

Effect of pregnancy on some hematological and biochemical parameters in pregnant Chinese water deer (*Hydropotes inermis*)

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Abstract

The hematological and biochemical parameters of endangered wildlife during gestation can provide knowledge of normal physical conditions and recognition of every event, thus contributing to conservation and management. For better breeding management of a Chinese water deer (*Hydropotes inermis*) reintroduced population, a series of hematological parameters were tested and analyzed, viz. white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), packed-cell volume (PCV), platelet count (PLT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean red blood cell distribution width coefficient of variation (RDW), and different serum biochemical parameters, e.g. total protein (TP), albumin (ALB), globulin (GLB), albumin to globulin ratio (A/G), total bilirubin (TBIL), alkaline phosphatase (ALP), γ -glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), AST/ALT, creatinine, urea (BUN), uric acid (URIC), total cholesterol (TC), triglyceride, creatine kinase (CK), lactate dehydrogenase (LDH), and cortisol. The WBC value of pregnant does was significantly lower than that of non-pregnant does, while the concentrations of TC and TBIL were both significantly higher than those of non-pregnant does. The changes in hematological and serum biochemical parameters during pregnancy can be not only used for health monitoring and disease diagnosis and reference for Chinese water deer, but can also be referred for other cervids.

Keywords: Chinese water deer; pregnancy; white blood cells; total cholesterol; total bilirubin.

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can be found on page 7

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Study contribution

The Chinese water deer is a vulnerable species on the International Union for Conservation of Nature (IUCN) Red List due to its sharply shrunk population. Better breeding management with clear physical and biochemical information about the deer can help the population recovery. However, there is little information about the changes in the effects of hematological and serum biochemical parameters in the Chinese water deer upon pregnancy. This study preliminarily established a reference of blood parameters in pregnant Chinese water deer that can be used for health management and clinical diagnosis. We found that pregnant does have higher total cholesterol and total bilirubin concentrations, and lower white blood cells than non-pregnant does. To our knowledge, this is the first report on the hematological and serum biochemical parameters in pregnant Chinese water deer.

Introduction

Assessing physiological conditions is imperative for the effective management of wild animal populations, as well as for evaluating health during pregnancy. To master the physiological status of animals, the detection of hematological and biochemical parameters has been widely used in health management, disease diagnosis, and reproduction monitoring.⁽¹⁻⁷⁾ The changes in hematological and biochemical parameters after pregnancy have been clarified in more and more animals, especially livestock, for health monitoring. In different species, even different breeds, the changes may differ, for example, the red blood cells (RBC), packed-cell volume (PCV), and hemoglobin (HGB) increased, while total white blood cells (WBC) and segmented neutrophils increased during the gestation period in Sahiwal cows;⁽⁸⁾ the glucose and cholesterol concentrations decreased significantly during pregnancy in Sahel goats;⁽⁹⁾ and the RBC, and packed-cell volume (PCV) decreased significantly during pregnancy in Baladi goats.⁽¹⁰⁾ For monitor the health condition of endangered species in the breeding season, it is necessary to obtain the basic hematological and biochemical parameters to understand the changes during the gestation period. Only a few studies on the Cervidae have provided hematology and biochemistry reference values. In red deer (*Cervus elaphus*) and fallow deer (*Dama dama*), the biochemical parameters of glutamate aminotransferase, triglyceride, total cholesterol (TC), and creatinine in the pregnant groups were significantly increased compared with the non-pregnant groups.⁽⁷⁾

As the only species in Hydropotinae, belonging to Cervidae, the Chinese water deer (*Hydropotes inermis*) is a vulnerable species in the IUCN Red List due to its sharply decreased population,^(11,12) and is mainly distributed in East China and the Korean peninsula.⁽¹³⁾ A group of Chinese water deer was reintroduced to recover the local population in Shanghai, China, where the deer disappeared a century ago. More physiological information, such as hematological and biochemical parameters, will be helpful to ensure the health of the Chinese water deer during pregnancy and contribute to enlarging the population. However, the Chinese water deer is a timid animal that can be easily frightened. They are difficult to catch and drawn off the blood for regular monitoring. We collected blood samples when transferring the deer and tested the hematological and serum biochemical parameters in pregnant does for long-term health monitoring.

