Short Communication
Erythrocytic parameters as indicators for differentiating between the pregnant and pseudopregnant bitches in Nigeria

Ajala O.O., Fayemi, O.O. and Kolawole, T.O.

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

Summary: The erythrocytic parameters during pregnancy and pseudopregnancy in bitches were studied and compared in 8 bitches aged 2 -3 years and weighing 10–12 kg. Blood samples were collected from the bitches before mating, during the three trimesters of pregnancy and the post partum period. The packed cell volume (PCV %), haemoglobin concentration (Hb gm/dl), red blood cell count(x10^6/µl) were determined using standard methods. The mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were then calculated. Six of the bitches were pregnant and 2 were pseudopregnant. The results showed that in pregnant bitches, the PCV decreased significantly from the premating values of 51.37$\pm$0.94% to 34.00$\pm$8.04% during the third trimester of pregnancy (P<0.05). There was also a significant decrease in Hb values (P<0.05) from the premating period (16.30 $\pm$ 0.20gm/dl) to the third trimester of pregnancy (11.25$\pm$1.80gm/dl). The values of Red blood cells (RBCx10^6/µl) during the premating period (12.70$\pm$3.15) were not significantly different from the values during the first second and third trimesters (11.13$\pm$3.87, 10.38$\pm$4.54 and 12.24$\pm$3.15, respectively). The trend of decrease in PCV and Hb values were not observed in the bitches with pseudopregnancy. This shows that these erythrocytic parameters can be used to detect and differentiate between pregnancy and pseudopregnancy in bitches as early as the first 20 days post mating.

Keywords: Erythrocytic parameters, Pregnancy, Pseudopregnancy, Bitches.

Address for correspondence: oluwatoyinajala63@yahoo.com

INTRODUCTION

Dog breeding in Nigeria during the past 10 years has greatly increased in the numbers of different breeds of dogs, dog population, owners and even establishment of kennel clubs. Because of this, there is an increase in the demand by dog breeders for early differentiation between pregnancy and pseudo-pregnancy in their bitches. Pseudopregnancy is a condition in bitches in which all external signs such as abdominal distension, development of mammary glands with secretion of milk; nesting and adoption of inanimate objects as if they were puppies mimic those of pregnancy (Gobello et al., 2001, Romagnoli, 2009).

Palpation of embryos through the bitches' abdomen as early as 28 days after mating (Farricelli, 2009), abdominal radiography as from 45 days after mating (Morrow, 1986), ultrasonography as from 30 days of pregnancy (Farricelli, 2009) and relaxin assay

as from 20 days after luteinizing hormonal surge are some methods that have been used to detect and differentiate between pregnancy and pseudo-pregnancy in the dog. In Nigeria some of these methods are not readily available to veterinarians for pregnancy diagnosis in animals. Therefore there is a need for other methods that could be adapted to Nigerian situation.

The whole blood is an important and reliable medium for assessing the health status of animals because physiological and pathological conditions of animals can be evaluated (Anosa and Isoun, 1978). The number of various circulating blood cells can vary with normal physiological and pathological conditions. Nutrition, age, sex, breed, oestrous cycle, pregnancy, climate and diseases have been shown to affect the haematological parameters in animals (Kaneko and Cornelius, 1971, Ogunrinade et al., 1981, Kaggawa et al., 1984, Bobade et al.,1985

Fraser ,1986, Kelly, 1979, Omotainse et al., 1994, Ogunsanmi et al., 1994(a), Mshellia et al 2005).

It has been observed that during pregnancy, the maternal haematocrit slowly declines from a mean of 53% to 32% at term and then increased to 42% the next 6 to 8 weeks after parturition (Schams et al., 1975). According to Concannon et al (1977), there is a decrease in maternal PCV following implantation and is usually below 42% and by day 35 of pregnancy, it is below 35 percent. This anaemia during pregnancy in the bitch was thought to be due to the haemodilution effects of increased plasma volume during pregnancy (Concannon et al., 1977). As gestation progresses maternal blood volume increases by 20-40% with a resulting decrease in erythrocyte count and Haematocrit (Mollie, 1996). During the second trimester of pregnancy in the bitch, there is also a decrease in haemoglobin concentration and increase in erythrocyte sedimentation rate (Morrow, 1986). There is decrease in the erythrocyte, hemoglobin level and packed cell volume with a concomitant increase in erythrocytic sedimentation rates and platelet counts from the third week of gestation in the bitch (Schams et al., 1975; Morrow, 1986).

