

The Oestrous Cycle of the Nigerian Local Bitch

Oluwatoyin O. Ajala, O.E. Fayemi and M.O. Oyeyemi

Department of Veterinary Surgery and Reproduction,
Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria

Abstract: The Nigerian local breed of dog is useful for many purposes and therefore their oestrous cycle was studied using 10 Nigerian local bitches aged 18-36 months, weighing 10.25 ± 1.44 kg and kept at the kennel of the Department of Veterinary Surgery and Reproduction, University of Ibadan, Nigeria. The study involved vaginal cytology, macroscopic examination of the vulva, behavioural changes, haematological and biochemical parameters and hormonal profile. Four phases of the oestrous cycle, the proestrus, oestrus, dioestrus and anoestrus with periods of 9.0 ± 0.0 , 9.0 ± 0.0 , 62 ± 1.0 and 122.5 ± 2.5 days, respectively were identified. The Inter-oestrus interval was 211.5 ± 3.5 days. The physical and behavioural changes in the bitches were not consistent within bitches during the oestrous cycles studied. In conclusion breeders of this breed of bitches are advised not to only depend on these signs alone to identify the period of oestrus for optimum breeding time (OBT) but should be combined with vaginal cytology as this may lead to infertility.

Key words: Nigerian Local Breed • Bitch • Oestrous Cycle • Ibadan

INTRODUCTION

In domestic dogs, sexual maturity begins at about 6 to 12 months of age for both male and female [1] although, this can be delayed until about 24 months for some large breeds [2]. This is the time at which female dogs have their first reproductive or oestrous cycle [3]. The reproductive activity in the bitch is different from the polycyclic pattern of other species in that there are no frequent re-occurring periods of heat. All bitches have a prolonged period of anoestrus or sexual quiescence between successive heat irrespective of whether they are pregnant or not [4]. The average interval between successive heats irrespective of whether they are pregnant or not is seven months but it may vary [5]. Some small breeds may have 3 – 4 oestrous cycles in a year while the large breeds have only one.

The intervals between oestrus are 37 weeks (9.25 months) and 26 weeks (6.5 months) in Rough Collies and German shepherd breeds respectively [6, 7] recorded an average of 8.1 months for Greyhound bitches [8] while 31 weeks (7.75 months) have been recorded for bitches generally [5].

The oestrous cycle is divided into two phases, the oestrogenic or follicular phase comprising of proestrus and oestrus and the progesterone or luteal phase comprising the dioestrus, while the anoestrus is the phase of quiescence [2, 9-13].

The oestrous cycles of exotic bitches such as German shepherd, Rottweiler have been described by many researchers using vaginal histology [14], exfoliated vaginal cytology [3,13-16], hormonal profiles [3], bacteria flora [17-20] and haematological and biochemical parameters [20] but there has not been any record for the Nigerian local breed of bitches in Ibadan.

This study was carried out to investigate the oestrous cycle of the Nigerian local bitches in Ibadan using macroscopic examination of the vulva and vagina, behavioural changes, vaginal cytology, 17 beta oestradiol and progesterone profiles, haematological and biochemical parameters.

MATERIALS AND METHODS

Experimental Animals: Ten adult bitches of the Nigerian local breed were used for this study. They aged between 18-36 months and averaged weighed 10.25 ± 1.44 kg. They were acclimatized for 1 month and during this period; they were vaccinated against rabies, using CANVAC© and Leptospirosis using CANVAC© and DHLPP vaccine. They were dewormed at 22mg/kg body weight orally daily for 3 consecutive days and with praziquantel at the rate of 20.0mg/kg body weight once orally every three months. They were washed with Amitrax © against ectoparasites monthly. Other veterinary attentions were given as at when indicated during the

experiment. All dogs were clinically examined and confirmed free from any obvious reproductive and systemic disorders. They were fed on compounded ration with 26.36% crude protein and 2.82k cal/g/DM energy. The bitches were fed once per day in the morning at about 8:00am in their separate kennels. After feeding, they were allowed to exercise around the central space in the kennel from 8:30am to 6:00pm before they were returned to the kennel.

Macroscopic Study of the Vulva and the Vaginal:

The quantities of the vaginal discharges were scored as: 0 – Dry, 1 – Slight, 2 – Intermediate, 3 – Large and the colour was scored as bright red, chocolate red or whitish. The consistency of the vaginal discharge was scored as serosanguinous or cheesy. The vulva of the bitches were also examined daily and scored as: no oedema, oedematous and firm, oedematous and soft.

Behavioural and Structural Changes of the Vulva During the Oestrous Phase:

The following attributes were observed in the bitches during two consecutive oestrous periods per bitch: keen interest in the adjacent kennel where the male dogs were kept, interest and acceptance of the male, frequent micturition, deviation of the tail to the side (Flagging), vaginal discharge and oedematous and soft vulva lips. Each of these attributes displayed was regarded as numerical score of 3 and hence a maximum of 6, if the same attribute was observed during the two consecutive oestrus periods. When an attribute was not displayed by the bitch it was scored zero for each oestrus periods. The total score if all attributes were displayed during the two oestrous periods was equal to 36 points. The percentages of the attributes were then calculated per bitch.