Materials and methods

Ethical statement

The authors confirm that the ethical policies of the journal, as noted in the journal's author guidelines page, have been followed. This study was approved by the University Committee on Animal Research Protection of East China Normal University (license No: Zhang 20190601) and written consent was obtained from the institutions and farms before the study.

Sample collection

The blood samples of pregnant does ($n = 48$) were collected from the Huaxia Park of Shanghai and the Zhoushan archipelago of Zhejiang Province, China. Data of the non-pregnant does were from Nie et al., 2020,⁽¹⁾ whose samples had been collected from the same locations.

The climate type of each place is subtropical monsoon. The body mass was between 7.25 and 20.23 kg. The deer were fed soybean meal once a day and freely reached grass on the ground in Shanghai; the deer were fed soybean meal and sweet potato vines in Zhoushan archipelago. Water was available *ad libitum*. All of them were deemed clinically healthy by veterinary inspection. All blood samples were collected before transportation in March and April 2016. No anesthetics were used. The managers caught the deer through a net. The eyes were covered as soon as the deer were caught to ensure that they stayed calm. The blood was collected by venipuncture of the hindquarter into 2.7 ml tubes containing EDTA for hematology, and into 5 ml empty serum collection tubes (Becton Dickinson and Company, Devon, UK) for serum biochemistry. All samples were transported to Shanghai Labway Clinical Laboratory Company in an icebox for hematological and serum biochemical parameters testing. The tests were completed within 24 hours.

Hematological analysis

Nine different blood parameters were analyzed using an Automated Hematology Analyzer (Sysmex XE-5000, SYSMEX, Japan). They included white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), packed-cell volume (PCV), platelet count (PLT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), and mean red blood cell distribution width coefficient of variation (RDW).

Serum biochemical analyses

Eighteen different biochemical parameters were analyzed using Automatic Biochemical Analyzer (Cobas-c702, Roche, Switzerland), and Automatic Biochemical Analyzer (Cobas 8000-e602, Roche, Switzerland). They were total protein (TP), albumin (ALB), globulin (GLB), albumin to globulin ratio (A/G), total bilirubin (TBIL), alkaline phosphatase (ALP), γ -glutamyl transferase (GGT), cortisol, alanine aminotransferase (ALT), aspartate aminotransferase (AST), AST/ALT, creatinine, urea (BUN), uric acid (URIC), total cholesterol (TC), triglyceride, creatine kinase (CK), and lactate dehydrogenase (LDH). The method employed is an electrochemical luminescence immunoassay.

Statistical analysis

The median and quartile were used to represent the baseline values when the result showed a non-normal distribution with a $P > 0.05$ by Shapiro-Wilk analysis. Therefore, the significant differences were investigated using the Wilcoxon signed-rank test. All P-values were corrected using the Bonferroni method. R software (R 3.4.4) was used for statistical analyses.⁽¹⁴⁾

Results

The results of 48 pregnant does' blood indicators compared to those from non-pregnant does⁽¹⁾ are both shown in [Table 1](#). By comparing the results during pregnancy and non-pregnancy, several significant changes were found among all indicators. The WBC ($P = 0.006$) of pregnant does was significantly lower than that of non-pregnant does; the TC ($P = 0.046$) and TBIL ($P = 0.014$) of pregnant does were significantly higher than those of non-pregnant does ([Table 1](#)).

Alternatively, when comparing the groups of pregnancy and non-pregnancy, no significant differences were found in RBC, HGB, MCV, PCV, MCH, MCHC, RDW, and PLT among the hematological parameters, and no significant differences were found in triglyceride, CK, LDH, GGT, cortisol, BUN, creatinine, URIC, TP, ALB, GLB, A/G, ALT, AST, AST/ALT, and ALP among serum parameters as well ([Table 1](#)).

