Hormonal, biochemical and hematological changes during gestation in rabbit does synchronized with prostaglandin F_2 alpha

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Abstract

Assessment of physiological parameters such as hormonal, biochemistry and hematology of animals at different stages of gestation are helpful to monitor the health and nutritional status of animals. This study therefore, evaluated the changes in hormonal levels, biochemical and hematological parameters during gestation in domestic rabbit (*Oryctolagus cuniculus*) does following estrous synchronization with prostaglandin F_2 alpha ($PGF_2\alpha$). Eight nulliparous, sexually mature intact New Zealand rabbit does with mean weight of 1.9 ± 0.1kg were used for the study. They were distributed into eight hutches, and were synchronized with 0.7 mg/kg BW im injection of $PGF_2\alpha$ prior to mating. After 48 hours, the eight does were naturally mated with four bucks each (within two hours each doe was allowed to mate with four bucks to maximize chances of pregnancy). Does were examined for pregnancy using ultrasonography seven days after mating. Blood was sampled from the jugular vein before mating (BM), seven days after mating (7DAM), 14 days after mating (14DAM), 21 days after mating (21DAM), 28 days after mating (28DAM) and three days post-parturition (3DPP), respectively. Blood plasma progesterone, follicle stimulating hormone (FSH), estrogen and prolactin were assayed using enzyme linked immunosorbent assay. Hematological and biochemical parameters determined were packed cell volume (PCV), hemoglobin (Hb) concentration, red blood cell (RBC) count, white blood cell (WBC) count, cholesterol, triglycerides, high density lipoproteins (HDL) and low density lipoproteins (LDL). Data obtained for hormone and serum biochemistry were subjected to descriptive statistics, while other data were subjected to analysis of variance using general linear model procedure of statistical software. Results revealed that mean values for progesterone, FSH, estrogen and prolactin during gestation significantly varied at different periods of the experiment ($p<0.05$). Progesterone secretion during gestation peaked at 14DAM (32.1 ± 0.27 ng/ml). Estrogen secretion was 857.2 ± 3.22 ng/ml BM, 857.5 ± 3.80 ng/ml 14DAM and 866.6 ± 2.17 ng/ml at 28DAM but subsequently declined to 850.7 ± 6.04 ng/ml at 3DPP. Prolactin increased from 92.3 ± 0.13 ng/ml at BM to 92.8 ± 0.06 ng/ml at 7DAM, but decreased to 91.8 ± 0.36 ng/ml at 14DAM then increased to 92.5 ± 0.20 ng/ml at 3DPP. Cholesterol, triglyceride and LDL were not significantly ($p>0.05$) influenced by the period of sampling. The PCV, RBC, Hb, and WBC significantly varied from BM to 3DPP ($p<0.05$). The RBC, PVC, Hb and WBC counts decreased gradually from BM to 28DAM and subsequently increased until 3DPP. The study concluded that there were changes in hormonal parameters, PCV, RBC, Hb, WBC, and LDL while cholesterol, triglyceride, HDL and the WBC differential counts showed no changes during the study period.

Keywords: Gestation, New Zealand white, progesterone, mating, parturition

Introduction

Developing countries including Nigeria are rapidly growing in human population, and as such the demand for protein source is increasing. Most of the world population is fed on small farm products which are becoming insufficient as the human population pressure increases.[1] This has led to the need for alternative protein sources that are cheap, readily available and pose minimal competition to man.[2] Rabbits are small mammals in the family *Leporidae* of the order *Lagomorpha*, found in several parts of the world. Domestic rabbits have been used as sources of food and wool, research subjects, and as pets. Some rabbits are bred mainly for meat production, while others are bred for laboratory and exhibition purposes.

Rabbits compared to other laboratory animals are more advantageous as research subjects because of their unique lipid metabolism which is similar to that of humans.[3] They have a relatively short

gestation period averaging 30-31 days, early maturity, fast growth rate, high genetic selection potential, high feed conversion efficiency, high prolificacy and economic utilization of space.[1,4] The rabbit's adaptation to tropical regions, their feeding and low costs of production are also added advantages which could make their use for research economical and easily affordable.[5]

Successful reproduction is an orderly sequence of events involving puberty, cyclicity, copulation, pregnancy, postpartum, lactation and recovery.[6] Maintenance of pregnancy as a reproductive event and the initiation of parturition are under endocrine control. These involves the secretion of reproductive hormones progesterone, estrogen, luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin. During the pregnancy period, metabolic changes may occur that may alter physiological range of blood constituents in the animal's body.[6] Therefore, assessment of the physiological parameters such as hormonal, biochemistry and hematology of animals at different stages of gestation are helpful to monitor the health and nutritional status of animals.

