

1 Journal of Equine Veterinary Science

2 Type of article: Original article

3

4 Title: Hematological and blood biochemical characteristics of newborn heavy draft foals after dystocia

5

6 Akiko CHIBA^a, Takahiro AOKI DVM, PhD^{a,b,†}, Megumi ITOH DVM, PhD^a, Norio YAMAGISHI DVM,

7 PhD^a and Kenichi SHIBANO DVM, PhD^a

8

9 a. Department of Applied Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine

10 b. Research Center for Global Agro-Medicine, Obihiro University of Agriculture and Veterinary Medicine

11

12 Corresponding author[†]: Takahiro AOKI DVM, PhD

13 Research Center for Global Agro-Medicine, Obihiro University of Agriculture and Veterinary Medicine

14 (Nishi 2-11, Inada-cho, Obihiro, Hokkaido, 080-8555, Japan)

15 E-mail: aokit@obihiro.ac.jp

16

17

18 **Abstract**

19 The negative impact of equine dystocia on hematological and serum biochemical profile of neonatal foals
20 remains unknown, particularly in heavy draft horses that show high incidence of dystocia. This study aimed
21 to reveal the hematological and serum biochemical profile of the foals born in normal delivery and examine
22 the effect of dystocia on blood properties in heavy draft newborn foals. In the normal birth group ($n = 23$),
23 stage II labor was <30 min, with spontaneous or assisted delivery with mild traction by one or two people. In
24 the dystocia group ($n = 13$), stage II labor was ≥ 30 min, with strong traction by more than three people or
25 mechanical tools with or without correcting fetal displacement. Blood samples were collected from the
26 jugular vein at 0, 1, and 12 hr and 1 and 2 days after foaling. Red blood cells, hemoglobin concentration, and
27 packed cell volume remained significantly lower in the dystocia group than in the normal birth group. The
28 white blood cell count was significantly higher in dystocia foals (1 day: $P < .05$). Dystocia foals had
29 significantly higher cortisol (1 hr: $P < .05$), urea nitrogen (1 hr: $P < .05$), and creatine kinase activities (1 hr:
30 $P < .01$, 12 hr: $P < .05$). This study revealed that dystocia foals were more likely to be affected by anemia,
31 physical stress, and muscle damage than normal birth foals.

32

33 **Key Words:** dystocia, foal, anemia, stress, muscle damage

34

35 ***1. Introduction***

36 Dystocia is a difficult labor that can result in neonatal death without assistance by humans [1]. The incidence
37 rate of dystocia has been found to be 4%–10% in horses, and dystocia occurs more frequently in heavy draft
38 horses than in light breed horses [2]. Most dystocia cases are caused by fetal displacement [3]. Parturition is
39 divided into three stages: the first stage of parturition is associated with cervical dilation and uterine
40 contractions, the second stage includes the time from the rupture of the chorioallantoic membrane to the end
41 of fetal delivery, and the third stage is associated with discharge of the placental and fetal membranes [4].
42 The progression of equine parturition occurs more rapidly than that in other farm animals. Stage II lasts for
43 only 20–30 min in mares [5]. A recent study reported that prolonged labor (Stage II \geq 30 min) is associated
44 with a higher risk of stillbirth [6]. In other studies, the morbidity and mortality in dystocia foals have been
45 found to be higher than those in normal birth foals [7–8]. The cortisol concentration in saliva [9] and blood
46 [10] has been reported to be higher in dystocia calves, leading to metabolic changes such as increased blood
47 glucose (Glu) and cholesterol levels [10]. The negative impact of equine dystocia on hematological and
48 serum biochemical profile of neonatal foals remains unknown, particularly in heavy draft horses that show
49 high incidence of dystocia. Understanding the effects of dystocia on neonatal foals would contribute to the
50 development of nursing and treatment procedures. This study aimed to reveal the hematological and serum
51 biochemical profile of foals born via normal delivery and examine the effect of dystocia on blood properties
52 in heavy draft newborn foals.