Discussion

Pregnancy requires physiologic adaptations in all maternal systems, including physiology and biochemistry of blood. There was a significant reduction in the WBC of pregnant Chinese water deer, which was similar to the results from pregnant red deer and opposed to that of fallow deer in pregnancy.⁽⁷⁾ The function of WBC is to defend the body against pathogens and to remove damaged cells, toxins, and other waste products.⁽¹⁵⁾ The WBC value is generally elevated during pregnancy in humans and other mammals, such as mares and goats.^(9,16,17) Immunity is generally considered intact during pregnancy, but cell-mediated immunity is significantly suppressed, and upregulation of innate immunity may be a compensatory mechanism.^(18,19) A major change in innate immunity is an increased WBC count, mainly due to neutrophilia upon pregnancy.⁽¹⁹⁻²⁴⁾ However, there were differences among cervids. WBC was significantly increased in pregnant red deer,⁽⁷⁾ but significantly decreased in pregnant fallow deer⁽⁷⁾ and Chinese water deer. However, the sample size of pregnant red deer ($n = 4$) and fallow deer ($n = 4$) were minimal compared with the non-pregnant red deer ($n = 36$) and the non-pregnant fallow deer ($n = 26$).⁽⁷⁾ The reasons for the decline in WBC in cervids are not well understood, and more cervid species and more samples are needed for further study.

Other hematological parameters, including RBC, HGB, MCV, PCV, MCH, MCHC, RDW, and PLT showed no significant changes in pregnant Chinese water deer. In mares, pigs, sheep, and dogs, their RBC and PCV declined significantly,⁽²⁵⁾ which may be a hemodilution effect caused by increased plasma volume in the later stages of pregnancy.^(26,27) Hemodilution that occurs in pregnancy could physiologically reduce blood viscosity, and greatly increases blood flow in the capillaries.⁽²⁸⁾

Table 1. Comparison of hematological and serum biochemical parameters in the sera of pregnant and non-pregnant Chinese water deer (*Hydropotes inermis*)

Measurement	Pregnancy (n = 48)		Not pregnancy (n = 24)	
	Median	(Q1, Q2)	Median	(Q1, Q2)
WBC(10 ⁹ /L)	1.91	(1.61, 2.68)	3.89	(2.12, 77.8)**
MCV(fl)	39.45	(38.3, 41.53)	40.55	(38.20, 49.83)
RBC(10 ¹² /L)	13.24	(12.53, 13.77)	13.33	(11.24, 14.19)
HGB(g/L)	200	(186.25, 209.75)	179.5	(163.75, 207.25)
PCV(%)	53.25	(49.55, 55.43)	53.85	(49.08, 62.08)
MCHC(g/L)	380	(359.5, 389)	368	(358.5, 401)
MCH(pg)	15	(14.73, 15.3)	14.9	(14.28, 15.1)
RDW(%)	33.3	(30.78, 34.6)	34.45	(32.23, 35.38)
PLT(10 ⁹ /L)	192	(138.5, 218.25)	172	(116, 291.5)
TC(mmol/L)	1.96	(1.77, 2.26)	1.56	(1.4, 1.91)*
Triglyceride(mmol/L)	0.56	(0.22, 0.76)	0.59	(0.36, 1.63)
CK(U/L)	1056	(609.75, 2014)	1935.5	(1228.75, 4401.5)
LDH(U/L)	759	(636, 942.5)	1124	(925.5, 2043)
GGT(U/L)	182	(134.5, 250.5)	213	(166.5, 272)
Cortisol(nmol/L)	308.5	(189.5, 346.25)	253	(159.25, 362.5)
BUN(mmol/L)	15.05	(11.9, 20.43)	17	(14.95, 19.4)
Creatinine(umol/L)	103	(89, 110.75)	91	(70, 118)
URIC(umol/L)	2	(1, 4)	5	(1, 12)
TP(g/L)	69.5	(66, 72)	71	(66, 74)
ALB(g/L)	37	(35, 38)	37	(33, 39)
GLB(g/L)	32	(29, 35)	33	(29.5, 37)
A/G(%)	1.18	(1.01, 1.35)	1.11	(0.88, 1.31)
TBIL(umol/L)	3	(2.28, 4.15)	1.4	(0.9, 3.6)*
ALT(U/L)	23	(18.25, 28.75)	23	(18.5, 29.5)
AST(U/L)	83	(65.25, 118.25)	80	(63.5, 133)
AST/ALT	3.96	(3.13, 5.48)	3.83	(2.62, 5.48)
ALP(U/L)	54.5	(33.5, 89.5)	70	(46.5, 115.5)