Vaginal Cytology: The vaginal cytology of the bitches was done everyday to characterize the reproductive activities of the bitches beginning from the first day of one vulvar discharge to another for two complete reproductive cycles according to the method described by Schuttle [15].

Characterization of the Epithelial Cells: The main types of cells and the estimated occurrence were scored as percentages of total number of cells. The cells were categorized as parabasal cells, small intermediate cells, large intermediates cells, superficial epithelial cells, Red blood cells and white blood cells. Indications for the different phases of oestrous cycle were characterized as

reported by Davol [3]. Proestrus was identified as when there were more than 75% parabasal cells and small intermediate cells with lots of red blood cells. Oestrus as when there were more than 75% superficial epithelial cells, very few red blood cells and lots of large intermediate cells. Dioestrus as the time when there were lots of small intermediate epithelial cells, lots of parabasal cells and white blood cells and anoestrus, as when there were some parabasal cells with cell debris.

Haematological, Biochemical and Hormonal Studies:

Blood was collected through the cephalic vein at the first and last days of the serosanguinous vulvar charge and later weekly till the beginning of another cycle. Two milliliter of blood was collected into heparinized bottles for haematological studies while 5ml of blood was collected, allowed to clot and serum separated. The sera samples were used for the biochemical and hormonal studies.

The haematological parameters such as packed cell volume (PCV), red blood cell count, white blood cell count, the erythrocytic indices and the differential leucocyte count were determined using standard methods described by Schalm *et al.* [21]. The analyzed biochemical parameters were; total protein, albumin, globulin using the methods described by Reinhold [22], aspartate aminotransferase (AST), serum alkaline phosphatase, serum total cholesterol, triglycerides (TRIG), high and low density, lipoproteins, calcium and inorganic phosphate by methods described by Gomori and Trinder [23, 24].

The hormones analysed were 17-beta oestradiol using the enzyme linked immunosorbent assay (ELISA) method and progesterone using the direct serum progesterone enzyme linked immuno assay (EIA) method. Using kits from Fortress Diagnostics Limited, BT 41 1 QS, United Kingdom for 17-beta oestradiol analysis and Immunometrics (United Kingdom) Limited, 280 Munster road, London SW6 6 BQ, United Kingdom, for progesterone analysis. For the validation of these kits, the optical density (OD) values were processed by manual construction of a standard curve and the values of the control samples and the unknown samples were read directly from the standard curve.

Statistical Analysis: The mean \pm SEM of the different epithelial cell counts, haematological and biochemical parameters were calculated at different phases as identified by vaginal cytology. In order to establish any difference in the parameters the means \pm SEM were

subjected to Analysis of variance (ANOVA) and multiple comparison table using least significant difference model (LSD). The mean±SEM of the 17beta oestradiol and progesterone values were calculated and then plotted against the different phases of the oestrous cycle as indicated by vaginal cytology.

RESULTS

Four phases of the oestrous cycle of the Nigerian local bitches were identified using the vaginal cytology. Five types of cells recognized in the vaginal smear were parabasal epithelial cells (the smallest epithelial cells, spherical in shape and had the largest nuclear- cytoplasmic ratio). The small intermediate epithelial cells were bigger than the parabasal cells, roundish in shape with smaller nuclei. The large intermediate epithelial cells had angulated borders, small nuclei and small nuclear-cytoplasmic ratio. The superficial epithelial cells were the largest cells in the vaginal smear with angulated borders and pyknotic nuclei (Fig 1, plates 1-4). With the relative population of these epithelial cells, the following phases were identified:

Proestrus: The population of the nucleated epithelial cells (parabasal and small intermediate cells) were very high about 100% of the epithelial cells in the smear. There was also a very high population of red blood cells (Fig. 1, Plate 1). The average number of days for proestrus obtained in this study was 9.0±0.0 days (Table 1).

Oestrus: The population of superficial epithelial cells was between 80-100% of the epithelial cells with a reduction in the population of red blood cells (Fig. 1, Plate 2). The average number of days for oestrus obtained in this study was 9.0±0.00 days (Table 1).

Dioestrus: There was a dramatic change in the population of the epithelial cells from superficial epithelial cells to small intermediate and parabasal epithelial cells with a high population of white blood cells (Fig. 1, Plate 3). The average number of days for dioestrus obtained in this study was 62.00±1.001 days (Table 1).

Anoestrus: The smear during the anoestrus phase was made up of parabasal cells and lots of debris which affected the clarity of the smear (Fig. 1 and Plate 4). The anoestrus phase averaged 122.5±2.5 days (Table 1).