Different authors have worked on the reproductive hormone profile of rabbit does during mating and during pregnancy.[7-9] The significance and variation in the biochemical and hematological indices observed among breeds of rabbits were also described by different researchers.[10,11] However, it appears that little or no work has been carried out on the variation in the hormonal, biochemical and hematological indices all through the different stages of gestation in rabbits.

Therefore, this study was designed to investigate the effect of gestation on different levels of hormones, some biochemical parameters and hematology of rabbit does.

Materials and methods

Animals

Eight nulliparous sexually mature intact non-pregnant New Zealand rabbit does with mean weight of 1.9 ± 0.1kg and four sexually mature bucks with mean weight of 1.9 ± 0.1kg were used for the experiment. They were purchased from a breeder located within the Abeokuta metropolis. Each of the rabbits was kept individually in a wooden hutch, housed in a naturally ventilated building. The experimental animals were fed a pelleted grower ration in the morning and thereafter with forage (*Tridax procumbens*), and water was supplied *ad-libitum*. Prior to commencement of study, all the animals were acclimatized for two weeks during which they were dewormed with albendazole syrup at 22 mg/kg/BW. All the animals were confirmed healthy based on the result of complete blood count and physical examination before the commencement of the study. Ethical approval for this study was obtained from the Research Ethics Committee, College of Animal Science, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Experimental design and procedure

The does were synchronized with 0.7mg/kgBW single im injection of PGF$_2\alpha$ (Lutalyse®, Upjohn Pharmaceutical Limited, Crawley, Sussex, UK) administered at the follicular phase of their cycle when they were non-receptive to bucks. All the does were thereafter naturally mated with four bucks 48 hours after synchronizing them, during which all of them became receptive to the bucks. On the day of mating, the does were transferred to the cage of the bucks, two does were introduced to one buck for a period of 30 minutes during which mating was observed. This procedure was repeated amongst all the does while rotating them with each of the bucks ensuring that all the bucks bred all the does, the whole breeding lasted for 24 hours.

Pregnancy diagnosis

Does were examined for pregnancy seven days after mating with the use of portable ultrasound machine with a 7.5 MHz transducer (Kaixin KX 2000®, Xuzhou, China). The ultrasonography was performed transcutaneously. Prior to scanning, the doe was restrained manually in dorsal recumbency to prevent her from moving. The hair on the abdominal region was clipped and scanning gel was applied at the examination site so that the space between the probe and the

animal's skin is free of air. The probe was positioned externally against the abdominal wall, and moved from right to left with the probe in the sagittal orientation and the image was viewed on the screen.

Blood sampling
         Three milliliters of blood were obtained from each rabbit doe via the jugular vein before mating (BM), seven days after mating (7DAM), 14 days after mating (14DAM), 21 days after mating (21DAM), 28 days after mating (28DAM) and three days postpartum (3DPP) from all does. Blood samples meant for hematological analysis were collected into plastic bottles containing ethylene-diamine-tetra- acetic acid (EDTA) while those that were meant for biochemical and hormonal assays were collected into lithium heparin bottles and taken to the laboratory for analysis. The plasma was obtained by centrifugation at 3000rpm in a refrigerated centrifuge, (4°C) for 15 minutes and stored at -20°C for hormone assay.

Hematological determination
         Hematological analysis was carried out using Mindray® BC-2800Vet auto hematology analyzer. This machine operates on the principle of measuring the impedance or light dispersion of EDTA blood (1ml). The parameters measured include RBC count, Hb concentration, PCV, WBC count and WBC differentials.