53

54 ***2. Materials & Methods***

55 *2.1 Animals*

56 Heavy draft foals (Percherons and crossbreeds between Percheron, Belgian, and Breton heavy draft
57 horses) born from January 2013 to January 2015 at three stud farms (Tokachi, Hokkaido, Japan) were
58 included in the study. Prepartum dams showing signs of foaling were monitored. Foaling events such as
59 rupture of the chorioallantoic membrane, appearance of the fetal sac, and delivery of foals were recorded.
60 Cases were excluded from the study if there was foaling in the absence of witnesses, abortion, premature
61 birth, or cesarean section.

62

63 2.2 Definition of normal birth and dystocia

64 In our study, dystocia was defined as prolonged labor with strong fetal traction with or without fetal
65 displacement. If stage II was ≥ 30 min and the labor did not progress, traction was applied to the fetus. In the
66 normal birth group ($n = 23$), stage II labor was < 30 min, with spontaneous or assisted delivery with mild
67 traction by one or two people. In the dystocia group ($n = 13$), stage II labor was ≥ 30 min, with strong traction
68 by more than three people or mechanical equipment with or without correcting fetal displacement.

69

70 2.3 Physical examination and blood sampling

71 Physical examination and blood sampling were conducted at 0 hr (within 5 min after birth), 1 hr (before
72 suckling colostrum), 12 hr, and 1 (24-48 hr) and 2 days (48-72 hr) after birth. The foal's vitality was
73 assessed immediately after birth using advanced APGAR score (seven items, each 2-point scale, a total of 0-
74 14 points) [11]. Rectal temperature, heart rate, respiratory rate and appearance of visible mucous membrane
75 were recorded. Peripheral blood was collected into 7 ml vacuum tubes (Venoject II VP-P070K, Terumo
76 Corp., Tokyo, Japan) and 5 ml vacuum tubes containing ethylenediaminetetraacetic acid (EDTA) (Venoject
77 II VP-NA050K, Terumo Corp.) by jugular venipuncture using 21 gauge $\times 1\frac{1}{2}$ inch needles (MN-2138MS,
78 Terumo Corp.). All blood samples were stored on ice until transfer to the laboratory and processed within 3
79 hr. The samples containing EDTA were used for complete blood counts. Tubes without EDTA were
80 centrifuged for 12 min at 3,000 rpm after incubation (37°C , 90 min). Serum was withdrawn and frozen at $-$
81 30°C for serum amyloid A (SAA), cortisol, and other biochemical analyses at a later date.

82

83 2.4 Hematological and serum biochemical analysis

84 The numbers of white blood cells (WBCs) and red blood cells (RBCs), hemoglobin (Hb) concentration,
85 packed cell volume (PCV), mean cell volume (MCV), mean cell Hb (MCH), mean cell Hb concentration
86 (MCHC), and platelet count were determined using an automated hematology analyzer (Celltac alpha
87 MEK-6358, Nihon Kohden Corp., Tokyo, Japan). In each sample, the levels of Glu, free fatty acid (FFA),
88 total cholesterol, triglyceride (TG), total protein, albumin, urea nitrogen (UN), creatinine (Cre), aspartate

89 aminotransferase, gamma-glutamyltransferase, alkaline phosphatase, creatine kinase (CK), lactate
90 dehydrogenase, iron, calcium, inorganic phosphate, magnesium, sodium, potassium, and chlorine were
91 measured using an automated clinical chemistry analyzer (TBA-120FR, Toshiba Medical Systems Corp.,
92 Otawara, Japan). The SAA level was measured using commercially available enzyme-linked immunosorbent
93 assay (ELISA) kits (Tridelta Phase Range Kit, Tridelta Development Ltd., Kildare, Ireland) according to the
94 manufacturer's instructions. The serum cortisol level was assessed by chemiluminescence enzyme
95 immunoassay in a commercial clinical laboratory (Obihiro clinical laboratory Inc., Obihiro, Japan).