It has been suggested that these erythrocytic features characteristic of pregnancy may be used diagnostically to differentiate between pregnancy and pseudopregnancy as early as the first trimester (1-20days after mating) and these tests are easily available in Nigeria but this is yet to be established experimentally by evaluating the erythrocytic parameters during pseudopregnancy.

This study was therefore designed to evaluate and compare erythrocytic parameters of pregnant and pseudopregnant bitches to ascertain the suitability of the erythrocytic parameters as an early differential tool between pregnancy and pseudopregnancy.

MATERIALS AND METHODS

Eight bitches and one stud were used for this study. They aged between 2-3 years and weighed 10 -12kg. All the dogs were apparently healthy with no clinical manifestation of any disease during sampling. The bitches were fed once per day in the

morning at about 8.00a.m in their separate kennels from the first trimester to mid-gestation and then twice daily till the end of gestation with compounded ration of 26.36% crude protein. They were allowed enough exercise and were mated naturally at the second day of their acceptance of the male which coincided with the second day of estrus as indicated by vaginal cytology. Six of these bitches were pregnant while two were pseudopregnant. Blood sample (2ml) was collected from the bitches before mating, at the different trimesters of pregnancy (1-20th day after mating as first trimester, 21st – 42nd day after mating as 2nd trimester and 43rd till parturition as the 3rd trimester) into heparinized bottles. The Packed cell volume (PCV), Erythrocyte count (RBC), Haemoglobin concentration (Hb) were determined using standard methods as described by Schalm et al., (1975). The erythrocytic indices (MCV, and MCHC) were then calculated.

Statistical Analysis

The mean $\pm$ SEM haematology parameters were calculated at different trimesters. In order to establish any difference in the parameters the mean $\pm$ SEM were subjected to analysis of variance (ANOVA) and multiple comparison table using least significant Difference (LSD) model (Bamgboye, 2006).

RESULTS

Six out of the eight bitches mated were pregnant and whelped while two were pseudopregnant. Like the pregnant bitches, the pseudopregnant bitches showed signs such as, abdominal distension, mammary gland enlargement, nesting and milk let down but no whelping after the required number of days for normal gestational bitches.

In the pregnant bitches, the mean PVC decreased significantly (P<0.05) from the premating values of 51.37 $\pm$ 0.94% to 42.33 $\pm$ 7.51%, 40.75 $\pm$ 4.99% and 34.00 $\pm$ 8.04% during the first, second, and third trimesters of pregnancy respectively (Table 1). There was a significant decrease (P<0.05) in the values of haemoglobin concentration of the pregnant bitches

Table 1:

The Haematological Parameters of the Bitches during pregnancy

Haematological Parameter	Premating	1st Trimester	2nd Trimester	3rd Trimester	2 Weeks Post Partum
PCV (%)	51.37 $\pm$ 0.94	42.33$\pm$7.51*	40.75$\pm$4.99*	34.00$\pm$8.04*	30.75 $\pm$ 8.302*
Haemoglobin (gm/dl)	16.30 $\pm$ 0.20	12.80$\pm$1.34*	12.08$\pm$1.06*	11.25$\pm$1.80*	9.75$\pm$2.783*
RBC (x10^{6}/μl)	12.70 $\pm$ 3.15	11.13 $\pm$ 3.87	10.38 $\pm$ 4.54	12.24 $\pm$ 3.15	9.34$\pm$3.953*
MCV (fl)	40.45 $\pm$ 1.72	40.10$\pm$10.54	43 $\pm$ 13.41	29.00$\pm$5.71*	32.92$\pm$0.30*
MCHC(gm/dl)	31.73 $\pm$ 0.67	30.67$\pm$13.50	31.25 $\pm$ 1.71	32.25 $\pm$ 1.71	31.71+0.70

Values are expressed as means $\pm$ S.E.M. * P<0.05 significantly different from the premating value. 1st trimester = 1st – 20th day after mating, 2nd trimester= 21st -42nd day after mating, 3rd trimester – 43rd – 63rd day after mating

