Perinatal veterinary medicine-related evaluation in hematological and serum biochemical profiles of experimental beagles throughout pregnancy and parturition

Tohru Kimura¹ | Kazuhiko Kotani²

¹Laboratory Animal Science, Joint Faculty of Veterinary Medicine, Yamaguchi University, Yoshida, Yamaguchi, Japan
²Division of Community and Family Medicine, Center for Community Medicine, Jichi Medical University, Yakushiji, Shimotsuke, Tochigi, Japan

Correspondence
Tohru Kimura, Laboratory Animal Science, Joint Faculty of Veterinary Medicine, Yamaguchi University, Yoshida, Yamaguchi, Japan.
Email: kimura1@yamaguchi-u.ac.jp

Abstract

Background: The aims of this study were (a) to ascertain age-related changes in the reference values in hematological 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.

Methods: Clinicopathological parameters were measured in young beagles at 6, 9 and 12 months and in adult beagles aged from 24 to 60 months. Likewise, pregnant beagles were investigated throughout the pregnancy and after parturition.

Results: Apparent age-related changes were found in erythrocytic parameters during the growth and development of beagles. Most of the parameters (total protein, albumin, blood urea nitrogen, creatinine, urate, alkaline phosphatase (ALP) and creatine kinase (CK)) exhibited age-dependent transitions. White cell count significantly increased after 30 days of pregnancy. The values of erythrocytic parameters moderately decreased during the second half of the pregnancy. Triglycerides, total cholesterol, free cholesterol and phospholipid concentrations increased in the mid- and late stages of pregnancy. ALP, lactate dehydrogenase, CK and cholinesterase activities markedly increased during pregnancy and/or after parturition. C-reactive protein (CRP) concentrations gradually increased and reached a maximum after 30-40 days of pregnancy. Serum amyloid A (SAA) levels markedly increased at 30 days of pregnancy before subsiding, and then increased again 3 days after parturition. Reactive oxygen metabolites (d-ROMs) showed significant increases after 30 and 40 days of pregnancy.

Conclusions: Reference values for hematological and serum biochemical examinations should be used for health evaluation of dogs, taking sex, age and the stage of pregnancy into consideration. Measurements of CRP, SAA and d-ROM levels are also useful for assessing maternal conditions in mid-pregnancy.

KEYWORDS
beagles, hematology, parturition, pregnancy, reactive oxygen metabolites, serum biochemistry
**1 | INTRODUCTION**

Complete follow-up clinicopathological data by sex and age group are not available for experimental dogs (beagles) in laboratory animal medicine. Although routine estimation of inflammatory and stress markers has become widespread in clinical veterinary medicine, there are very few reports on multiphasic reference values including newly developed parameters. It is essential to formulate these criteria for proper diagnosis based on daily hematological and serum biochemical examinations.

There are several past reports in the literature on reference values of hematological and serum biochemical examinations in experimental beagles. The reference values in these studies are taken from previously published experimental data and the parameters and measuring methods are considerably different from those carried out in the current studies. Relatively little information is available on clinicopathological profile changes during pregnancy and after parturition in dams, considering the influence of pregnancy on their life cycle. The availability of such limited data for standard clinicopathological evaluation is a very significant problem in perinatal veterinary medicine and laboratory animal medicine.

Newer in-house veterinary analyzers have made it possible to run complete hematological and serum biochemical panels with small volumes of blood. In order to correctly interpret clinicopathological data for each stage of the growth and life cycle of beagles, it is necessary to establish baseline profiles (reference values) of current laboratory test parameters measured by the new reliable methods.

The acute phase response, namely increases in acute phase proteins, plays an important role in the innate defense system of animals against infection, inflammation and tissue damage. Previous investigations in pregnant bitches of associations between pregnancy and acute phase proteins have been inconclusive, based on the analysis of C-reactive proteins (CRP), serum amyloid A (SAA) proteins, hepatoglobulins, ceruloplasmins and fibrinogens. Although pregnancy is characterized by naturally occurring physical changes, this condition is known to induce occasional critical disorders such as pregnancy-induced hypertension and gestational diabetes mellitus in human beings. The changes in oxidative stress during the pregnancy period remain to be elucidated in animals and human beings. Accurate measurement of acute phase proteins and oxidative stress markers is important for investigating pathophysiological changes in pregnant carnivorous beagles. It is expected that both these parameters could be potentially useful in evaluating a crisis during an unusual pregnancy.

In this study, the hematological and serum biochemical profiles of experimental beagles reared in a clean environment were examined using current laboratory methods. The purpose of this study was twofold. Experiment 1 aimed to ascertain reference values for hematological and serum biochemical examinations of beagles, incorporating effects of sex and age in the profiles. Experiment 2 aimed to clarify the changes in hematological and serum biochemical profiles, compared with data obtained from Experiment 1, including acute phase proteins and oxidative stress, throughout pregnancy and after parturition.

**2 | MATERIALS AND METHODS**

**2.1 | Experiment 1**

**2.1.1 | Animals**

Fifteen healthy male 6-month-old beagles and 15 healthy aged-matched female beagles were used in this study. Fifteen healthy male and fifteen female beagles ranging from 24 to 60 months were also used as an advanced age group. Animals were housed in pen house cages (W100 x D210 x H250 cm) in animal rooms at the Breeding Division of Hongo Beagle Farm, Kitayama Labes Co., Ltd (Yamaguchi, Japan). The animal rooms were controlled at 23 ± 2°C and 55 ± 10% relative humidity, with 12-15 exchanges of 100% fresh air per hour and a 12 hours light/dark cycle (changing at 6.00 AM/6.00 PM). The dogs were fed a commercial dry dog food (DS-E, Oriental Yeast Co., Ltd, Tokyo, Japan) 200 g/dog per day and water ad libitum.