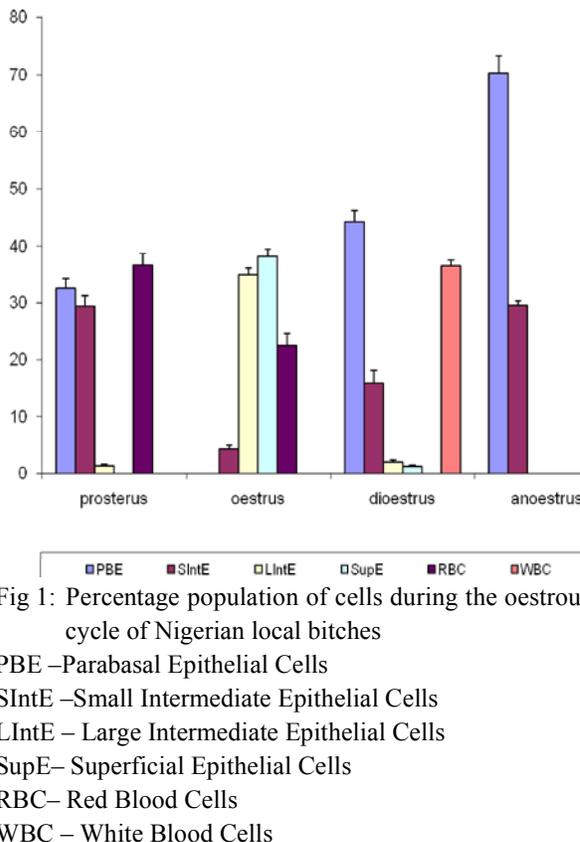
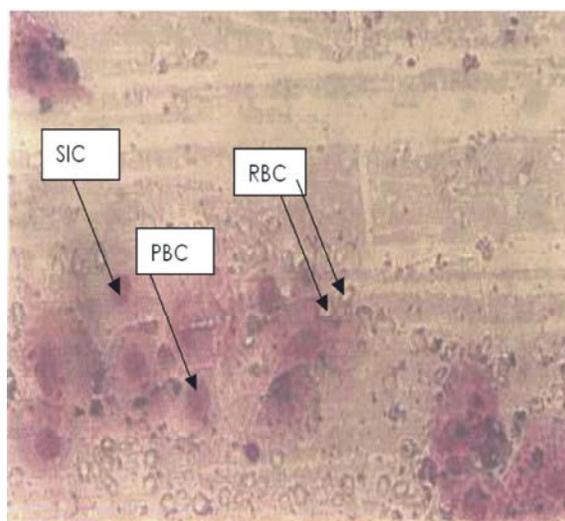


Fig 1: Percentage population of cells during the oestrous cycle of Nigerian local bitches

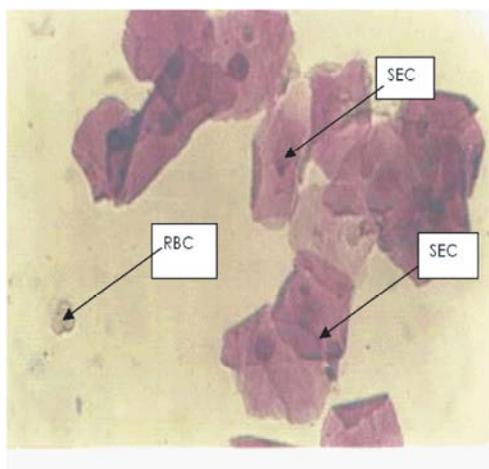
PBE –Parabasal Epithelial Cells
 SIntE –Small Intermediate Epithelial Cells
 LIntE – Large Intermediate Epithelial Cells
 SupE– Superficial Epithelial Cells
 RBC– Red Blood Cells
 WBC – White Blood Cells



Prooestrus: Parabasal cells (PBC) Small Intermediate Cells (SIC), Red Blood Cells (RBC) dirty background (Giemsa, x300)

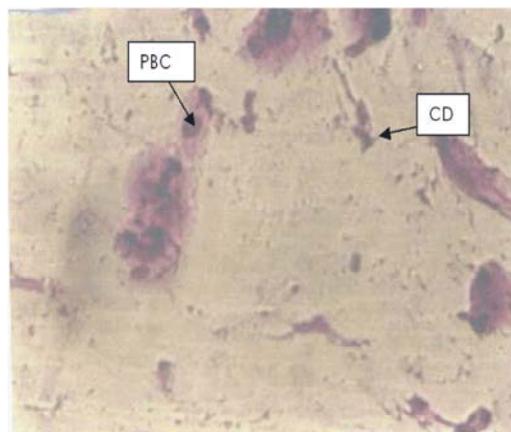
Plate 1: Vaginal cells during proestrus

Interoestrus Interval: The Interoestrus interval was calculated to be 211.5±3.5 days. The clarity of the vaginal smear was disturbed by the presence of lots of red blood



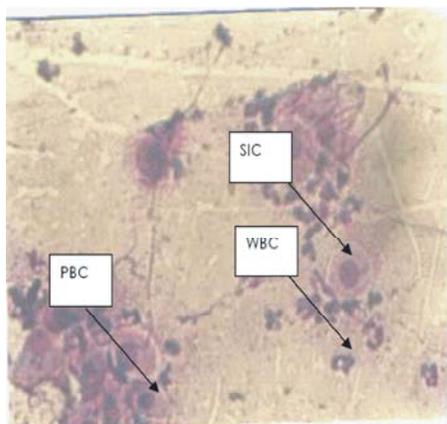
Oestrus Superficial Epithelial Cells (SEC) with very clear background (Giemsa x300)

Plate 2: Vaginal cells during oestrus



Parabasal Cells (PBC) with Cell Debris (CD)(Giemsa, x300)

Plate 4: Vaginal cells during anoestrus



Parabasal Cells (PBC) Small Intermediate Cells (SIC) White Blood Cells (WBC) with dirty background (Giemsa, x 300)

Plate 3: Vaginal cell during late dioestrus

cells during proestrus. During oestrus the smear was very clear with very little or no red blood cells. During dioestrus and anoestrus the smear appeared dirty with a lot of white blood cells and debris (Plate 1-4).