96

97 2.5 Statistical analysis

98 The sequence of postnatal data was analyzed with repeated-measures analysis of variance (ANOVA).
99 When significant differences or interactions between the two groups were observed, Student's or Welch's
100 *t*-test were used to identify differences between the groups at each sampling period. Results with *P*-value
101 $< .05$ were considered significant, and those with $P < .1$ were considered to have a tendency (marginal
102 difference). These statistical analyses were conducted using Statcel3 (OMS Ltd, Saitama, Japan).

103

104 **3. Results**

105 There is a marginal difference ($P < .1$) in the APGAR score between the normal birth group (mean: 10.6,
106 SD: 1.4, range: 8-13) and the dystocia group (mean: 9.4, SD: 2.3, range: 6-13). There were no significant
107 differences in other physical examination findings between the two groups (Table 1). Significant differences
108 or interactions between the two groups were observed for WBC and RBC counts; Hb concentration; PCV;
109 and cortisol, UN, FFA, and CK levels by repeated-measures ANOVA. Significant differences were not
110 observed for the other parameters (Tables 2 and 3). Significant differences between the two groups in each
111 sampling period were examined using Student's or Welch's *t*-test, and the results are shown in Figures 1 and
112 2. The RBC count (0 hr: $P < .1$, 1 hr: $P < .05$, 12 hr: $P < .05$, 1 day: $P < .01$, 2 days: $P < .05$), Hb
113 concentration (12 hr: $P < .1$, 1 day: $P < .05$, 2 days: $P < .05$), and PCV (12 hr: $P < .05$, 1 day: $P < .01$, 2
114 days: $P < .01$) remained at significantly lower levels in the dystocia group than in the normal birth group.
115 Serum cortisol ($P < .05$), UN ($P < .05$), and CK ($P < .01$) levels at 1 hr; CK ($P < .05$) and FFA ($P < .1$) levels

116 at 12 hr; and the WBC count ($P < .05$) at 1 day were higher in dystocia foals than in foals in the normal birth
117 group. Although, a foal in the dystocia group died after the first day of sampling (n for day 2 in the dystocia
118 group = 12), the cause of death was unknown.

119

120

121 Table 1

122 Results of physical examinations of newborn heavy draft foals within 2 days after birth. Normal birth
123 group ($n = 23$). Dystocia group ($n = 13$). Data are shown as mean (standard deviation). Statistical
124 significance is denoted by ** ($P < .01$).

125

	Time after foaling					Repeated-measures ANOVA		
	0 hr	1 hr	12 hr	1 day	2 days	Time	Group	Interaction
Rectal temperature (°C)								
Normal	37.7 (0.7)	38.2 (0.5)	38.4 (0.3)	38.6 (0.2)	38.7 (0.2)	NS	NS	NS
Dystocia	38.0 (0.6)	38.1 (0.4)	38.5 (0.5)	38.7 (0.4)	38.8 (0.2)			
Heart rate (/min)								
Normal	86.0 (21.9)	98.8 (28.7)	105.4 (36.5)	92.0 (28.5)	98.4 (23.5)	**	NS	NS
Dystocia	78.7 (36.9)	116.1 (42.1)	118.0 (27.4)	99.4 (29.0)	98.0 (22.7)			
Respiratory rate (/min)								
Normal	75.2 (13.9)	56.0 (13.9)	67.1 (23.9)	67.7 (23.5)	67.2 (20.1)	**	NS	NS
Dystocia	72.1 (28.7)	50.2 (17.1)	58.2 (17.7)	62.8 (27.6)	53.3 (22.2)			

126

127

128

129 Table 2

130 Results of hematological analysis in newborn heavy draft foals within 2 days after birth. Normal birth
 131 group ($n = 23$). Dystocia group ($n = 13$). Data are shown as mean (standard deviation). Statistical
 132 significance is denoted by * ($P < .05$) or ** ($P < .01$).

