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Study on changes in haematological and serum biochemical parameters during mid gestation and late gestation in bitches

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Abstract
The aim of this study is to compare some blood haematological and serum biochemical parameters during mid and late gestation period in bitches. Samples obtained from 20 pregnant bitches to study Total erythrocyte count, Haemoglobin concentration, Packed cell volume. Total leucocyte count. Mean corpuscular haemoglobin concentration, Mean cell volume and Serum biochemical parameters like Alanine amino transferase, Aspartate amino transferase, Serum creatinine, Blood urea nitrogen. Total protein, Albumin. Results of the study showed significant difference in Haematological parameters the values decreased to lower values of reference range by the end of gestation and Serum biochemical parameters decreased to lower values, whereas the Serum creatinine and Blood urea nitrogen values increased but were within reference range by the end of gestation.

Keywords: Gestation period, bitches, haematology, serum biochemical parameters, reference range

1. Introduction
In India, there are 80 million of dogs and cats. At present, it is estimated that there are 9.1 million cats and 62 million dogs that are enrolled in India. This figure is increasing day-to-day due to increased awareness about the benefit of owning dogs in the civilized society. This development has triggered the idea of conducting study on canine breeding, puppy mortality and reduction measures.

Pet breeding have some risks that arise during pregnancy. Neonatal mortality in bitches represents a potential emotional and economic loss for pet owners and breeders.

Circulating Blood cells vary with Normal physiology as well as pathology during pregnancy. PCV is gradually reduced from 59 to 32% and increased to 42% in Next few weeks post-partum (Chaudhari and Mshelia, 2006, Concannon *et al*., 1977) [2, 4]. Total Leukocytic Count (TLC) and Differential Leukocytic Count (DLC) influenced by Age (Schalm *et al*., 1975) [21] observed changes during pregnancy. In mid-gestation, there is a reduced RBC count, haemoglobin (Hb.) concentration. increased erythrocytic sedimentation rate (ESR) and platelet count from third week of gestation (Mollie al., 1996) [17].

Ajala *et al*. (2011) [1] used erythrocytic during pregnancy and pseudopregnancy in bitches. results showed in pregnant bitches, PCV decreased significantly during the last trimester of pregnancy. There was a significant decrease in Hb values from premating period to the last trimester of pregnancy and values of RBC during premating period were not significantly differed during all trimesters and the trend of decrease in PCV and Hb.

Tohru and Kazuhiko (2018) [14] conducted study to (a) to ascertain age related changes in the reference values in haematological and serum biochemical examinations of beagles and (b) to clarify the changes in these findings. including acute phase proteins and oxidative stress, throughout pregnancy and after parturition and concluded that reference values for haematological and serum biochemical examinations should be used for health evaluation of dogs, taking sex, age and the stage or pregnancy into consideration.

2. Materials and Methods
2.1 Selection of Animals
The study was undertaken on 20 bitches (10 small breeds or < 15kg Body weight and 10 large breeds of > 15kg Body weight) with 30 to 35 days crossing history aged between 1-6 yrs. were presented to Veterinary clinical complex, Veterinary Gynaecology and Obstetrics ward,

College of veterinary Sciences, Rajendranagar. As gestation progresses maternal blood volume increases by will) a resulting decrease in erythrocyte count and Haematocrit (Mollie, 1996) [17].

Mean Corpuscular Haemoglobin Concentration (MCHC) (g/dl) is a in measurement of the average amount of haemoglobin in a single red blood cell (RBC) as it relates to the volume of the cell. In the present study. MCHC is maintained normally at mid-gestation and during late gestation. the values are decreased but within in Ille reference range itself. in both small breeds and large breeds. The MCHC test is one of a panel of tests called the RBC indices. which help to define different physical characteristics of RBC, as an assessment of the blood's ability to carry oxygen. the MCHC test (along with the other RBC indices) can be used to diagnose and classify disorders that affect the blood. like anaemia.

Mean cell volume (MCV) (Fl) is maintained in normal values at mid-gestation and decreased below the reference range during late gestation in both small breeds and large breeds. The MCV provides an indication of the status or size of erythrocytes and epitomizes either normal or abnormal cell division during erythropoiesis Nussey *et al*. (1995) [19], which were lower in pregnant bitches due to higher RBC number during pregnancy periods in canine populations.

On our observations from mid to late gestation of pregnancy, there is a drastic increase of WBC. Doxey (1966) [9] and Kimberley *et al*. (2006) [13] reported higher WBC count in the advanced stage of gestation but it was not increased beyond the normal reference range. A similar observation was made in the present study where all the groups exhibited a leucocyte count close to the higher margin of the reference range This high leukocyte count could be associated with the inflammatory environment at the time of parturition, characterized by decreased progesterone and increased cortisol and prostaglandins concentrations. (Dimco et 2013; Frehner *et al*. 2018) [8, 11].