**2.1.2 | Blood sample collection**

Before feeding, blood samples were collected from the cephalic vein of each animal using no anticoagulant. It took the whole morning to finish drawing canine blood. Twenty minutes after collection of blood samples, sera were separated by centrifugation at 1500 g for 10 minutes for biochemical analysis. For hematological samples, blood was collected into tubes containing K₂EDTA. In young dogs, blood samples were drawn at 6, 9, and 12 months of age. Beagles ranging from 24 to 60 months provided advanced age samples.

**2.1.3 | Hematology**

The following parameters were examined using an automated cell counter (Microsemi LC-662 Horiba Co. Ltd, Kyoto, Japan): red blood cell count (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), white cell count (WBC), platelet count (PLT), platelet distribution width (PDW) and mean platelet volume (MPV).

**2.1.4 | Serum biochemistry**

The following parameters were measured using a blood chemistry analyzer (Dry Chem NX 500V: Fuji Film Co. Ltd, Tokyo, Japan): total protein (TP), albumin (Alb), albumin/globulin (A/G) ratio, total bilirubin (T-Bil), urate (UA), blood urea nitrogen (BUN), creatinine (Cre), glucose (Glu), triglycerides (TG), total cholesterol (T-CHO), asparate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), cholineserase (ChE), leucine aminopeptidase (LAP), creatine kinase (CK), γ-glutamyl
transpeptidase (γ-GT, GGT), amylase (AMS), electrolytes (Na, K, Cl, Ca) and inorganic phosphorus (IP).

High density lipoprotein-cholesterol (HDL-CHO), free cholesterol (F-CHO), phospholipids (PL) and free fatty acids (FFA; NEFA, non-esterified fatty acids) concentrations were measured using the autochemistry analyzer method (HITACHI 7170S, Hitachi High-technologies Co., Ltd, Tokyo, Japan).

The acute phase proteins serum amyloid A (SAA) and C-reactive proteins (CRP) were evaluated to establish age-related characteristics. Serum CRP concentrations were measured by sandwich enzyme-linked immunosorbent assay and the aforementioned blood chemistry analyzer method. Serum concentrations of SAA were determined using a latex agglutination turbidimetric immunoassay (a trial reagent; Eiken Chemical Co., Ltd, Tokyo, Japan).

Reactive oxygen metabolites (d-ROMs) were assayed for the degree of oxidative stress with a Free Radical Elective Evaluator (FREE., Wismerll Co. Ltd, Tokyo, Japan).

### 2.1.5 Statistical evaluation

Values were expressed as the means ± standard deviation (SD) and statistical analysis was performed using one-way repeated measures analysis of variance (ANOVA), and a multiple comparison test for parametric data (Dunnett’s method) and for non-parametric data (Steel’s method). A comparison review of the aforementioned parameters in beagles at 6, 9 and 12 months and the initial age group (6-month-old dogs) was carried out and statistical significant was established at \( P < 0.05 \) or \( P < 0.01 \).

### 2.2 Experiment 2

#### 2.2.1 Animals

Fifteen healthy pregnant beagles ranging in age from 2 to 5 years were used to assess physiological changes in hematological and serum biochemical profiles throughout pregnancy and the post-parturition period. These pregnant beagles were also used to determine the properties of acute phase proteins (SAA and CRP).

The animals used in this study were kept under the same conditions as described in Experiment 1.

#### 2.2.2 Blood sample collection

Test procedures were performed in the same way as in Experiment 1. Blood samples and sera were collected from the pregnant beagles at 0, 10, 20, 30, 40, 50, and 60 days during pregnancy, and 3 and 7 days after parturition.

#### 2.2.3 Hematology

Hematological examinations were performed as described above. Hematological measurements were carried out 0, 30, and 60 days after pregnancy.

### 2.2.4 Serum biochemistry

Serum biochemical parameters were performed as described above and we examined changes in these parameters during the pregnancy. Serum biochemical measurements were carried out every 10 days throughout the pregnancy period and 3 and 7 days after parturition. Acute phase proteins (SAA and CRP) and d-ROMs were evaluated to clarify gestational stage-related characteristics. In addition, ALP isoenzymes were examined by measurement of the individual fractions following electrophoresis (polyacrylamide gel disc electrophoresis). ALP zymodeme was detected using several fractions derived from lever, bone, placenta and small intestine.

### 2.2.5 Statistical evaluation

Values were expressed as the means ± standard deviation (SD) and statistical analysis was performed using one-way repeated measures ANOVA, and multiple comparison tests for parametric data (Dunnett’s method) and for non-parametric data (Steel’s method). A comparison review of the aforementioned parameters in pregnant beagles (at 10, 20, 30, 40, 50, and 60 days of pregnancy and 3 and 7 days after parturition) and non-pregnant beagles (beagles at 0 day after pregnancy) was carried out. Statistical significance was established at \( P < 0.05 \) or \( P < 0.01 \).

In Experiments 1 and 2, all procedures involving animals were approved by the Institutional Animal Care and Use Committee of Yamaguchi University and followed the Guidelines of Animal Care and Experiments of Yamaguchi University. The animal care and use program for Advanced Research Center for Laboratory Animal Science in Yamaguchi University has been accredited by AAALAC International since 2018.