Plasma biochemistry and hormonal assay (progesterone, FSH, estrogen and prolactin)
         The plasma obtained was analyzed for concentration of cholesterol, triglycerides and lipoproteins using automated spectrophotometer (Agappe diagnostics®, Switzerland) using a conventional method. Plasma concentrations of progesterone, FSH, total estrogen and prolactin were however carried out using enzyme linked immunosorbent assay (ELISA, MyBioSource) as previously described.[12]

Statistical analysis
         Data obtained were presented as mean ± standard deviation and compared both between and within groups using ANOVA for repeated measures, with significance set at $P=0.05$. Data analysis was performed with Statistical Analysis System (SAS Institute, 2000).

Results
         All the eight does that were mated were confirmed pregnant with gestational sacs observed at day 7 after mating. The gestational sacs were characterized by oval shaped anechoic sac containing bipolar hyperechoic bands (Plate 1).
         The mean plasma progesterone concentrations varied from BM until three days PP (Figure 1). The plasma progesterone was at the lowest value (30.2± 0.11 ng/ml) BM, rose after mating until it reached the highest value of 32.1± 0.27 ng/ml at 14DAM and thereafter dropped significantly ($P<0.05$) from 28DAM until three days PP (Figure 1). Changes in mean plasma estrogen in rabbit does from before mating to three days PP is shown in Figure 2. There was no significant difference ($P>0.05$) in mean plasma estrogen concentration from BM up to 14DAM. It thereafter increased significantly ($P<0.05$) from 14DAM to 21DAM after which it significantly reduced from 28DAM to three days PP (Figure 2). The mean plasma FSH concentration had an initial significant increase ($P<0.05$) BM and 7DAM, it thereafter stabilized until 3 days PP (Figure 3). Figure 4 shows the changes in mean plasma prolactin BM to three days PP in rabbit does. Following an initial slight increase in mean plasma prolactin concentration after mating, it gradually reduced ($P<0.05$) between 7DAM and 14DAM. Changes in cholesterol level during gestation in rabbit does are shown in Figure 5. The highest cholesterol level was at 14DAM, all the other values obtained throughout the period of study were not significantly ($P>0.05$) different. Figure 6 shows the mean triglyceride changes as obtained in the rabbit does before they were mated up to three days PP. The result showed no significant ($P>0.05$) difference throughout the period of study. There was a significant increase ($P<0.05$) in the mean plasma HDL changes in rabbit does from before mating to 24DAM following which it significantly reduced until

28DAM (Figure 7). The changes in mean plasma LDL are presented in Figure 8 which showed that all the mean values obtained from before mating until 3 days PP were not significantly (P>0.05) different. The hematological changes from BM through mating until three days PP in the rabbit does are presented in Table 1. The mean values obtained for PCV, RBC, Hb, WBC and NEUT differ significantly (P<0.05) between days of sampling, while the mean values obtained for lymphocytes, monocytes, eosinophils and basophils showed no significant (P>0.05) differences between the days of sampling. Red blood cells, Hb, PCV, WBC and neutrophils significantly decreased during the last week of gestation. Hemoglobin increased significantly from BM to 7DAM and subsequently decreased till 28DAM. Packed cell volume increased from BM until 7DAM and subsequently decreased until 28DAM and then increased until 3 days PP.

Discussion

Pregnancy in rabbits has three stages: the period of fertilization and implantation, organogenesis and fetal growth.[13] During these periods, different hormones are secreted in pulses, which play a role in the maintenance of the pregnancy and also in preparing the mammary gland for lactation.[13] Progesterone action on the endometrium is essential for embryo implantation and pregnancy maintenance. The progressive increase in progesterone secretion obtained in this study after mating to mid-gestation could be attributed to the important role of progesterone in maintenance of pregnancy.[14] Follicle stimulating hormone plays a key role in the development and functions of the reproductive system. It is necessary for the follicular selection and growth and for the production of estrogens from androgen substrates.[14] There was a decrease in FSH synthesis from 7DAM which almost remained the same until three days PP. There was a slight increase in estrogen production at 14DAM which was maintained at a plateau until 28DAM. The main role of this estrogen could be attributed to its indispensable luteotropic role in the pregnant rabbit.[15] Prolactin levels observed in this study were high at the first and last stage of pregnancy. The initial gradual increase may be attributed to its central role in the development of the mammary glands and in the initiation and maintenance of lactation after parturition.[16] Cholesterol levels recorded in the present study increased progressively from 7DAM to 14DAM and then decreased to 28DAM, and triglyceride increased gradually to 14DAM then slightly reduced until 28DAM during the period of gestation. This result agrees with that reported by Wells et al during the gestation period in New Zealand Whites, although they recorded an outstanding increase on day 19 compared to the result observed in this study.[17] Hematological parameters pass through a series of changes and are helpful to determine the health and nutritional status of animals.[18] The reduction in RBC, PCV and Hb up to 28DAM could be attributed to physiological anemia that might occur due to hemodilution.[19] The reduction in the Hb may be due to mobilization of the dam's hemoglobin into fetal circulation and also due to dilution of blood which occurs sequel to plasma volume increase.[19] The RBC and Hb results correlate with that reported by Wells et al,[17] who recorded downward trends in RBC and Hb towards the end of organogenesis. The results for eosinophils, basophils and monocytes obtained in this study agrees with those reported by Kim et al who reported an increase in these parameters from day 0-12 and then decreased to its lowest level on day 24 of gestation.[20] In conclusion, the results obtained in this study have shown that physiological parameters (hormonal, biochemical and hematological) are at variance during gestation as compared to before mating. The data obtained in this study could serve as useful tool to accurately assess and adjudge the health status of rabbit does during pregnancy.

