{a}	0.27 ± 0.01^{a}	0.39 ± 0.06^{a}	0.83 ± 0.35^{b}
CRT	2.10 ± 0.21^{a}	3.03 ± 0.07^{bc}	2.55 ± 0.35^{ab}	3.37 ± 0.09^{c}
CLT	2.10 ± 0.10^{a}	2.73 ± 0.15^{b}	2.97 ± 0.19^{bc}	3.37 ± 0.09^{c}
LRE	1.47 ± 0.18^{a}	2.50 ± 0.06^{b}	2.10 ± 0.00^{b}	2.57 ± 0.19^{b}
LLE	2.50 ± 0.36^{a}	2.20 ± 0.10^{a}	2.20 ± 0.12^{a}	2.67 ± 0.12^{a}

Mean of different supercript in the same row are significantly different ($P<0.05$)
WRTE = weight of right testes and epididymis
WRT = weight of right testes
WRE = weight of right epididymis
WLTE = weight of left testes and epididymis
WLT = weight of left testes
WLE = weight of left epididymis
CRT = circumference of right testes
CLT = circumference of left testes
LRE = length of right epididymis
LLE = length of left epididymis

Testicular and epididymal parameters

Table II shows the mean testicular and epididymal parameters of the animals. All the testes and epididymal parameters showed highest and lowest mean values at 24 weeks and 12 weeks of age respectively. There was significant difference ($p<0.05$) in the values of WRTE, WRT, WLTE, WLT, CRT, CLT, and LRE while there was no significant difference in the values of WRE, WLE and LLE ($P > 0.05$) between age groups observed in the dogs. The result further showed that as the age of the animal increases, there was a relative increase in the testicular and epididymal parameters.

Mean testicular biometric and histometric data

The testicular biometric and histometric data are shown in Table III.

The highest (2.45 ± 0.04) and lowest values (0.69 ± 0.09) for testicular weight were recorded at 24 weeks and 12 weeks respectively. The highest (100.72 ± 4.85) and least values (65.43 ± 0.94) for tubular diameter were recorded at 20 weeks and 12 weeks respectively. The correlation between Testes weight (g) and Testicular diameter is 0.491 Square of the value when approximated to 1 decimal place (0.5) = 0.25. To determine what percentage of variability is shared, then multiply by 100 = 25%, hence, Testes weight shares about 25% of its variability with tubular diameter. As can be observed, testicular weight showed a lower rate of growth from 12 weeks to 20 weeks of age and increased dramatically/significantly in the puppies from 20weeks to 24 weeks of age.

TABLE III: Mean biometric and testicular histometric data

AGE (weeks)	BODY WEIGHT (kg) Mean+/SEM	TESTICULAR WEIGHT (kg) Mean+/SEM	TUBULAR DIAMETER (μm) Mean+/SEM
12	3.00 ± 0.00^{a}	0.69 ± 0.09^{a}	65.43 ± 0.94^{a}
16	4.50 ± 0.76^{a}	1.01 ± 0.09^{ab}	87.21 ± 1.47^{b}
20	5.17 ± 0.60^{a}	1.26 ± 0.20^{b}	100.72 ± 4.85^{c}
24	7.33 ± 0.88^{b}	2.45 ± 0.04^{c}	99.12 ± 2.82^{c}

Mean of different letters in the same row are significantly different ($P<0.05$)

TABLE IV: Mean testicular percentage to body weight

Parameters	Age (weeks) Mean+/SEM			
	12	16	20	24
Weight of animal(kg)	3.00 ± 0.00^{a}	4.50 ± 0.76^{a}	5.17 ± 0.60^{a}	7.33 ± 0.88^{b}
Weight of both testes(kg)	0.69 ± 0.09^{a}	1.01 ± 0.09^{ab}	1.26 ± 0.20^{b}	2.45 ± 0.04^{c}
Percentage (%)	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.02 ± 0.00^{a}	0.03 ± 0.00^{a}

Mean of different supercript in the same row are significantly different ($P<0.05$)

Testicular percentage to body weight

Table IV shows the mean weight of both testes (kg) and the mean testicular percentage to body weight (%).

The highest mean testicular weight (2.45±0.04) was recorded at 24 weeks while the least mean testicular weight (0.69 ± 0.09) was recorded at 12 weeks. There was significant difference ($P < 0.05$) in the values of the body weight of the animals between groups. There was also significant difference ($P < 0.05$) in the values of the weight of both testes to the body weight while there was no significant difference ($P > 0.05$) in the mean percentage of the testes to the body weight.

The relationship among weight of animal, weight of both testes and corresponding testes percentage was shown in regression analysis. The magnitude of the association of R^2 (Coefficient of determination) = 0.963 = 96.3% of the variability of testicular weight and corresponding testicular percentage that can be explained by weight of the dogs. Explaining over 96% represents good fit considering that other factors are not put into consideration. R (Correlation coefficient) = 0.981 means weight of dog tends to predict testicular weight and corresponding percentage with linear association among weight of dog, testicular weight and corresponding percentage.

$y = a + b_1x_1 + b_2x_2$.

x_1 = weight of both testes

x_2 = testicular percentage

y = weight of animal

ab = constant value

y (weight of animal) = 5.18 + 3.62 (weight of testes) – 192.50 (testicular percentage).

Testosterone profile of dogs before and after castration

Table V presents the testosterone level (U/L) in the dogs before and after castration.