The colour of the mucous membrane of the vulvar lips varied from slightly pink during proestrus to deep pink during oestrus. It was pale during dioestrus and anoestrus phases (Table 1).

The size of the vulvar lips increased from slightly oedematous at the start of proestrus to grossly oedematous and firm to touch at the end of proestrus. During oestrus the oedema was still maintained but became soft to touch thus, making the orifice of the vulva more accessible. At dioestrus and anoestrus phases, the oedema was lost and the vulvar lips returned to their small sizes as before proestrus (Table 1).

The colour of the vaginal discharge was bright red, serosanguinous with increasing volume at proestrus while it was dark red (chocolate) with a reduced volume at oestrus. At dioestrus the vaginal discharge was whitish, sticky or cheesy and the volume was very small. There was no discharge during the anoestrus period.

The following clinical characteristics were displayed during the oestrus of the Nigerian local bitches:

- Keen interest in the kennel where the studs were kept
- Interest and acceptance of the stud (male)
- Frequent micturition
- Deviation of the tail (Flagging)
- Chocolate red coloured vaginal discharge
- Oedematous and flaccid vulvar lips

As shown in Table 2, 50% of the bitches expressed keen interest in the kennel of the male during the first oestrus phase while 75% of them expressed such behavior during the second oestrus phase. All the bitches (100%) had interest and accepted male during the two oestrus phases. All the bitches (100%) expressed frequent micturition during the two oestrus phases. Fifty percent of the bitches flagged their tails during the first oestrus while 75% of the bitches flagged their tails during the second oestrus phase. Chocolate red coloured vaginal discharge was observed in only 75% of the bitches during the first and the second oestrus phases. All the bitches (100%) had oedematous and flaccid vulva throughout the first and second oestrus phases.

Table 1: Changes in vaginal discharge, vulvar lips and the average number of days of the oestrous cycle of the Nigerian local bitches

Phases	Colour of Vulval mucous membrane	Consistency of vulval lips to touch	Quantity, Colour, consistency of vaginal discharge	Average number of days for different phases
Proestrus	Slightly-deep pink	Oedematous and firm to touch	Serosanguinous Quantity=large	9.00±0.00
Oestrus	Deep pink	Slight oedema and soft	Chocolate red Quantity=small	9.00±0.00
Dioestrus	Pale	No oedema	Whitish Quantity=sparingly	62.00±1.00
Anoestrus	Pale	No oedema	Dry	122.50±2.50

Table 2: Observed oestrus characteristics of bitches

Animal Grp.	1 st Oestrus						2 nd Oestrus						Total (36) (%)	
	(3) K	(3) I	(3) F	(3) D	(3) V	(3) O	(3) K	(3) I	(3) F	(3) D	(3) V	(3) O		
A	0	3	3	0	3	3	3	3	3	3	3	3	30	83.3%
B	3	3	3	3	3	3	3	3	3	3	3	3	36	100%
C	0	3	3	0	0	3	0	3	3	0	0	3	18	50%
D	3	3	3	3	3	3	3	3	3	3	3	3	36	100%
Total (12)	6	12	12	6	9	12	9	12	12	9	9	12		
%	50%	100%	100%	50%	75%	100%	75%	100%	100%	75%	75%	100%		

KEY:

K = Keen interest in the kennel where the studs were kept.

I = Interest and acceptance of the stud.

F = Frequent micturition.

D = Deviation of the tail (flagging).

V = Vaginal discharge chocolate red colour.

O = Oedematous and flaccid vulvar lips.

Table 3: Haemogram of the dogs during the four phases of the oestrous cycle

Phases of	PCV%	Hbg/dl	RBC x 10 ⁶ /μl	MCV fl	MCHCg/dl	MCHpg	WBC x 10 ³ /μl	Differential Absolute Leucocyte					Platelets	
								Neut	Lymphocyte	Mono	EOSI			
Oestrus	Mean±SEM	Mean±SEM	μl Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM
Proestrus	47.63±1.28 ^a	14.95±0.70 ^a	7.49±0.2 ^a	64.40±1.27 ^{ac}	30.57±0.64 ^a	19.45±0.45 ^a	7.93±0.90 ^a	47.13±2.77 ^a	51.23±3.21 ^a	1.83±0.60 ^a	2.00±0.58 ^a	2.25±0.33 ^a		
Oestrus	51.37±0.94 ^b	16.30±0.20 ^b	8.33±0.1 ^b	61.77±1.72 ^a	31.81±0.67 ^a	19.61±0.19 ^b	9.34±1.5 ^b	48.00±5.35 ^a	47.13±5.17 ^b	2.33±0.60 ^a	2.25±0.32 ^a	2.11±0.08 ^a		
Dioestrus	50.92±0.45 ^b	16.48±0.18 ^b	8.42±0.08 ^b	60.45±0.72 ^a	32.37±0.18 ^{ac}	19.71±0.13 ^a	8.34±0.61 ^b	44.33±2.41 ^a	53.52±5.17 ^c	2.04±0.17 ^a	1.71±0.28 ^{ab}	1.57±0.09 ^a		
Anoestrus	50.75±0.75 ^b	16.95±0.46 ^b	8.51±0.21 ^b	59.77±1.76 ^{ab}	33.39±0.55 ^{ad}	19.95±0.57 ^a	7.68±0.77 ^a	37.25±3.79 ^a	58.50±3.28 ^d	2.50±0.50 ^a	3.00±0.41 ^{ac}	1.76±0.38 ^a		