### 3 RESULTS

#### 3.1 Experiment 1

Hematological results for all beagles are shown in Table 1. There were no significant differences in hematological values between sexes for the experimental beagles during the course of their growth to 60 months old. In contrast, apparent age-related changes were found in erythrocytic parameters during the growth and development of the dogs. Three variables (RBC, Hb, and PCV) gradually increased in parallel with an increase in age and statistical differences were noted in 12-month-old and 24- to 60-month-old dogs compared with those in 6-month-old dogs (\( P < 0.01 \)). WBCs remained unaffected until 12 months old, and then slightly decreased at 24-60 months old. The slight downward tendency of PLTs, PDW, and MPV in male dogs was correlated with increasing age.

Serum biochemical results for all beagles are shown in Table 2. There were no significant sex differences during the follow-up study period, but most of the parameters exhibited age-dependent transitions. Although Alb concentrations remained unchanged in this study, TP concentrations gradually increased, resulting in an elevated...
A/G ratio. The age-related increases in TP concentration and A/G ratio were statistically significant during the growth phase of the beagles ($P < 0.01$). The increases in BUN, Cre and UA levels were also significant, similar to those in TP concentrations, during the dogs' growth period ($P < 0.05$ or $P < 0.01$). There were no significant changes in the lipometabolic profiles of the beagles.

Regarding the enzyme activity measurements, there were significant age-related decreases in ALP and CK compared with activity in the 6-month-old beagles ($P < 0.01$). The other enzyme activities remained stable over the course of this study of canine growth.

In the case of serum electrolytes, Na, K, and Cl levels were largely unaffected by the growth process during the study period, but both Ca and IP levels slowly declined with an increase in age.

Under the healthy conditions of the dogs in this study, the levels of acute phase proteins (CRP and SAA) and d-ROM showed no marked changes associated with maturation and aging.

### 3.2 | Experiment 2

Pregnant beagles were normally delivered of healthy puppies and litter size was 5.4 ± 1.3. The hematological results for the pregnant beagles are shown in Figure 1. There were few changes in hematological profiles from early to mid-pregnancy (first 30 days of pregnancy). In late pregnancy (after 50 days of pregnancy), WBCs significantly increased to approximately two times the level before pregnancy and at 30 days of pregnancy ($P < 0.01$). The values of erythrocytic parameters (RBCs, Hb concentrations, and PCV ratio) moderately decreased compared with those during the first half of the pregnancy period ($P < 0.01$). The number of PLTs was significantly elevated in late pregnancy ($P < 0.01$).

Serum biochemical results for the pregnant beagles are shown in Figures 2, 3 and 4. TP concentrations declined markedly by about 10 g/L in late pregnancy and the levels remained lower up to 7 days after parturition ($P < 0.01$). Alb concentrations gradually decreased from 33.7 ± 3.5 g/L to 24.2 ± 3.4 g/L throughout the pregnancy and the low concentrations were subsequently maintained after parturition. Significant decreases in Alb concentrations were observed from 30 days of pregnancy ($P < 0.05$ or $P < 0.01$). The A/G ratio also decreased gradually in conjunction with the changes in the TP and Alb parameters ($P < 0.05$ or $P < 0.01$, respectively). Three and 7 days after parturition, BUN levels were mildly elevated by a mean of 2-3 mmol/L, whereas Cre levels gradually decreased during the study.
**TABLE 2** Serum biochemical profiles in healthy beagles (means ± SD)