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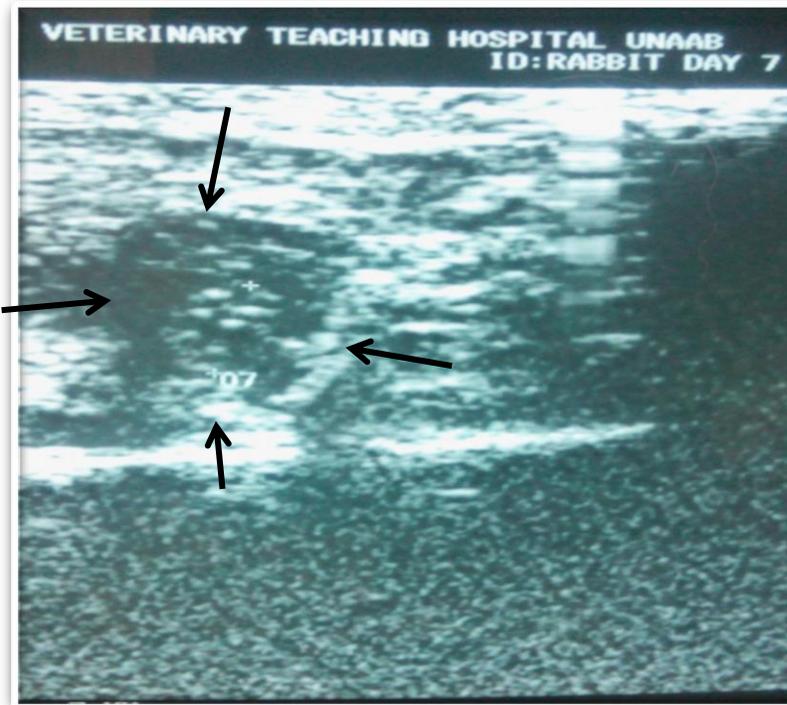
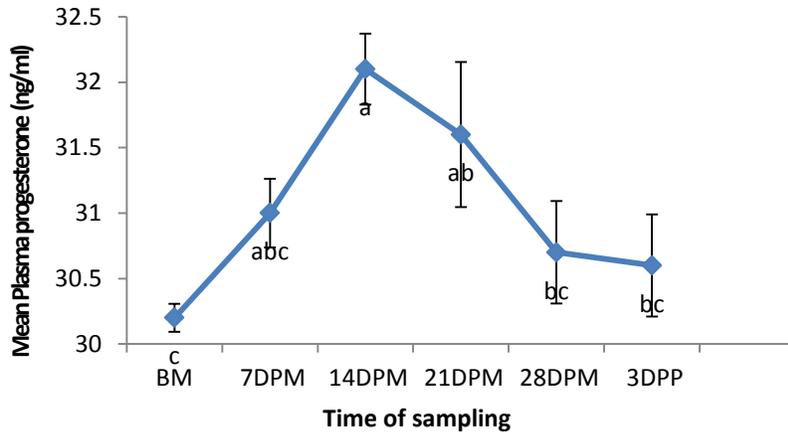**Plate 1:** 7 days old gestational sac of pregnant doe