Table 2:
Haematological Parameters of Pseudopregnant Bitches

Parameter	Premating	0–20 days Postmating	21–42 days Postmating	42–63 days Postmating
PCV (%)	51.37±0.94	50.92±0.45	50.75±0.75	50.75±0.75
Haemoglobin (gm/dl)	16.30±0.20	16.48±0.18	16.95±0.45	16.95±0.46
RBC (x10^6/µl)	8.33±0.10	8.42±0.08	8.51±0.21	8.51±0.21
MCV(fl)	61.77±1.72	60.45±0.72	59.77±1.76	59.77±1.77
MCHC(gm/dl)	31.81±0.67	31.37+0.18	33.39±0.55	33.39±0.55

Values are mean ± S.E.M. There was no significant difference in the values of parameters measured.

from the premating period through the three trimesters to the end of gestation (Table 1).

There was no significantly difference (P<0.05) in the RBC value before mating and the values during the first, second and third trimesters of pregnancy (Table 1). The MCV value before mating was significantly higher than the values during the third trimester and two weeks post partum (P<0.05), but was not significantly different from the values during the first and second trimesters (Table 1). There was no significant difference in the values of MCHC before mating through to the end of gestation.

In the pseudopregnant bitches, there was no significant difference in the mean values of PCV, RBC, Hb, MCV and MCHC before mating to the end of the pseudopregnancy period (Table 2).

DISCUSSION

The heamatology result showed a decrease in the PCV of the pregnant bitches in the 1st, 2nd, and 3rd trimesters, but was most notable in the 3rd trimester thus showing that there was anaemia in the 1st, 2nd and 3rd trimesters but most notable in the 3rd trimester of pregnancy. This is what is often called the physiological anemia during pregnancy. This observation agrees with the result from earlier works done by Concannon, (1986); Concannon and Lein, (1989); Concannon, (2002); Mshelia et al, (2005). Concannon and Lein, (1989) stated that the PCV below 40% was seen 31 to 40 days after fertile mating while PCV below 35% was seen 53 days after fertile mating. This was explained as due to the haemodilution effects of increased plasma volume, because total blood volume increases alongside body weight increase of 20 to 55% over the course of gestation.

In this study the PCV value of 30.75 ± 8.30% was noticed two weeks after parturition compared with the pre-mating PCV of 51.37± 0.94 %. This agrees with the report of Concannon, (2002) that there is a progressive, anaemia starting between day 25 and 30 of pregnancy which becomes maximal near term and recovers over an 8 -12 week period post partum. This anaemia post partum might be due to the stress of

parturition and nursing of the puppies.

The decrease in the value of haemoglobin during pregnancy in this study agrees with the reports by Fisher and Fisher (1981); Concannon and Lein (1989); Mshelia et al, (2005) in which they stated that during pregnancy, there is a decrease in haemoglobin levels. The RBC, MCV, and MCHC values before mating were not significantly different from the values during the 1st and 2nd trimesters showing that the anaemia observed at this period in this study was normochromic normocytic. This observation agrees with the report of Concannon (2002), that there is a progressive normochromic normocytic anaemia starting between day 25 and 30 of pregnancy.

There were no significant changes in the erythrocytic parameters of pseudopregnant bitches as compared with the anaemia observed in the pregnant bitches during the period of study. This may be explained as due to the fact that since the pseudopregnant bitches were not actually pregnant and so no implantation that may lead to haemodilution that is thought to be causing the anaemia in the pregnant bitches (Concannon and Lein, 2005). This observation confirmed the suggestion of Morrow (1986) who emphasized that the anaemia seen in late canine pregnancy might be useful for the differential diagnosis of pregnancy and pseudopregnancy. It can be concluded therefore that the normochromic normocytic anaemia observed when considering the erythrocytic parameters such as PCV, Hb, RBC, MCV and MCHC of bitches can be used to detect and differentiate between pregnancy and pseudopregnancy states as early as the first trimester (1 -20 days after mating).

REFERENCES

Anosa,V.O. and Isoun, T. T. (1978). Haematological studies on domestic animal in Nigeria: Factors influencing the haematocrits of sheep and goats Zentrblt. Fer. Vet. Med. Ass. 25: 640 – 646.

Bamgboye, A. E. (2006). A companion of medical statistics. 1st edn. Ibipress and publishing co. Ibadan pp. 216.

Bobade, P. A., Oduye, O. O., Aghomo, Helen, O. (1985) Haemogram of clinically normal dogs with particular references to Local (Nigerian) and German shepherd dogs Nigerian Veterinary Journal. 14 (1): 7-11.