| Parameters | Units | Sex   | Age   |       |       |       |       |
|------------|-------|-------|-------|-------|-------|-------|-------|
|            |       |       | 6 M   | 9 M   | 12 M  | 24-60 M |       |
| TP         | g/L   | Male  | 54.6 ± 2.0 | 60.7 ± 3.6** | 65.1 ± 4.3** | 71.5 ± 4.0** |
|            |       | Female| 54.7 ± 2.8 | 58.9 ± 3.3** | 64.8 ± 4.5** | 65.1 ± 3.6** |
| Alb        | g/L   | Male  | 32.7 ± 1.2 | 33.1 ± 1.6 | 33.3 ± 1.6 | 32.9 ± 2.0 |
|            |       | Female| 33.7 ± 1.7 | 34.9 ± 2.3 | 34.6 ± 2.3 | 34.7 ± 1.8 |
| A/G ratio  |       | Male  | 1.50 ± 0.11 | 1.21 ± 0.18** | 1.07 ± 0.16** | 0.87 ± 0.15** |
|            |       | Female| 1.61 ± 0.15 | 1.48 ± 0.22** | 1.2 ± 0.28** | 1.17 ± 0.22** |
| T-Bil      | μmol/L| Male  | 0.55 ± 0.20 | 0.76 ± 0.29 | 0.68 ± 0.25 | 0.82 ± 0.29 |
|            |       | Female| 0.60 ± 0.24 | 0.98 ± 0.21 | 1.11 ± 0.37 | 0.78 ± 0.24 |
| BUN        | mmol/L| Male  | 4.4 ± 1.1 | 5.0 ± 0.7* | 5.3 ± 0.7** | 5.1 ± 0.9* |
|            |       | Female| 4.4 ± 0.7 | 5.0 ± 0.7* | 5.5 ± 1.0** | 5.0 ± 0.8* |
| UA         | μmol/L| Male  | 13.9 ± 2.9 | 16.3 ± 2.7** | 20.6 ± 4.4** | 20.6 ± 3.1** |
|            |       | Female| 14.5 ± 2.7 | 17.5 ± 1.5** | 20.2 ± 3.0** | 15.5 ± 3.8** |
| Cre        | μmol/L| Male  | 49.8 ± 5.5 | 54.9 ± 5.8** | 58.5 ± 7.8** | 57.9 ± 6.8** |
|            |       | Female| 48.6 ± 7.2 | 56.6 ± 6.2** | 55.7 ± 5.9** | 54.6 ± 9.1** |
| Glu        | mmol/L| Male  | 5.27 ± 0.27 | 5.11 ± 0.22 | 4.91 ± 0.34 | 4.82 ± 0.25 |
|            |       | Female| 5.62 ± 0.29 | 5.19 ± 0.20 | 4.69 ± 0.28 | 4.94 ± 0.28 |
| TG         | mmol/L| Male  | 0.31 ± 0.07 | 0.30 ± 0.12 | 0.21 ± 0.07 | 0.25 ± 0.07 |
|            |       | Female| 0.30 ± 0.09 | 0.25 ± 0.08 | 0.27 ± 0.10 | 0.34 ± 0.12 |
| T-CHO      | mmol/L| Male  | 4.40 ± 0.66 | 4.53 ± 0.78 | 3.70 ± 0.69 | 3.27 ± 0.75 |
|            |       | Female| 4.20 ± 0.45 | 3.85 ± 0.75 | 4.26 ± 0.90 | 4.55 ± 1.06 |
| HDL-CHO    | mmol/L| Male  | 3.22 ± 0.46 | 3.40 ± 0.58 | 2.92 ± 0.52 | 2.60 ± 0.54 |
|            |       | Female| 3.14 ± 0.29 | 3.00 ± 0.54 | 3.30 ± 0.63 | 3.43 ± 0.64 |
| F-CHO      | mmol/L| Male  | 1.20 ± 0.18 | 1.19 ± 0.20 | 0.95 ± 0.17 | 0.84 ± 0.20 |
|            |       | Female| 1.14 ± 0.13 | 1.00 ± 0.20 | 1.06 ± 0.21 | 1.17 ± 0.26 |
| NEFA       | mmol/L| Male  | 0.66 ± 0.22 | 0.65 ± 0.20 | 0.70 ± 0.15 | 0.54 ± 0.19 |
|            |       | Female| 0.66 ± 0.12 | 0.65 ± 0.20 | 0.70 ± 0.15 | 0.54 ± 0.19 |
| PL         | mmol/L| Male  | 4.09 ± 1.13 | 4.32 ± 0.56 | 3.78 ± 0.52 | 3.38 ± 0.61 |
|            |       | Female| 4.18 ± 0.39 | 3.86 ± 0.64 | 4.17 ± 0.66 | 4.37 ± 0.76 |
| AST        | U/L   | Male  | 44.1 ± 25.4 | 30.1 ± 4.3 | 34.5 ± 7.6 | 40.1 ± 7.9 |
|            |       | Female| 35.1 ± 3.7 | 32.9 ± 3.3 | 36.4 ± 5.8 | 28.2 ± 1.9 |
| ALT        | U/L   | Male  | 40.0 ± 10.4 | 36.4 ± 9.0 | 41.1 ± 11.4 | 64.9 ± 64.0 |
|            |       | Female| 42.1 ± 9.8 | 38.3 ± 8.9 | 35.4 ± 7.0 | 35.5 ± 11.3 |
| ALP        | U/L   | Male  | 490.7 ± 111.1 | 301.3 ± 76.1** | 201.5 ± 53.2** | 188.2 ± 100.8** |
|            |       | Female| 464.9 ± 84.3 | 285.3 ± 42.9** | 221.5 ± 43.2** | 84.1 ± 103.6** |
| GGT        | U/L   | Male  | 4.8 ± 0.9 | 4.3 ± 1.0 | 4.3 ± 1.0 | 6.1 ± 1.9 |
|            |       | Female| 4.5 ± 0.6 | 4.4 ± 0.6 | 4.2 ± 0.9 | 4.9 ± 1.4 |
| LDH        | U/L   | Male  | 97.9 ± 33.4 | 122.4 ± 58.0 | 111.5 ± 41.5 | 113.8 ± 70.3 |
|            |       | Female| 80.5 ± 19.5 | 102.8 ± 28.9 | 136.7 ± 72.7 | 114.5 ± 96.4 |
| ChE        | U/L   | Male  | 9.1 ± 1.7 | 8.9 ± 1.9 | 8.7 ± 3.0 | 8.5 ± 1.9 |
|            |       | Female| 9.9 ± 1.7 | 7.6 ± 1.5 | 9.3 ± 2.1 | 8.5 ± 3.0 |
| LAP        | U/L   | Male  | 59.7 ± 10.9 | 49.6 ± 9.6 | 50.3 ± 11.5 | 39.7 ± 10.7 |
|            |       | Female| 59.3 ± 11.2 | 48.7 ± 11.4 | 52.1 ± 14.3 | 43.7 ± 24.6 |
| AMY        | U/L   | Male  | 807.1 ± 158.5 | 879.5 ± 228.2 | 1024.5 ± 208.8 | 943.4 ± 230.5 |
|            |       | Female| 764.0 ± 148.1 | 776.8 ± 141.9 | 826.5 ± 162.1 | 790.7 ± 353.3 |

(Continues)
Pregnancy had little effect on Glu concentrations in this study. Regarding lipid metabolism, TG concentrations gradually increased during the pregnancy and there were significant increases at 40, 50, and 60 days of pregnancy (P < 0.01). Subsequently, TG concentrations returned to their baselines after parturition. Significant increases in T-CHO concentrations were found at 30, 40, and 50 days of pregnancy (P < 0.01), followed by significant decreases after parturition (P < 0.01). In contrast, SAA levels increased significantly after 30 days of pregnancy (P < 0.01) and subsequently fell below the initial values. NEFA concentrations decreased after 30 days of pregnancy and declined again after parturition.