Note: Means±SEM along the same vertical column with different superscripts are significantly different at P < 0.05

Table 4A: Serum biochemical parameters of Nigerian local dogs during the oestrous cycle

Parameters	Proestrus	Oestrus	Dioestrus	Anoestrus
Phosphate mg/100ml(dL)	7.92±2.88 ^a	6.10±0.25 ^a	4.80±0.63 ^a	5.59±0.16 ^a
Calcium mg/100ml(dL)	9.00±0.20 ^b	9.56±0.50 ^b	9.54±0.29 ^b	9.49±0.18 ^b
Total Protein (TP) g/100ml(dL)	6.41±0.12 ^d	6.26±0.14 ^d	6.41±0.14 ^d	6.22±0.18 ^d
Albumin (A) g/100ml(dL)	3.06±0.12 ^e	3.15±0.22 ^e	3.10±0.18 ^e	2.99±0.12 ^e
Globulin (G) g/100ml(dL)	3.35±0.061 ^f	3.11±0.083 ^f	3.31±0.18 ^f	3.22±0.22 ^f

Note: All means±SEM along the same row with different superscripts are significantly different at P<0.05.

Table 4B: Serum enzymes and lipids of the Nigerian local dogs during the oestrous cycle

Parameters	Proestrus	Oestrus	Dioestrus	Anoestrus
Alkaline Phosphatase (AP) Iu/L	38.27±5.16 ^a	34.96±4.61 ^a	30.04±7.20 ^a	32.43±3.67 ^a
Aspartate Aminotransferase (AST) Iu/L	22.38±5.92 ^b	14.50±1.21 ^d	19.92±1.43 ^b	18.92±1.91 ^b
Alanine Aminotransferase (ALT) Iu/L	32.13±6.34 ^c	21.88±2.09 ^e	21.92±1.80 ^e	21.84±4.08 ^e
Cholesterol mg/dL	121.13±9.49 ⁱ	123.75±9.75 ⁱ	122.75±5.10 ⁱ	112.59±5.12 ⁱ
Triglycerides mg/dL	150.88±34.44 ^j	196.50±39.48 ^j	114.75±1.71 ^j	134.75±7.68 ^j
High density Lipids (DDL) mg/dL	35.50±10.01 ^k	39.38±7.40 ^k	34.33±4.46 ⁱ	32.33±3.47 ^k
Low density Lipids (LDL) mg/dL	59.00±6.64 ^l	50.88±4.22 ⁱ	58.34±13.72 ^l	53.33±6.47 ^l

Note: All means±SEM along the same row with different superscripts are significantly different at P<0.05.

The PCV (%) value during proestrus was significantly lower than the values during oestrus, dioestrus and anoestrus ($P < 0.05$). There was a significantly lower haemoglobin content during proestrus than during the oestrus, dioestrus and anoestrus phases ($P < 0.05$). The mean red blood cell counts ($\times 10^6$ /dl) was significantly lower during proestrus when compared with the values at oestrus, dioestrus and anoestrus ($P < 0.05$). The mean corpuscular haemoglobin (MCH) values, were not significantly different between the phases of oestrous cycle but the mean corpuscular haemoglobin concentration (MCHC) value during proestrus was significantly lower ($P < 0.05$) than the values during dioestrus and anoestrus but not different from the value during oestrus. The leucocytic counts (WBC) at oestrus, dioestrus were not significantly different but the values for proestrus and anoestrus were significantly lower than the values for oestrus and dioestrus ($P < 0.05$). A significant difference ($P < 0.05$) was only observed with the value of eosinophils during dioestrus and anoestrus. The platelets values were also not significantly different from each other throughout the four phases of the oestrous cycle.

The phosphate, calcium, liver enzymes (ALP, AST, ALT), cholesterol, triglycerides, high and low density lipids values were not significantly different from each other throughout the four phases of the oestrous cycle (Table 4A and B).