Concannon, P. W., Powers, M. W., Holder, W., Hansel, W. (1977). Pregnancy and parturition in the bitch. Biology of Reproduction. 16: 317 – 318.

Concannon, P. W. (1986). Physiology and endocrinology of canine pregnancy. In: Current therapy in Theriogenology, Vol. 2, Murrow, D.A.(ed). W.B. Saunders Philadelphia, USA, pp: 491- 497.

Concannon, P. W. and Lein, O.H. (1989). Hormonal and clinical correlates of ovarian circles, ovulation pseudopregnancy and pregnancy in dogs. In; Current Veterinary therapy: X. Small animal practice. W.B. Saunders Co. Philadelphia, USA, pp: 1269-1282.

Concannon, P. W. (2002). Physiological and clinical parameters of pregnancy in dogs. 27 WSAVA Congress Proceeding. http://www.vin.com/ proceeding. p/x? C/D=WSAVA 2002 œ PID=2681œ print=1. Retrieved 21-03-2005.

Farricelli, J. (2009). Signs of phantom pregnancy in dogs. http:/www.helium.com/item/1517965-dog-pseudo-pregnancy-dog-pseudo-pregnancy-dur--- .Retrieved 20 -03 2011.

Fisher and Fisher, D. (1981). Serum assay for canine pregnancy testing. Mod. Vet. Pract. 62: 466.

Fraser. M. (1986). Haematology In: The Merck Veterinary Manual, 6th edn. Rahway, Merck and Co. Inc. pp882-885.

Gobello, C., Concannon, P.W., Verstegen, J. (2001). Canine pseudopregnancy: A Review In: Recent advances in Small animal reproduction, P. W. Concannon, G. England and J. Verstegen (Eds) International Veterinary Information Services (www.ivis.org), Ithaca, New York USA Retrieved 20- 03- 2011.

Kaggawa, E., Munyna, W. K., Mugera, G. M. (1984). Pathogenicity of trypanosome brucei in dogs.

Bull Anim. Hlth Prod. Afr. 32: 360 – 366.

Kaneko, J. J. and Cornelius, C. E. (1971). Clinical biochemistry of domestic animals 2nd ed. Academic press New York pp. 236.

Kelly, W. R. (1979). Veterinary Clinical Diagnosis. 2nd Edn. Baillier Tindall, London, U.K. pp261-300.

Mollie, W. (1996). Anaesthesis for the pregnant bitch. Current therapy in theriogenology 2nd ed. D. Morrow (edn.) W. B. Saunders Co. Philadelphia. Pp . 502 – 504.

Morrow, D. A. (1986) Current therapy in theriogenology: Diagnosis, treatment and percentage of reproductive diseases in small and large animals. 2nd ed. W. B. Saunders co. Philadelphia. Pp 450 – 502.

Mshellia, G. D., Amin,J. D. and Chaudhari, S. U. R. (2005). Haemogram of Nigerian Mongrel Bitch at different stages of the reproductive cycle. Pakistan Vet. J., 25 (1): 22-24.

Ogunrinade, A., Fajimi, J., Adenaike, A. (1981). Biochemical indices in White Fulani (Zebu) Cattle in Nigeria, Rev. Elev. Med. Vet. Pays Trop. 34 (4): 413 – 415.

Ogunsanmi, A. O., Akpavie, S. O. and Anosa, V. O. (1994a). Haematological changes in ewe experimentally infected with trypanosome brucei. Rev. Elev. Med. Vet. Pays. Trop. 47(1): 53 – 57.

Omotainse, S. O., Anosa, V. O. and Falayo, C. (1994). Clinical and biochemical changes in experimental Trypanosoma brucei infection in dogs. Isreal journal of Vet. Med. 49 (1): 36 – 39.

Romagnoli, S. (2009). An update on pseudopregnancy. 34th World Small Animal Veterinary Association. 2009 WSAVA congress. Sao Paulo Brazil. July 21-24 2009. http://www.vin.com/proceeding/Proceeding.plx?cid=WSAVA2009. Retrieved 20- 03- 2011

Schalm, O. W., Jain, N. C. and Carrol, E. J. (1975). Veterinary haematology 3rd ed. Lea and Febiger Philadelphia pp. 51 – 81.