Figure 1: Plasma progesterone changes from before mating (BM) through post mating (PM) to three days post parturition (PP).
Means with different letters are significantly (P<0.05) different
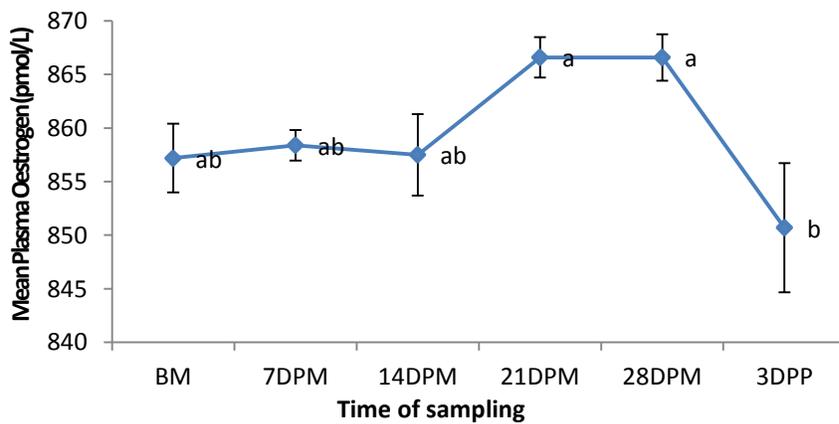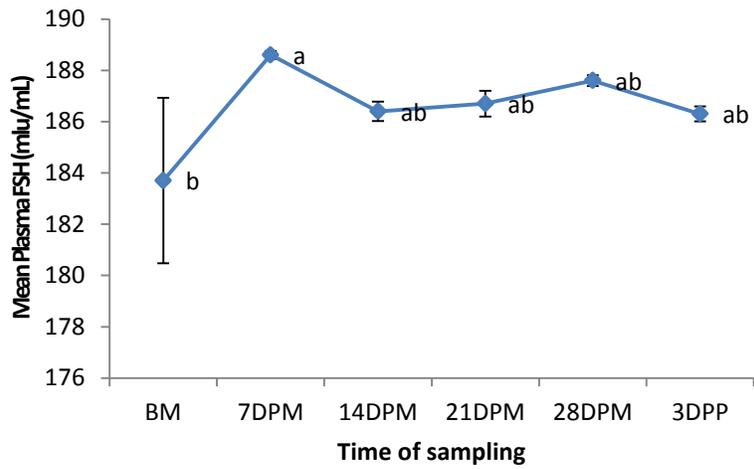

Figure 2: Plasma estrogen changes from before mating (BM) through post mating (PM) to three days post parturition (PP).
Means with different letters are significantly (P<0.05) different

Figure 3: Plasma Follicle Stimulating Hormone (FSH) changes from before mating (BM) through post mating (PM) to three days post parturition (PP).
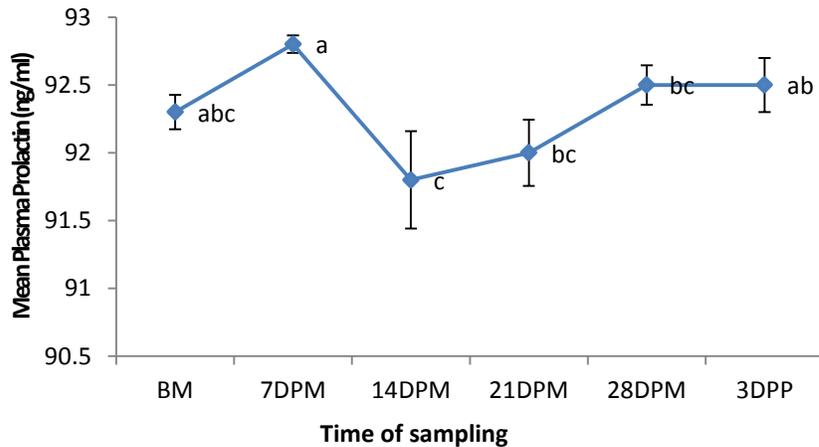Means with different letters are significantly (P<0.05) different