Regarding enzyme activities, AST levels increased only at 7 days after parturition, while ALT activities declined moderately at and after the 30th day of pregnancy but were elevated again 7 days after parturition. ALP activities increased markedly by the 30th day after pregnancy and remained elevated during the pregnancy and after parturition (P < 0.01). In particular, ALP activities at 30 and 40 days of pregnancy were more than twice as high as the levels before pregnancy. LDH levels after 50 and 60 days of pregnancy increased 2-3 times over the initial levels (P < 0.01). CK activities began to increase from 50 days of pregnancy and the changes were even more pronounced after parturition (P < 0.01). ChE activities were also significantly elevated in the postpartum period (P < 0.01). ALP zymograms showed that ALP activity in pregnant beagles consisted almost entirely of hepatic ALP isozymes (>94.7%). The levels of serum electrolytes (Na, K, Cl, Ca, electrolytes; NEFA, non-esterified fatty acids; PL, phospholipids; SAA, serum amyloid A; T-Bil, total bilirubin; T-CHO, total cholesterol; TG, triglycerides; TP, total protein; UA, urate).

**Significantly different from 6 months old (6 M) (P < 0.05).**

**Significantly different from 6 M (P < 0.01).**
obtained from adult dogs. According to several reports, erythrocytic parameters in beagles start to increase from about the first 2 months of life, generally reaching adult levels by approximately 6 months to 1 year of age. One report asserts that beagles become mature at about 1 year of age and adulthood continues until about 5 years of age, at which time clinical signs of aging are manifested. Our study demonstrated that, while there were no sex differences in the hematological profiles, moderate increases were found in these measurements of beagles throughout the course of their growth to 60 months of age. Compared with previous reference ranges of WBCs ($7 \times 10^9 / L$), experimental beagles in our controlled environment showed limited variation in leukocyte levels. There were slight differences in platelet parameters between sexes and slight changes with advancing age.

In the literature reporting serum biochemical variables in puppies, TP, Alb, Cre, Na, and Cl values in puppies were lower than those in adult dogs. In addition, these reports stated that higher values in puppies compared with adults were found for IP, K, BUN, ALP, and CK levels. A previous work has shown that serum chemistry values obtained from beagles (4, 8 and 12 months of age) varied as follows: increases were recorded for TP, BUN, AST and AMS, decreases for Alb, UA, Glu, IP and ALT, and no changes for T-CHO, T-Bil and Cl. Another investigator has reported that no significant differences with sex and age were observed in hematological and serum biochemical measurements. Harper et al showed that there were significant effects of age on hematological and plasma biochemical profiles in beagles aged from 22 days to 15 years old. Recent studies reported that markedly significant age-related

**FIGURE 1** Hematological changes (means ± SD) during pregnancy in beagles. RBC, red blood cell count; Hb, hemoglobin concentration; PCV, packed cell volume; WBC, white cell count; PLT, platelet count. Marked changes in hematological parameters are found after 50 d of pregnancy. *Significantly different from Day 0 ($P < 0.05$). **Significantly different from Day 0 ($P < 0.01$).
changes in hematological and serum biochemical variables were noted in neonatal and geriatric dogs.\textsuperscript{12,14,20,22}

The present study demonstrated that while no significant sex differences were found in the serum biochemical profiles of our dogs, there were significant differences with an advancing age, with increases in TP, A/G, BUN, UA, and Cre, and decreases in ALP and CK in beagles ranging from 6 to 60 months old. Our results demonstrated that the hematological and serum biochemical profiles in puppies were different from those in adolescence, showing the onset of puberty at 6 months of age. These increased parameters were attributed to developmental changes, such as changes in plasma volume, hepatic or renal functions, and protein synthesis as a result of the growth process. The increased TP concentrations were composed of higher globulin concentrations along with unchanged Alb concentrations. These findings have been previously described in older dogs\textsuperscript{20} and were due to elevated IgA concentrations. Previous studies reported that increased urea concentrations were present in geriatric dogs.\textsuperscript{20,21} The increasing trend in BUN levels over the course of our series of measurements agrees with these previous results. However, our results showing increasing UA values differ from those reported in a previous investigation using beagles.\textsuperscript{1} In human beings, UA levels tend to rise with an increase in age,\textsuperscript{23} and this description accorded with our results obtained from experimental beagles in a healthy environment. The changes in Cre concentration can be explained by a lower muscle mass to body size ratio in young animals.

Considering previous reports,\textsuperscript{13-15,24-26} it was likely that the increased ALP activities seen in our 6-month-old beagles were related to their growing bone tissues. Several studies\textsuperscript{13,25} have stated that serum CK activities were elevated in puppies compared with adult dogs and that CK activities in pups less than 6 months old were double those of adult dogs. Similar age-related changes in CK activities were found in our study. It is likely that these age-related changes in CK values were associated with muscle enhancing processes during the growing period.

The concentrations of the acute phase proteins (CRP and SAA) did not differ between males and females and their low levels remained stable in the course of this study on canine growth stages. Acute phase protein concentrations increase as a result of inflammatory and infectious processes. Because CRP and SAA levels do not vary widely in healthy beagles, these parameters are useful for detecting slight changes in body conditions. It has been reported in tests on healthy dogs that mean d-ROMs levels are 73.9 ± 8.73 U. CARR, ranging from 56.4 to 91.4 U. CARR.\textsuperscript{27} The present d-ROMs data accords with the abovementioned report. The reference range in dogs is less than one-third of that detected in human beings (250-300 U. CARR). This difference between species can be attributed to the ability of dogs, unlike humans, to produce ascorbic acids in the body.