The hormonal profiles (Fig. 2) showed that during proestrus 17 beta oestradiol concentration was 50.00 ± 30.68 pg/ml while progesterone concentration was 7.80 ± 1.07 ng/ml. During oestrus 17 beta oestradiol concentration was 13.11 ± 5.89 pg/ml and progesterone concentration was 28.00 ± 8.79 ng/ml. There was an interjection on the graphs of both the 17 beta oestradiol and progesterone profiles at about 2 days into oestrus (Fig. 2) when the values of 17 beta oestradiol and progesterone were 20.5 pg/ml and 20.5ng/ml, respectively. This might be suggestive of the period of ovulation. During dioestrus 17 beta oestradiol (pg/ml) and progesterone (ng/ml) concentrations were 5.39 ± 2.83 and 52.36 ± 11.96 respectively and at anoestrus phase 17- beta oestradiol (pg/ml) and progesterone ng/ml concentrations were 4.80 ± 1.46 and 13.00 ± 1.14 , respectively.

DISCUSSIONS

Four distinct phases of the oestrous cycle were identified in the Nigerian local bitches using vaginal cytology. They were; proestrus (9.00 ± 0.0 days), oestrus (9.00 ± 0.0 days), dioestrus (62.00 ± 1.0 days) and anoestrus (122.5 ± 2.5 days) similar to the reports of some workers on other breeds of dogs [2, 3, 25, 26]. The Inter-oestrus period was 211.5 ± 3.5 days and this was longer than 183 ± 52 days reported by [27] for German Shepherd in Kenya, but was comparable to the reports of [5], who reported that the average interval between successive heats was 217 days.

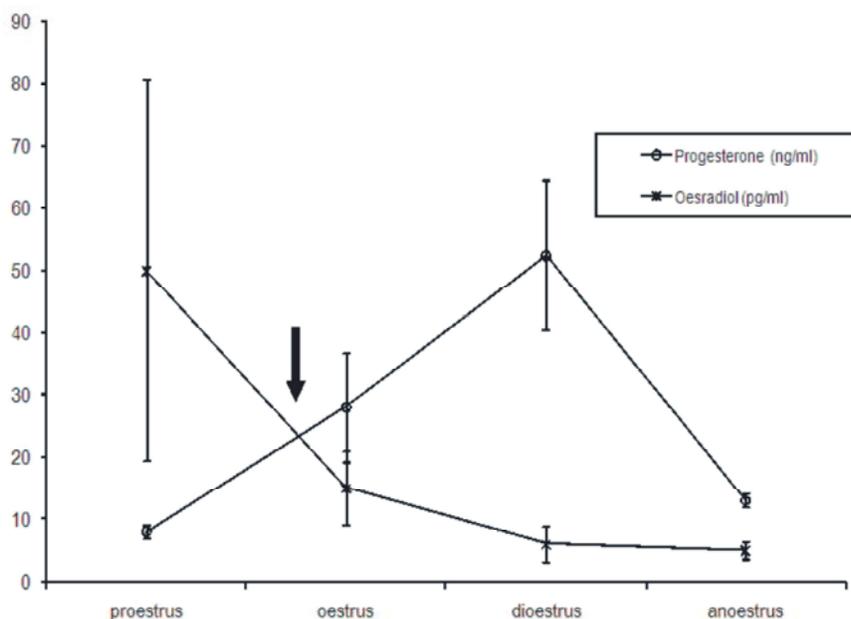


Fig 2: Oestrogen and Progesterone profiles during Oestrous Cycle of Nigerian local bitch Is the indicative of the likely period of Ovulation

The different behavioural characteristics that were observed in the local bitches used for this study during oestrus were keen interest in male, acceptance of the male, frequent micturition and flagging of the tail which varied from bitch to bitch and from oestrus to oestrus in the same bitch. These inconsistencies have been observed by some workers on other breeds [2, 26]. The other physical characteristics observed during oestrus in this study were oedema of the vulva and change in the quantity and colour of the vaginal discharge. These were more constant characteristics during oestrus in all the bitches as was reported in other breeds [2, 26]. To obtain the optimum breeding time (that is, period during oestrus when mating will result in maximal litter size) in the bitch, the veterinarian should not depend solely on these behavioural and physical changes because they are not constant. They must be combined with the vaginal cytology of the bitch because it is based on the morphological changes in the vaginal epithelial cells in relation to the circulatory levels of oestrogen [3].

It was discovered that there were changes in the colour of the mucous membrane of the vulva from deep pink during late proestrus to very pale during the anoestrus period and this was consistent in all the bitches in this study. This can be used to identify the phases of the oestrous cycle in the bitch, though qualitatively. The changes in the colour of the mucous membrane might be due to the amount of blood supply to the reproductive organs and the effect of oestrogen on their smooth muscles at the different phases of oestrous cycle. It was also discovered that the changes in the sizes of the vulvar lips through the four phases of the oestrous cycle was consistent in all the bitches and throughout all the oestrous cycles studied. This observation is similar to that of Davol, [2, 3, 26, 28, 29] in other breeds of dogs. The quantity, colour and consistency of the vaginal discharge throughout the oestrous cycles in all bitches is consistent with the reports of some previous workers on other breeds of dogs [3, 13, 25, 26].