133

	Time after foaling					Repeated-measures ANOVA		
	0 hr	1 hr	12 hr	1 day	2 days	Time	Group	Interaction
White blood cells ($10^3/\mu\text{L}$)								
Normal	74.0 (11.7)	64.2 (12.1)	82.1 (17.9)	57.5 (26.1)	60.9 (19.1)	NS	NS	*
Dystocia	72.8 (19.6)	60.9 (15.0)	89.2 (25.3)	79.4 (25.5)	69.8 (18.5)			
Red blood cells ($10^4/\mu\text{L}$)								
Normal	1129.7 (92.0)	1131.8 (87.8)	1093.6 (115.4)	1036.9 (108.9)	994.9 (107.2)	*	*	*
Dystocia	1071.2 (95.0)	1049.3 (89.5)	989.8 (105.1)	925.3 (84.2)	891.3 (102.4)			
Hemoglobin concentration (g/dL)								
Normal	14.9 (1.0)	15.3 (1.1)	14.5 (1.2)	13.8 (1.3)	13.4 (1.1)	NS	*	*
Dystocia	14.8 (0.9)	14.7 (1.0)	13.8 (1.3)	12.8 (1.1)	12.3 (1.2)			
Packed cell volume (%)								
Normal	49.5 (3.3)	49.7 (3.7)	47.3 (3.3)	44.6 (3.8)	42.3 (3.4)	*	**	NS
Dystocia	48.5 (3.1)	47.6 (3.0)	44.0 (3.9)	40.7 (3.1)	38.6 (4.0)			
Mean cell volume (fL)								
Normal	43.9 (2.5)	45.3 (8.1)	43.4 (2.5)	43.2 (2.6)	42.8 (3.4)	*	NS	NS
Dystocia	45.5 (2.7)	45.1 (2.6)	44.6 (2.7)	44.0 (2.7)	43.5 (2.9)			
Mean cell hemoglobin (pg)								
Normal	13.2 (0.9)	13.9 (2.4)	13.3 (0.9)	13.4 (1.0)	13.5 (1.2)	NS	NS	NS
Dystocia	13.9 (1.1)	14.1 (1.1)	13.9 (0.8)	13.9 (1.0)	13.8 (1.0)			
Mean cell hemoglobin concentration (g/dL)								
Normal	30.1 (1.3)	32.2 (6.4)	30.8 (1.4)	31.1 (1.2)	31.6 (1.3)	*	NS	*
Dystocia	30.5 (1.1)	31.2 (1.4)	29.1 (7.7)	31.5 (1.3)	31.8 (1.4)			
Platelet count ($10^4/\mu\text{L}$)								
Normal	35.1 (9.8)	36.9 (10.4)	32.1 (13.1)	29.2 (12.4)	31.6 (13.9)	NS	NS	NS
Dystocia	31.2 (6.6)	33.4 (5.2)	29.9 (6.6)	29.3 (4.8)	29.3 (6.2)			

134

135

136

138 Results of serum biochemical analysis in newborn heavy draft foals within 2 days after birth. Normal
 139 birth group ($n = 23$). Dystocia group ($n = 13$). Data is shown as mean (standard deviation). Statistical
 140 significance is denoted by * ($P < .05$) or ** ($P < .01$).