A previous study reported that some changes in the values of TP, BUN, T-CHO, Cre, ChE, and ALP were seen during the estrous cycle.\textsuperscript{7} Our study showed no apparent changes in these parameters during the estrous cycle, and the significance of the estrous cycle cannot be discerned using the present hematological and serum biochemical examinations.

In our hematological and serum biochemical profiles, there were few differences between the dams at day 0 of pregnancy in Experiment 2 and age-matched female beagles in Experiment 1. These 2- to 5-year-old female beagles were often used as reproductive dams in the breeding facilities. In previous studies,\textsuperscript{1,5,7,8,16,28} the erythrocytic elements of the profiles (RBCs, PCV, and Hb concentrations) have been shown to decrease throughout gestation, reaching a low point at term, with a gradual rise thereafter. The reports noted that increases in WBCs corresponded to these changes in erythrocytic elements during gestation. During lactation, erythrocyte and leukocyte parameters returned to reference ranges.

Our results showed that hematological changes in healthy pregnant beagles were significant in the late stage of pregnancy. In addition, MCV, MCH, and MCHC remained unaffected during the pregnancy period, whereas the number and size of platelets were influenced by the pregnant state. Allard et al\textsuperscript{28} reported that the age of the dams did not significantly correlate with the hematological profiles at any gestation phase. Our findings from the dogs used in this study revealed that the age of the female beagles (2-5 years old) and litter size had no direct influence on hematological profiles or serum biochemical profiles.

The decreases in TP and Alb concentrations and the A/G ratio during pregnancy reported here agree with previous studies.\textsuperscript{7,8,29} These changes resulted from the increase in circulatory blood (serum) volumes with advancing pregnancy. The levels of BUN, Cre, and UA were concomitantly affected by the changes in protein metabolism.

Serum lipids consist mainly of four substances (CHO, TG, PL and NEFA). Several investigators have shown that dogs develop increased levels of T-CHO or TG during pregnancy.\textsuperscript{7,19,29} A previous study reported that during gestation, plasma T-CHO concentrations were depressed early on, and then increased in the later stages under the influence of diets given.\textsuperscript{30} Our measurements demonstrated that T-CHO, TG, F-CHO, NEFA, and PL gradually increased in the late pregnant beagles. It is likely that the changes in these lipid metabolism parameters are associated with changing levels of estrogen with advancing pregnancy. The results show that it is useful to examine lipid metabolism parameters for correct diagnosis of the normal progression of pregnancy. In our previous study, we reported that dogs could very efficiently use lipid metabolism components for energy requirements during fasting.\textsuperscript{31} In this study, we stated that ketogenesis was enhanced by pregnancy, and thus late pregnancy in dogs was not accompanied by changes in the absolute rates of gluconeogenesis or glycogenolysis.\textsuperscript{32} The present study suggests that healthy pregnant dogs should be capable of activating adipokinesis without severe changes in blood glucose concentrations. In pregnant beagles, lipid metabolism components are available as a maternal energy resource.

Relatively little is known about the role of glycoprotein ChE produced in the liver of pregnant beagles. A previous study reported
FIGURE 2  Serum biochemical changes (means ± SD) during pregnancy and after parturition of beagles. TP, total protein; Alb, albumin; A/G, albumin/globulin ratio; BUN, blood urea nitrogen; UA, urate; Cre, creatinine; Glu, glucose; TG, triglycerides; T-CHO, total cholesterol; HDL-CHO, high density lipoprotein-cholesterol. Marked decreases in protein metabolism (Alb and A/G ratio) are noted during pregnancy. Significant increases in lipid metabolism (TG and T-CHO) are observed in mid- and late stages of pregnancy. *Significantly different from Day 0 (P < 0.05). **Significantly different from Day 0 (P < 0.01). △, parturition
FIGURE 3  Serum biochemical changes (means ± SD) during pregnancy and after parturition of beagles. F-CHO, free cholesterol; NEFA, non-esterified fatty acids; PL, phospholipids; ALP, alkaline phosphatase; GGT, γ-glutamyl transpeptidase; LDH, lactate dehydrogenase; ChE: cholinesterase; CK, creatine kinase. Marked increases in lipid metabolism (F-CHO and PL) are observed at 30-50 d of pregnancy. Enzyme activities (ALP, LDH, ChE, and CK) are also changed. *Significantly different from Day 0 ($P < 0.05$) **Significantly different from Day 0 ($P < 0.01$). $\triangle$, parturition
that serum ChE activities increased from the 10th day of pregnancy to parturition. The present results have shown that canine ChE activities are influenced under pregnant and parturient physiological conditions.

Although increases in the activity of AST, ALT, GGT, and LAP were generally noted through damage to livers, bile ducts or biliary tracts, these enzymes did not change significantly throughout this study. In contrast, the main elements in the increase in ALP activities were hepatic ALP isozymes from the middle stage of pregnancy. These results confirmed that the increase in ALP activity in pregnant beagles resulted from hepatic hematogenesis in canine fetuses and was different from ALP isozyme activity originating in placental functions in human beings. It is probable that in the late pregnant and postpartum periods, the increase in LDH activity in pregnant beagles was also associated with hepatic hematogenesis in fetuses. The elevation of CK activities just before or after parturition was caused by tissue damage to the birth canal.