The haemogram of the bitches obtained in this study were comparable to the values, PCV $46.10 \pm 4.16\%$, Hb 15.39 ± 1.99 and RBC 7.57 ± 0.90 reported by Bobade *et al.* [30] for Nigerian Local dogs. However, the WBC values (7.93 ± 0.90 to $9.34 \pm 1.5 \times 10^3/\mu\text{l}$) were lower than (18.52 ± 2.51) reported by Bobade *et al* [30] but compares favourably with the reference ranges of $6-17 \times 10^3/\mu\text{l}$ [31]. The result of this study also showed that PCV, Hb and RBC values were lowest during the proestrus phase of the oestrous cycle and the values of these parameters were elevated during estrus but there was no significant

difference ($P > 0.05$) between these values throughout oestrus, dioestrus and anoestrus phases of the cycle. This is similar to the report by Mschelia *et al.* [32] that RBC values were high during anoestrus and dropped gradually during proestrus. The above observation might be due to the action of oestradiol, with increased concentration during the phase of proestrus on the blood vessels of the vagina allowing the red blood cells to leak into the vagina resulting in the serosanguinous discharge during this period. The PCV started increasing during oestrus when these leakages started to reduce due to a reduction in the concentration of oestradiol after ovulation [33]. The observation of an increase in WBC during proestrus ($7.93 \pm 0.9 \times 10^3/\mu\text{l}$) and oestrus ($9.34 \pm 0.9 \times 10^3/\mu\text{l}$) and the slight decrease during dioestrus ($8.3 \pm 0.6 \times 10^3/\mu\text{l}$) is similar to the report by Mshelia *et al.* [32]. The values of phosphate ions (4.80 ± 0.63 to 7.92 ± 2.88 mg/100ml (dl) in this study were higher than the reference ranges for dogs of both exotic [34, 35] and local [36] breeds while the mean calcium values (9.00 ± 0.20 to 9.56 ± 0.50 mg/100ml (dl) were within the reference ranges for the exotic breeds [31, 34] and Nigerian local breeds [36]. The total serum protein, albumin and globulin values determined in this study were within the values of the reference ranges [32, 34-37]. The Liver enzymes ALP, AST and AST values were lower than values obtained by Awah and Nottidge [36] in the same breed of dogs. In this study, the phases of oestrous (proestrus, oestrus, dioestrus and anoestrus) only had a slight influence on these parameters mentioned above. All changes observed in the biochemical parameters were within reference values.

The patterns of the hormonal profiles obtained for $17-\beta$ oestradiol and progesterone were as reported by Olson and Nett [25] but the values recorded were higher than their values.

Some of the haematological parameters of the Nigerian local bitches have been found to be influenced by the different phases of the oestrous cycle. The interpretation of the results of such parameters as indices for medical conditions should always be with caution. In conclusion it can be stated that the oestrous cycle of the Nigerian local bitches in Ibadan compares favourably with those of other breeds in literature.