According to results. total RBC. Haemoglobin, PCV. MCHC. MCV was normal in pregnant bitches at early stage and later on at last stage of gestation there is reduced values compared to their reference ranges. Total Leucocyte count (10^3/ uL) is increased above their reference range from) mid-gestation to late gestation in small and large breeds. This is due 10 increase in maternal immunity as pregnancy advances.

2.2. Serum Biochemistry

The following parameters were measured using a blood chemistry analyser (Fully Automated Clinical Chemistry Analyzer-TurboChef Neo) are total protein (TP), albumin (Alb), albumin/globulin (A/G) ratio. total bilirubin (T-Bil), blood urea nitrogen (BUN), creatinine (Cre) aspartate aminotransferase (AST), alanine aminotransferase (A LT).

Among Pregnant bitches which were normally delivered of healthy puppies with haematological results have few changes in haematological profiles from mid to late pregnancy (first 30 days of pregnancy), there is serum biochemistry such as LFT and KFT, recorded as normal values are there within a reference range, some are increased and some are reduced from mid to late gestation.

In Liver Function Test (LFT), ALT is high at mid-gestation and decreased during late gestation in small breeds and high at mid-gestation and decreased during late gestation in large breeds. AST is high at mid-gestation and decreased during late gestation in both small and large breeds.

Albumin concentrations gradually decreased during pregnancy and the low concentrations were subsequently maintained significantly were observed from 30 days of pregnancy.

Serum Albumin (g/L) is high at mid-gestation and decreased during late gestation in large breeds and high at mid-gestation and decreased during late gestation in small breeds. Serum Globulin (g/L) is high at mid-gestation and decreased during late gestation in both small and large breeds, The A/G ratio also decreased gradually in conjunction with the changes in the TP and Alb parameters

Total Protein (g/L) is high at mid-gestation and decreased during late gestation in both small and large breeds, Serum biochemical results for the pregnant bitches are showed declined TP concentrations markedly in late pregnancy.

In Kidney Function Test (KFT). Serum Creatinine (mg/dL) values are within the reference range i.e., low at mid-gestation and increased during late gestation in small and large breeds. Blood Urea Nitrogen (mg/dL) are within the reference range i.e., normal at mid-gestation and decreased during late gestation in large breeds and low at mid-gestation and increased during late in small breeds,

veterinary science, Rajendranagar, Hyderabad. The estimation or haematological parameters (CBP) and serum biochemical parameter (LFT & KFT) by various laboratory procedures.

2.3. Blood Sampling

Two blood samples were collected from each dog in the BD Vacutainers without anticoagulant (levaram life sciences Pvt. Ltd. India) and BD Vacutainers with EDTA (levaram life sciences Pvt. Ltd. India). The blood is collected from the cephalic vein of the bitches on 30th day and 60th post mating. The samples were processed within 2-3 hours after collection for haematological parameters with the help of ABX Micro ESV 60 fully automated veterinary haematology analyser manufactural by horiba Pvt. Lid, India in Department of VCC, C.V. Sc Rajendranagar, Hyderabad. The serum was separated Iron blood samples by centrifugation at 2000 rpm for 5 minutes and collected into serum collection vial and analysed in EM DENSITY I80 fully auto biochemical analyser supplied by Erba Mannheim Pvt. Ltd, Germany.

3. Results and Discussion

3.1 Hematological Values

Table 1: Mean values of hematologic alterations in bitches

S. No.	Parameter	Reference Range	Small breeds		Large breeds	
			mid-gestation	late-gestation	mid -gestation	Late-gestation
1	Total erythrocyte count (10^6/µL)	4.8-7.87	5.73±0.23	5.16±0.243	5.89±0.11	5.17±0.14
2	Haemoglobin (g%)	11.9-18.9	11.23±0.08	10.23±0.162	13.2±0.24	11.35±0.3
3	PCV (%)	35-57	39.75±0.9	32.91±0.7	38±0.06	32±0.01
4	Total Leucocyte count (10^3/ µL)	4.0-14.1	14.32±1.39	18.6±2.43	18.87±0.83	23.27±0.82
5	MCHC [g/dl]	32-36.3	35.95±0.44	33.2±0.44	34.98±0.72	33.03±0.32
6	Mean cell volume (MCV) [Fl]	66-77	66.95±0.68	62.35±0.81	70.07±0.94	64.4±1.05

Obtained haematological values from small and large breeds were presented in Table 1 the values for haematological comparison of study groups were determined as statistically significant. the values were observed as low in late gestation group when compared to mid gestation group. except for Total Leucocyte count (10^3/ gL) which is increased above their reference range from mid-gestation to late gestation in small and large breeds.