It is known that the levels of serum electrolytes such as Ca and IP change during pregnancy and after parturition in dogs, with an increase in Ca and a decrease in IP. Our tests showed that serum electrolyte levels remained stationary throughout this study, providing evidence that the pregnant beagles were well-nourished and in good condition.

Acute phase proteins are used as non-specific makers of inflammation in companion and farm animal medicine. In addition to their role in inflammatory responses, acute phase proteins are reported to be released in normal physiological conditions such as pregnancy. An increase in canine CRP concentration reported in a recent study is not as high as that detected in our previous report. Our study showed that canine CRP concentrations widely and markedly increased during the second half of pregnancy and after parturition. In contrast, canine SAA values were only elevated at 30 days of pregnancy, showing good agreement with the onset of embryonic implantation. SAA concentrations subsequently increased again with the initiation of parturition, with this change being attributable to inflammation of the injured birth canal. The present results obtained from experimental dogs maintained under hygienic controlled circumstances provide clear evidence that rises in CRP and SAA concentrations did not resulted from pregnancy disorders. Although neither CRP nor SAA are affected by pregnancy in human beings, the levels of both parameters were significantly influenced during pregnancy in the beagles. These species differences are highly relevant to the histological structure of the placentas, because the endotheliochorial placenta shows marked invasion of blood capillaries in the endometrium. Our results revealed that the canine species developed a remarkable pregnancy-associated inflammatory response.

In pregnant women, it is believed that oxidative stress fluctuates in the higher range during the pregnancy. Our study showed that canine d-ROMs values increased from 30 to 40 days of pregnancy. Because carnivorous placenta is a structure characterized by zonary placenta, syncytiotrophoblasts derived from villi invade endometrial tissue, resulting in severe inflammation and hemorrhage in maternal capillaries. It is probable that the significantly increased d-ROMs values coincided with hypoxic conditions during the period of embryonic implantation and placentation. Acute phase proteins play a role in the immune response and provide protection against oxidative stress generated in the course of an inflammatory response. The present correlations between CRP, SAA, and d-ROMs values at 30-40 days of pregnancy in our study support the view that CRP and SAA have some anti-oxidant effects. Our results reveal that measurements of CRP, SAA and d-ROMs levels are very useful for physiological diagnosis of normal pregnancy in beagles.
The present study revealed that age-related variations in hematological and serum biochemical profiles occurred during the first year (from puppy stage to adolescence), reflecting rapid growth and maturation of beagles. The interpretation of pregnant phase-related changes in these profiles makes it possible to diagnose accurately the condition of a pregnancy and provides important predictive information on parturition. Measurements of CRP, SAA, and d-ROMs levels are also useful to assess maternal conditions in mid-pregnancy in dogs. Reference values for hematological and serum biochemical examinations should be used in health evaluation of dogs, taking sex, age and the stage of pregnancy into account.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the researchers at Eiken Chemical Co., Ltd for their incredible technical and general support. In addition, we thank facilities staff in Kitayama Labes Co., Ltd. for their help with data collection.

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

Both listed authors meet the requirements for authorship. TK was in charge of the animal experiments and wrote the article. KK analyzed the results of the animal experiment. Both authors read and approved the final manuscript.