Figure 4: Plasma prolactin changes from before mating (BM) through post mating (PM) to three days post parturition (PP).
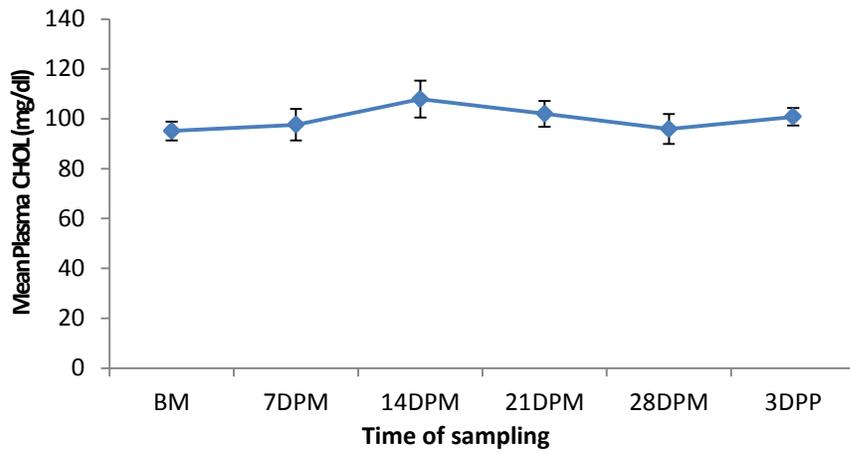Means with different letters are significantly (P<0.05) different

Figure 5: Plasma Cholesterol (CHOL) changes from before mating (BM) through post mating (PM) to three days post parturition (PP).
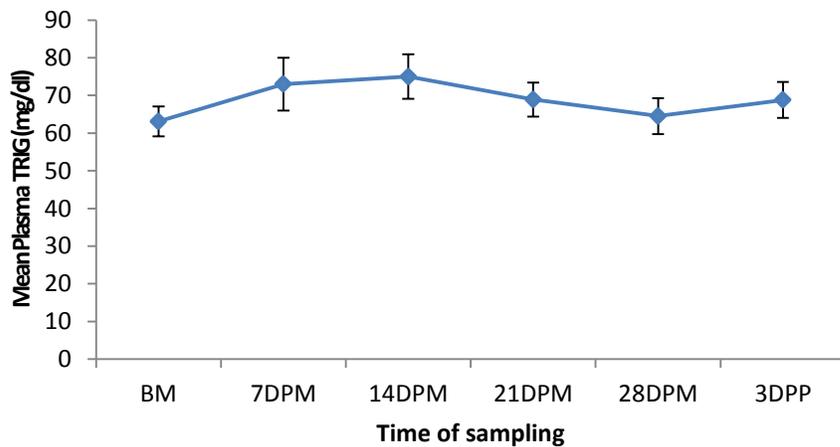