The highest and least mean values (1.43 ± 0.35 and 0.12 ± 0.07) respectively for testosterone before castration were recorded at 24 weeks and 18 weeks respectively. There was significant difference ($P < 0.05$) in the values of testosterone before and after castration. The testosterone level increased in the animals with respect to age with values of 0.70 ± 0.06 at 12 weeks before castration and 1.43 ± 0.35 at 24 weeks before castration. The mean testosterone

TABLE V: Mean testosterone profile (u/l)

Parameters	Age (weeks) Mean+/SEM						
	12	14	16	18	20	22	24
Before castration	0.70 ± 0.06^{abc}	0.27 ± 0.03^{a}	0.20 ± 0.06^{a}	0.12 ± 0.07^{ab}	1.13 ± 0.44^{bc}	1.20 ± 0.40^{bc}	1.43 ± 0.35^{c}
After castration	0.00^{a}	0.13 ± 0.03^{b}	0.10 ± 0.00^{ab}	0.17 ± 0.03^{b}	0.10 ± 0.00^{ab}	0.43 ± 0.07^{c}	0.13 ± 0.03^{b}

Mean of different superscripts in the same row are significantly different ($P<0.05$)

levels of the animals decreased after castration although there were haphazard fluctuations observed with the highest mean testosterone (0.043 ± 0.07) at 22 weeks and drops drastically to 0.13 ± 0.03 at 24 weeks. When the mean testosterone values before castration was compared with values after castration, it was observed that there was a significant decrease ($P<0.05$) ranging from 0.27 ± 0.03 (before castration) to 0.13 ± 0.03 (after castration) and 1.43 ± 0.35 (before castration) to 0.13 ± 0.03 (after castration).

DISCUSSION

Taking into consideration, factors such as feeding and exercise, there was a positive relationship between the age and body weight of the experimental animals used in this study. The dogs grew approximately linearly from 7 weeks up to 24 weeks of age. This is in agreement with the findings of Burns *et al.* (2008). The body weight of dogs tends to increase with increase in age.

The highest and lowest values of the testicular and epididymal parameters were recorded at 24 weeks and 12 weeks of age respectively. As the age of the animal increases, there was a relative increase in the testicular and epididymal parameters. The right testes had higher values of the testicular and epididymal parameters compared to the left testes. This is in agreement with the report of England (1991).

Body weight was significantly ($p<0.05$) higher in dogs aged 24 weeks than those aged between 7 weeks and 20 weeks. A similar trend was observed in testicular measurement. This is expected as there is a linear relationship between body weight, testicular measurement and age. The testicular weight in the NLD shares about 25% of its variability with tubular diameter. Thus, as body weight and age increased, testicular weight and tubular diameter also increased. This is at variance with the findings in the cat (Franca and Godinho, 2003) in which there was no significant correlation between tubular diameter and testes weight.

Investigations into the mammalian body and testicular biometrics are important for various aspects of reproduction. These studies help characterize puberty and sexual maturity and enable inferences about spermatogenesis. Testicular biometry is an important component of monitoring the testes for normality and gauging potential sperm production.

Tubular measurement is the approach traditionally used as indicator of spermatogenic activity in investigations of testicular function (Navarro *et al.,* 2004; Souza *et al.,* 2009 and Caldeira *et al.,* 2007). The study of testicular morphophysiology and body biometry allows the establishment of physiological and behavioural patterns important to understanding reproductive biology of various species (Caldeira *et al.,* 2010).

The testicular percentages (%) to the body weight (kg) of the Nigeria Local Dogs

(NLD) used in this study were between 0.02% and 0.03%. The relationship between body weight and testicular percentage to the body weight of the animals shows that, weight of the animals tends to predict testicular weight and the corresponding percentage. There is a linear relationship among weight of dog, testicular weight and corresponding percentage. Thus, for every unit increase in the weight of the dogs, there was a corresponding increase in the testicular weight and a decrease in testicular percentage.

During this study, there were fluctuations in the levels of testosterone before castration. This could be attributed to the fact that the blood samples were collected at different times of the day. Testosterone level varies throughout the day - they are highest in the morning, decrease over the course of the day, and rise again in the evening. Plasma testosterone concentrations decreased after castration (De Gier *et al.,* 2011). There was a decrease in the testosterone levels after castration. This is because castration results in rapid decrease in circulating testosterone concentration (Amann and Walker, 1983). A possible explanation for the relatively low testosterone concentration in the peripheral circulation is due to the transfer of testosterone from the venous outflow of the testes to the testicular artery. This is in agreement with Baumans, (1985).who reported that the intratesticular androgen which appeared to be mainly testosterone is 5000-fold concentration higher than that in serum.

The absence of mature sperm cells in the seminiferous tubules and the semen of the NLD at the end of this study showed that this breed of dog like several other small and medium sized breeds of dogs, do not reach sexual maturity on or before 24 weeks of age. This finding is supported by Kawakami, (1991) in which spermatozoa were observed in the testes of all the beagle dogs used in his study at 28 weeks of age. Further study may reveal the same finding if more time is allowed.

CONCLUSION

It was concluded in this study that Nigerian local dogs do not have spermatozoa present in their semen and have very low serum testosterone level before 24 weeks of age, therefore puberty in the Nigerian local dog does not occur before 24 weeks of age and results of this study can be used as a baseline data in the further study of the classification and characterization of the NLD.

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