REFERENCES

1. Dewey, T. and S. Bhagat, 2002. *Canis Inpus familiaris*. Animat. Diversity Web. Retrieved, 06-01-2009.

2. Nelson, R.N. and C.G. Couto, 2003. *Small Animal Internal Medicine* 3rd Ed. Mosby 11830 Westline Industrial Drive St. Louis Missouri 63146: 847-933.
3. Davol, P.A., 2000. Canine Reproduction Part 1. Reproduction and the Bitch pdavol@labbies.com file: //A:/canine Reproduction part 1.htm. Retrieved 26-02-2010.
4. Arthur, G.H., D.E. Noakes and H. Pearson, 1982. *Veterinary Reproduction and Obstetrics, (Theriogenology)* 5th sdn. Bailtere Tindall, London, pp: 454.
5. Christie, D.W. and E.T. Bell, 1971. Endocrinology of the Oestrous cycle in the bitch. *J. the small Animal Practice*, 12: 383-389.
6. Sokolowski, J.H., D.G. Stover and F. Van-Ranvenswaa, 1977. Seasonal Incidence of estrus and interestrous Interval for Bitches of Seven Breeds. *J. American Veterinary Medical Association*, 171(3): 271-273.
7. Prole, J.H.B., 1973. Some Observations on the Physiology of Reproduction in the Greyhound Bitch. *J. Small Animal Practice*, 14: 781-784.
8. Hancock, J.L. and I.W. Rowlands, 1949. *The Physiology of Reproduction in Dogs*. *Veterinary Records*, 61: 771-779.
9. Bucker, R.C., 1979. The genital system. In *Canine Medicine* 4th edition Vol. 1. Edited by E.J. Catcott. American Veterinary Publications Inc. Drawer K.K. Santa Barbara California, pp: 501-632.
10. Allen, D.G., S.A. Kruth and M.S. Garvey, 1991. *Small Animal Medicine*. J.B. Lippincott Company Philadelphia, pp: 763-790.
11. Allen, E.D., E. Noakes and J.P. Penfon, 1995. *Canine Medicine and Therapeutic*. 3rd Edn. D.J Thompson edn. Blackwell Scientific Pty Ltd Australia, pp: 659-697.
12. Dyce, K.M., W.O. Sack and C.J.G. Wensing, 1996. *Text book of Veterinary Anatomy*. W.B. Saunders Company Philadelphia, pp: 435-453.
13. Wright, P.J. and Chu Po-yin, 2001. The Oestrous Cycle of the Bitch: Oestrus Induction and Suppression. <http://www.greyhoundvet.co.uk/downloads/AGVAOL.HTM> Retrieved, 19-08-2003.
14. Fowler, E.H., M.K. Feldman and W.F. Loeb, 1971. Comparison of Histologic Features of Ovarian and Uterine Tissues with Vaginal Smears of the Bitch. *American J. Veterinary Research*, 32: 327-334.
15. Schuttle, A.P., 1967. Canine Vaginal Cytology III. Compilation and Evaluation of Cellular Indices. *J. Small Animal Practice*, 8: 313-317.
16. Phemister, R.D., P.A. Holst, J.S. Spano and M.L. Hopwood, 1973. Time of Ovulation in the Beagle Bitch. *Biology of Reproduction*, 8: 74-82.
17. Olson, P.N. and E.C. Marther, 1978. Canine Vaginal and Uterine Bacterial Flora. *J. American veterinary Medical Association*, 172: 708-709.
18. Baba, E., H. Hata, T. Fukata and A. Arakawa, 1983. Vaginal and uterine microflora of the adult dogs. *American J. Veterinary Research*, 44: 606-610.
19. Larsen, B., A.J. markovertz and R.P. Galask, 1976. Bacterial Flora of the Vagina of Rodents. *J. Infectious Diseases*, 134: 486-490.
20. Mshelia, G.D., J.D. Amin and S.U.R. Chaudhary, 2001. Vaginal Bacterial flora of Nigeria Local Bitches during different Stages of Reproductive Cycle. *International J. Agriculture and Biology*. 32: 193-185. <http://www.ijab.org> Retrieved 04-11-2009.
21. Schalm, O.W., N.C. Jain and E.J. Carrol, 1975. *Veterinary Haematology*. 3rd edn. Lea and Febiger. Philadelphia, pp: 51-81.
22. Reinhold, J.G., 1953. Total Protein, Albumin and Globulin. *Standard Methods of Clinical Chemistry*. Edited by D. Seligson. Academic Press Inc. New York, pp: 1-88.
23. Gomori, G., 1942. A Modification of the Colorimetric Phosphorus Determination for the use of the Photoelectric Colorimeter. *J. Lab. Clin. Med.*, 27: 995-960.
24. Trinder, P., 1960. Colorimetric Micro Determination of Calcium in Serum. *Analyst*, 85: 889-894.
25. Olson, P.N. and T.M. Nett, 1986. *Reproductive Endocrinology and Physiology of the Bitch*. *Current Therapy in theriogenology*. 2nd Ed. Edited by Morrow D.A. W.B. Saunder Company. Philadelphia pp: 1196-1206.
26. Beimborn, K.S., H.L. Tarpley, P.J. Bain and K.S. Latimer, 2003. The canine estrous cycle staging using vaginal cytological examination. File //E:/The % 20 canine % 20 Estrous % 20 cycles % 20 staging % 20 using % 20 vaginal % 20 cyto.... Retrieved, 27-01-2001.
27. Mutembei, H.M., E.R. Mutiva and V.T. Tsuma, 2000. A Retrospective Study on Some Reproductive Parameters of German Shepherd Bitches in Kenya. *S. Afri. Vet. Assoc.*, 71(2): 115-117.
28. Correa, J.E., 2002. *Canine breeding and Reproduction*. Food and Animal Science. Alabama, A. and M. University. Alabama Cooperative Extension System. UNP-52 www.aces.edu Retrieved, 01-06-2009.

29. Root-Kustritz, W.V., 2001. Comments on the Small Animal Reproduction. Discussion List. Cafereprod web site. Retrieved, 06-02-2001.
30. Bobade, P.A.O.O. and O. Oduye Aghomo Helen, 1985. Haemogram of clinically normal dogs with particular references to Local (Nigerian) and German shepherd Dogs. *Nigerian Veterinary J.*, 14(1): 7-11.
31. Merck's Veterinary Manual, 2005. Reproductive system 8th Ed. Merck and Co. Inc. White House Station. N.J., USA in cooperation with Merial Ltd, pp. 977-1048.
32. Mshelia, G.D., J.D. Amin and S.U.R. Chaudhary, 2005. Haemogram of Nigerian Mongrel Bitch at Different Stages of Reproductive Cycle. *Pakistan Veterinary J.*, 25(1): 22-24.
33. Floss, J.L., 1993. Reproductive Management of Brood Bitch. File: //A:/G9922. Reproductive Management of the Brood Bitch. htm. Retrieved, 29-03-2005.
34. Kelley, D.F., V.M. Leicke and C.J. Gaskell, 1982. Notes on Pathology for Small Animal Clinicians. John Wright and Sons Ltd. England.
35. Bush, B.M., 1991. Interpretation of Laboratory Result for Small animal Clinicians. Blackwell Science Publication. London Butterworth Heinmann.
36. Awah, J.N. and H.O. Nottidge, 1998. Serum biochemical parameters in clinically healthy dogs in Ibadan. *Trop. Vet.*, 16: 123-129.
37. Coles, E.H., 1986, Serum Biochemistry. In: *Veterinary Clinical Pathology* 4th Ed. W.B. Saunders Co. Philadelphia, pp: 562.