In the present study, Total erythrocyte count, Haemoglobin, PCV. MCHC. MCV values are decreased from mid gestation to late gestation, on comparison with normal reference ranges in both small and large breeds.

In haematology, Total erythrocyte count (106/gL) is normal at mid-gestation and decreased during late gestation in both small breeds and large breeds. The erythrocytic count may depends up on other related parameters such as Hb,, MCHC and MC V, PCV as in relationship. In pregnant bitches. RBC values were decreased and so there is an impact of decrease in value of PCV and haemoglobin (Concannon and Lien, 1989) [6]. Erythrocyte production increases during pregnancy while erythrocyte mass per unit of body weight remains constant throughout the entire pregnancy and haemoglobin and haematocrit progressively decrease into the third trimester (Lund and Donovan 1967: Peck and Arias. 1979; Heilmann, 1987) [15, 20, 12].

Haemoglobin (g%) is maintained normally at mid-gestation and decreased during late gestation in both small breeds and large breeds below the reference range. The 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) [4]. Physiological anaemia is solely due to a dilution decrease in haemoglobin concentration and relatively increased plasma volume of about 50% and red cell mass of about 18 to 25%, which was very much consistent with our findings. This is regarded as physiological anaemia observed during the pregnancy. The decrease in the value of haemoglobin during pregnancy in this study agrees with the reports by (Fisher and Fisher, 1981) [10]; Concannon and Lien (1989) [6]; Mshelia *et al*. (2005) [18] In which they stated that during pregnancy, there is a decrease in haemoglobin levels.

PCV (%) is maintained normally at mid-gestation and decreased below the reference range during late gestation in both small breeds and large breeds. In pregnancy, there is a low PCV. there is a reduced PCV from first to 3rd trimester. Moreover, PCV decreases in pregnant bitches due to a shorter life span of erythrocyte and hem dilatation (McFee, 1973) [16]. This observation agrees with the result from earlier works done by (Concannon. 1986; Concannon and Hon. 2002; Mshelia *et al*. 2005) [3, 5, 18]. Concannon *et al*. (1989) [6] stated that the PCV below 40% was seen 31 days after fertile mating while PCV below 35% was seen 53 days after fertile mating. This agrees with the report or (Concannon, 2002) [5] that there is a progressive. anaemia starting between clay 25 and 30 or pregnancy which becomes maximal near term and recovers over an 8 to 12 weeks period post-partum

3.2 Serum Biociiemistry

Table 2: Biochemical parameters bitches

S. No.	Parameter	Reference Range	Small breed bitches		Large breed bitches	
			Mid-gestation	late-gestation	mid -gestation	Late-gestation
1.	ALT (U/L)	10-100	47.37±7.07	40.4±3.59	26.23±3.17	22.95±2.68
2.	AST (U/L)	10-109	41.31±4.91	34.4±7.97	38.01±3.38	35.55±4.05
3.	Serum Albumin (g/L)	2.7 - 4.4	3.1 ±0.08	3.07±0.12	3.15±0.29	2.75±0.21
4.	Serum Globulin (g/L)	5.7 - 8.9	6.76+0.18	6.06±0.19	6.73±0.23	6.7±0. 17
5.	Total Protein (g/L)	5.0-7.4	6.73±0.22	6.16±0.13	6.57±0.31	6.45±0.2
6.	Serum Creatinine (mg/dL)	0.5 – 1.6	1.033±0.33	1.833±0.28	1.08±0.31	1.46±0.27
7.	Blood Urea Nitrogen (mg/dL)	6-25	11.16±0.72	17.39 ± 2.49	19.48±3.04	24.72±3.89

Obtained serum biochemical values from small and large breeds were presented in table 2. The values for serum biochemical value comparison of study groups were determined as statistically significant. the values of (ALT, AST, Serum Albumin, Serum Globulin, Total Protein were observed as low in late gestation group when compared to mid-gestation group. but Serum Creatinine and Blood Urea Nitrogen values increased.

As In the present study serum biochemical parameters includes Liver function tests such as ALT. AST, albumin and TP are said to be decreased from mid to late trimester of pregnancy and kidney function tests such as BUN and creatinine is said to be increased from mid to late trimester of' pregnancy. All these values are in accordance with Tohru kimura *et al*, (2018) [14].

4. Conclusion

It was assessed from the results that haematological and serum biochemical parameters were found to be significantly lower in mid-gestation when compared to late-gestation except for total leucocyte count, serum creatinine and blood urea nitrogen which increased in late gestation. Therefore, it is thought that monitoring of haematological and serum biochemical parameters would be beneficial for maintaining healthy pregnancy. So, if there are any adverse changes in these values required care and treatment has to be provided for maintenance of healthy pregnancy.

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