ORCID

Tohru Kimura http://orcid.org/0000-0002-2920-2840

REFERENCES

1. Andersen AC, Schalm OW. Hematology. In: Andersen AC, ed. The Beagle as an Experimental Dog. Iowa: The Iowa State University Press; 1970:261-281.
2. Bulgin MS, Munn SL, Gee W. Hematologic changes to 4 1/2 years of age in clinically normal beagles. J Am Vet Med Assoc. 1970;157:1064-1070.
3. Cramer MB, Turbyfill CL, Dewes WA. Serum chemistry values for the beagle. Am J Vet Res. 1969;30:1183-1186.
4. Earl FL, Melveger BE, Wilson RL. The hemogram and bone marrow profile of normal neonatal and weanling Beagle dogs. Lab Anim Sci. 1973;23:690-695.
5. Shifrine M, Munn SL, Rosenblatt LS, Bulgin MS, Wilson FD. Hematologic changes to 60 days of age in clinically normal beagles. Lab Anim Sci. 1973;23:894-898.
6. Whitaker J, Rogala AR, LeVine DN, Fletcher CA. The Laboratory Dogs. In: Kurtz DM, Travlos GS, eds. The Clinical Chemistry of Laboratory Animals, 3rd edn. Boca Raton: CRC Press; 2017:113-152.
7. Hayashi M. Composition of the blood of adult Beagle bitches during the estrous cycle, pregnancy and postpartum period. Jap J Anim Reprod. 1974:20:69-94.
8. Khan SA, Epstein JH, Olival KJ, et al. Hematology and serum chemistry reference values of stray dogs in Bangladesh. Open Vet J. 2011;1:13-20.
9. Eckensall PD, Bell R. Acute phase proteins: biomarkers of infection and inflammation in veterinary medicine. Vet J. 2010;185:23-27.
10. Ulutas PA, Musal B, Kiral F, Bildik A. Acute phase proteins levels in pregnancy and estrus cycle in bitches. Res Vet Sci. 2009;86:373-376.
11. Root Kustritz MV. Pregnancy diagnosis and abnormalities of pregnancy in the dog. Theriogenology. 2005;64:755-765.
12. O’Brien MA, McMichael MA, Boedec KL, Lees G. Reference intervals and age-related changes for venous biochemical, hematological, electrolytic, and blood gas variables using a point of care analyzer in 68 puppies. J Vet Emerg Crit Care (San Antonio). 2014;24:291-301.
13. Rosset E, Rannou B, Casseleux G, Chalvet-Monfray K, Buff S. Age-related changes in biochemical and hematologic variables in Borzoi and Beagle puppies from birth to 8 weeks. Vet Clin Pathol. 2012;41:272-282.
14. Rartveit R, Sævik BK, Eggertsdøttir AV, et al. Age-related changes in hematologic and serum biochemical variables in dogs aged 16-60 days. Vet Clin Pathol. 2015;44:47-57.
15. Harper EJ, Hackett RM, Wilkinson J, Heaton PR. Age-related variations in hematologic and plasma biochemical test results in Beagles and Labrador Retrievers. J Am Vet Med Assoc. 2003;223:1436-1442.
16. Rizzi TE, Meinkoth JH, Clinkenbeard KD. Normal Hematology of the Dog. In: Weiss DJ, Wardrop KJ, eds. Schalm’s Veterinary Hematology, 6th edn. Iowa: Wiley-Blackwell; 2010:799-810.
17. Mc Kelvie DH. Blood serum chemistry. In: Andersen AC, ed. The Beagle as an Experimental Dog. Iowa: The Iowa State University Press; 1970:281-284.
18. Eckersall PD. Proteins, proteinomics, and the dysproteinemias. In: Kaneko JJ, Harvey JW, Bruss ML, eds. Clinical Biochemistry of Domestic Animals, 6th edn. Burlington, MA: Academic Press; 2008:117-156.
19. Choi SY, Hwang JS, Kim IH, Hwang DY, Kang HG. Basic data on the hematometry reference values of stray dogs in Bangladesh. Int J Hematol. 2010;91:89-97.
20. Badakovich LB, Pannone SC, Truelove MP, Olver CS, Santangelo KS. Hematology and biochemical profile of healthy Labrador Retriever puppies. J Vet Emerg Crit Care (San Antonio). 2003;13:139-146.
21. Pati S, Panda SK, Acharya AP, Senapati S, Behera M, Behera SS. Evaluation of geriatric changes in dogs. Vet World. 2015;8:273-278.
22. Mundim AV, Coelho AO, Hortêncio SM, Guimarães EC, Espindola FS. Influence of age and sex on the serum biochemical profile of Doberman dogs in the growth phase. Comp Clin Patol. 2007;16:41-46.
23. Gephardt MC, Hanlon TJ, Matson CF. Blood uric acid values as related to sex and age. JAMA. 1964;189:1028-1029.
24. Pickrell JA, Schluter SJ, Belaisch JJ, et al. Relationship of age of normal dogs to blood serum constituents and reliability of measured single values. Am J Vet Res. 1974;35:897-903.
25. Aktas M, Auguste D, Concordet D, et al. Creatinine kinase in dog plasma: preanalytical factors of variation, reference values and diagnostic significance. Res Vet Sci. 1994;56:30-36.
26. Bush BM. White Blood Cells (WBCs). In: Bush BM, ed. Interpretation of Laboratory Results for Small Animal Clinicians. Oxford, UK: Blackwell Scientific Publications; 1991:132-195.
27. Pasquini A, Luchetti E, Marchetti V, Cardini G, Iorio EL. Analytical performances of d-ROMs test and BAP test in canine plasma. Definition of the normal range in healthy Labrador dogs. Vet Res Commun. 2008;32:137-143.
28. Allard RL, Carlos AD, Faltin EC. Canine hematological changes during gestation and lactation. Comp Anim Prac. 1989;19:3-6.
29. Evans WE. Proteins, Lipids, and Carbohydrates. In: Latimer KS, ed. Duncan and Prasse’s Veterinary Laboratory Medicine: Clinical Pathology, 5th edn. West Sussex: Wiley-Blackwell; 2011:173-209.
30. Wright-Rodgers AS, Waldron MK, Bigley KE, Lees GE, Bauer JE. Dietary fatty acids alter plasma lipids and lipoprotein distributions in dogs during gestation, lactation, and the perinatal period. J Nutr. 2005;135:2230-2235.
31. Kimura T. Effects of fasting therapy in obese dogs on body weight, and hematological, blood coagulation and serum biochemical values. West Indian Vet J. 2005;5:10-20.
32. Connolly CC, Holste LC, Aglione LN, et al. Alterations in basal glucose metabolism during late pregnancy in the conscious dog. Am J Physiol Endocrinol Metab. 2000;279:E1166-E1177.
33. Kuribayashi T, Shimada T, Matsumoto M, et al. Determination of serum C-reactive proteins (CRP) in healthy Beagle dogs of various ages and pregnant dogs. Exp Anim. 2003;52:387-390.
34. Vannucchi CI, Mirandola RM, Oliveira CM. Acute-phase proteins profile during gestation and diestrous: proposal for an early pregnancy test in bitches. Anim Reprod Sci. 2002;74:87-99.
35. Eckersall PD, Harvey MJ, Ferguson JM, Renton JP, Nickson DA, Boyd JS. Acute phase proteins in canine pregnancy (Canis familiaris). J Reprod Fertil Suppl. 1993;47:159-164.

How to cite this article: Kimura T, Kotani K. Perinatal veterinary medicine-related evaluation in hematological and serum biochemical profiles of experimental beagles throughout pregnancy and parturition. Animal Model Exp Med. 2018;1:282–294. https://doi.org/10.1002/ame2.12043