Figure 6: Plasma Triglyceride (TRIG) changes from before mating (BM) through post mating (PM) to three days post parturition (PP).
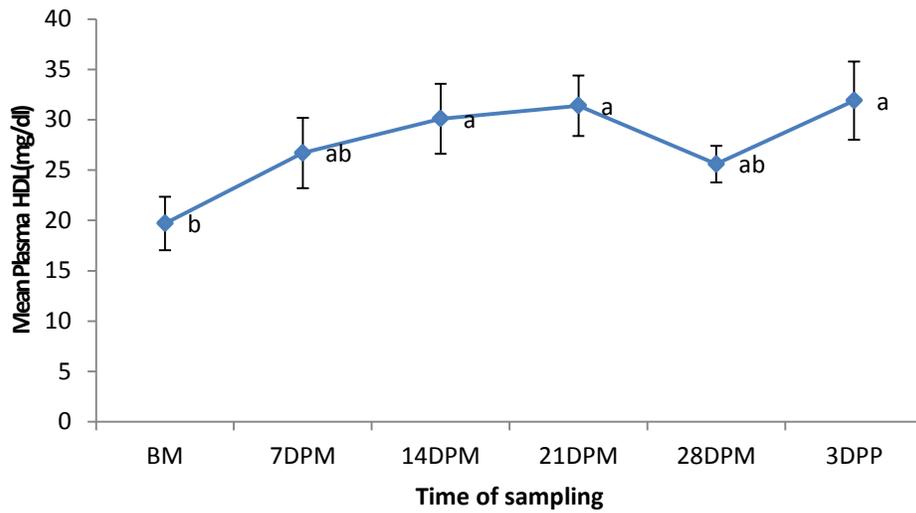
Figure 7: Changes in mean Serum High Density Lipoprotein (HDL) before mating (BM) through post mating (PM) to three days post parturition (PP).
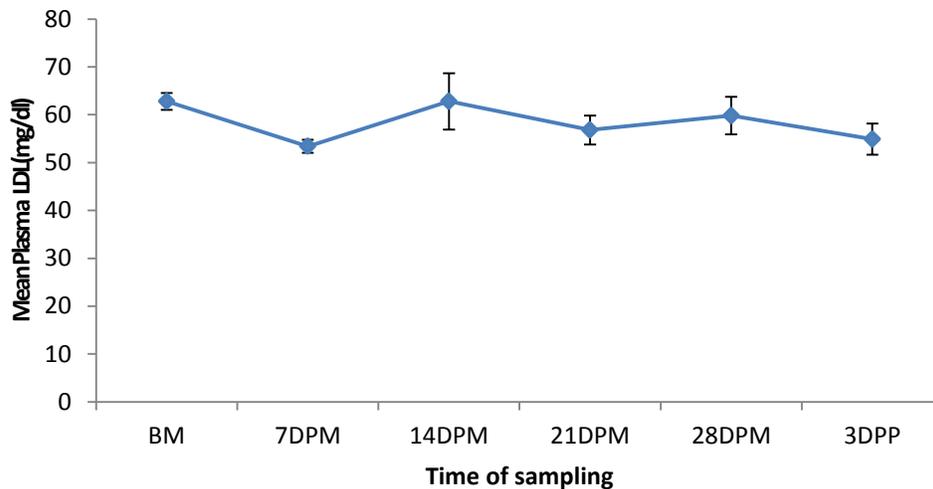Means with different letters are significantly (P<0.05) different

Figure 8: Changes in mean Low Density Lipoprotein (LDL) before mating (BM) through post mating (PM) to three days post parturition (PP).
Means with different letters are significantly (P<0.05) different

Table 1: Hematological changes from before mating (BM) through post mating (PM) to three days post parturition (PP).

Parameter	BM	7DPM	14DPM	21DPM	28DPM	3DP
RBC (x 10^6/ul)	5.40 ± 0.11^{ab}	5.47 ± 0.18^{a}	5.35 ± 0.23^{ab}	5.08 ± 0.21^{ab}	4.94 ± 0.14^{b}	5.54 ± 0.09^{a}
PCV (%)	34.63 ± 0.73^{ab}	35.13 ± 0.90^{a}	33.00 ± 1.57^{ab}	32.38 ± 1.15^{ab}	31.63 ± 0.84^{b}	34.25 ± 0.59^{ab}
Hb (g/dl)	11.58 ± 0.22^{ab}	11.81 ± 0.33^{a}	11.59 ± 0.52^{ab}	10.88 ± 0.39^{ab}	10.60 ± 0.31^{b}	11.44 ± 0.19^{ab}
WBC (x 10^3/ul)	$10.03\pm0,76^{bc}$	10.60 ± 0.61^{b}	9.66 ± 0.59^{bc}	7.81 ± 0.90^{c}	7.90 ± 0.35^{c}	12.91 ± 0.79^{a}
Lymphocyte (%)	68.63 ± 3.59	56.88 ± 5.89	61.38 ± 6.65	62.88 ± 6.08	74.57 ± 3.53	57.00 ± 6.90
Monocyte (%)	1.75 ± 0.31	0.75 ± 0.31	2.13 ± 0.79	1.25 ± 0.45	2.00 ± 0.46	1.63 ± 0.50
Eosinophil (%)	0.75 ± 0.31	1.00 ± 0.38	1.25 ± 0.41	1.13 ± 0.30	0.75 ± 0.31	0.63 ± 0.26
Basinophil (%)	0.00 ± 0.00	0.13 ± 0.13	0.13 ± 0.13	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Neutrophil (%)	28.00 ± 3.51^{ab}	41.25 ± 5.83^{a}	34.88 ± 6.99^{ab}	35.00 ± 6.06^{ab}	22.63 ± 3.65^{b}	40.75 ± 6.94^{a}

Means with different superscripts are significantly different.