a. Hematological parameters include white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), packed - cell volume (PCV), platelet count (PLT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean red blood cell distribution width coefficient of variation (RDW). b. Biochemical parameters include total protein (TP), albumin (ALB), globulin (GLB), albumin to globulin ratio (A/G), total bilirubin (TBIL), alkaline phosphatase (ALP), γ -glutamyl transferase (GGT), cortisol, alanine aminotransferase (ALT), aspartate aminotransferase (AST), AST/ALT, blood urea nitrogen (BUN), creatinine, uric acid (URIC), total cholesterol (TC), triglyceride, creatine kinase (CK), lactate dehydrogenase (LDH).

*: P < 0.05; **: P < 0.01

Therefore, hemodilution can improve blood flow through the blood vessels of the placenta, especially later in pregnancy, to increase nutrients and oxygen to the fetus,^(10,28,29) but our study did not find such changes in Chinese water deer.

ALT, AST, GGT, TBIL, TP, ALB, and ALP are related to liver function. There were no significant changes in pregnant Chinese water deer values of ALT, AST, GGT, TP, and ALB. However, TBIL increased significantly. Significantly reduced bilirubin levels have been found in pregnant women due to hemodilution.⁽³⁰⁾ A study found fetal hemoglobin lysis in Akkaraman sheep, resulting in elevated bilirubin.⁽³¹⁾ Another study in the pregnant mare also found a raise in TBIL. The authors explained that the change may be caused by the secondary cholestasis from the enlarged uterus.⁽³²⁾ The values of RBC were similar between pregnant and non-pregnant Chinese water deer, but the ALP of pregnant does was much lower than that of non-pregnant does without significance, which may be related to TBIL change in Chinese water deer.

The concentrations of ALB and ALT were both significantly increased in the pregnant fallow deer,⁽⁷⁾ but both showed no change in pregnant Chinese water deer. The activity of elevated aminotransferase suggests that rapid gluconeogenesis associated with pregnancy may result in damage to muscle (Cory cycle) and liver cells.⁽⁷⁾ Therefore, our study suggests that pregnancy has no serious effect on the liver function of Chinese water deer.

Our results also suggest that pregnancy has no serious effect on kidney function since the values of BUN, URIC, and CRE, which are related to renal function, showed no significant changes in pregnant Chinese water deer.

TC concentrations increased significantly in pregnant Chinese water deer. This result was similar to those in red deer, fallow deer,⁽⁷⁾ and Sahel goats.⁽⁹⁾ TC is central to maintaining the long-term regulation of ovarian steroidogenesis, which may have increased due to changes in endocrine function during pregnancy.⁽³³⁾ Notably, pregnant cows have higher concentrations of TC, presumably to maintain gestational homeostasis.⁽³⁴⁾ Female Chinese water deer may also need high TC concentration to maintain homeostasis during pregnancy. The triglyceride concentration may be affected by the nutritional condition, and was found to be significantly higher both in pregnant red deer and fallow deer.⁽⁷⁾ However, no significant changes were found here.

Conclusions

Pregnancy has some influence on the hemato-biochemical traits of Chinese water deer. This is reflected by the significant differences found in several indicators, including TC, TBIL, and WBC. Increased TC concentrations may be a common phenomenon after pregnancy in several ungulates. The significant increase in TBIL may be related to changes in liver function when the deer are pregnant. This study obtained the hematological and serum biochemical parameters of pregnant Chinese water deer, which may be used in health monitoring and disease diagnosis for reproduction in this species, but may also provide a reference for other evolutionarily similar species. There was a small sample size in the current study since these deer constitute an endangered species. To understand the Chinese water deer during gestation, better long-term data collection is necessary for further studies.

Data availability

The datasets analyzed or generated during this study are available from the corresponding authors on reasonable request.

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Conflicts of interest

None of the authors of this paper have any financial or personal relationships with other people or organizations that could inappropriately influence or bias the content of the paper.

Author contributions

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