141

	Time after foaling					Repeated-measures ANOVA		
	0 hr	1 hr	12 hr	1 day	2 days	Time	Group	Interaction
Cortisol ($\mu\text{g/dL}$)								
Normal	8.1 (0.8)	11.2 (2.1)	2.6 (1.5)	2.4 (2.2)	1.2 (0.5)	NS	NS	*
Dystocia	8.5 (2.2)	13.7 (4.5)	4.4 (3.8)	2.1 (0.7)	1.3 (0.5)			
Serum amyloid A ($\mu\text{g/ml}$)								
Normal	3.8 (12.0)	6.0 (22.7)	44.2 (57.7)	224.2 (215.1)	232.4 (585.6)	NS	NS	NS
Dystocia	1.0 (0.1)	1.0 (0.0)	67.0 (76.7)	216.4 (176.2)	200.9 (242.6)			
Glucose (mg/dL)								
Normal	72.0 (12.7)	61.0 (22.7)	136.9 (24.2)	130.3 (18.2)	134.1 (24.4)	*	NS	NS
Dystocia	86.5 (21.5)	74.7 (29.5)	122.0 (55.8)	127.6 (25.5)	134.5 (20.3)			
Free fatty acids ($\mu\text{Eq/L}$)								
Normal	142.8 (28.1)	586.7 (173.3)	439.8 (168.8)	298.1 (106.8)	302.0 (117.5)	*	NS	**
Dystocia	154.3 (31.1)	584.6 (212.8)	667.8 (433.1)	451.5 (419.2)	293.3 (108.0)			
Total cholesterol (mg/dL)								
Normal	132.6 (28.0)	150.0 (37.3)	179.9 (51.8)	212.4 (57.9)	198.4 (59.3)	*	NS	NS
Dystocia	149.7 (42.7)	163.9 (49.1)	188.9 (50.9)	209.8 (60.4)	192.1 (33.6)			
Triglyceride (mg/dL)								
Normal	8.1 (2.8)	8.7 (15.8)	11.6 (5.7)	40.5 (23.0)	78.3 (41.3)	*	NS	NS
Dystocia	10.4 (3.9)	5.8 (2.8)	15.9 (11.0)	81.5 (146.2)	62.0 (27.8)			
Total protein (g/dL)								
Normal	4.0 (0.3)	4.2 (0.5)	5.3 (1.1)	5.5 (0.9)	5.4 (1.0)	*	NS	NS
Dystocia	4.1 (0.7)	4.1 (0.7)	5.0 (0.9)	5.1 (0.8)	5.1 (0.8)			
Albumin (g/dL)								
Normal	3.2 (0.2)	3.3 (0.4)	3.0 (0.4)	3.0 (0.3)	3.0 (0.3)	*	NS	NS
Dystocia	3.2 (0.6)	3.3 (0.6)	3.1 (0.5)	2.9 (0.4)	2.9 (0.4)			
Urea nitrogen (mg/dL)								
Normal	18.3 (5.3)	17.9 (5.4)	20.9 (4.6)	13.9 (2.9)	13.2 (4.8)	*	NS	**
Dystocia	18.1 (6.4)	18.5 (6.0)	24.3 (6.4)	21.2 (11.6)	14.4 (4.8)			
Creatinine (mg/dL)								
Normal	2.1 (0.5)	1.9 (0.5)	1.6 (0.5)	1.1 (0.3)	1.0 (0.2)	*	NS	NS
Dystocia	2.1 (0.5)	2.1 (0.5)	1.8 (0.6)	1.2 (0.4)	1.0 (0.1)			
Aspartate aminotransferase (IU/L)								
Normal	76.0 (26.7)	84.0 (26.4)	158.3 (54.0)	177.2 (35.9)	200.5 (91.3)	*	NS	NS
Dystocia	73.5 (15.1)	81.3 (20.7)	151.9 (34.7)	182.8 (47.2)	188.8 (35.5)			

142

143

144

145 Table 3

146 Results of serum biochemical analysis in newborn heavy draft foals within 2 days after birth. Normal
 147 birth group ($n = 23$). Dystocia group ($n = 13$). Data is shown as mean (standard deviation). Statistical
 148 significance is denoted by * ($P < .05$) or ** ($P < .01$).

149

	Time after foaling					Repeated-measures ANOVA		
	0 hr	1 hr	12 hr	1 day	2 days	Time	Group	Interaction
Gamma-glutamyltransferase (IU/L)								
Normal	22.8 (8.5)	25.3 (7.4)	38.1 (14.0)	33.2 (11.2)	44.1 (37.9)	*	NS	NS
Dystocia	20.6 (6.1)	21.6 (6.2)	35.9 (13.9)	31.8 (7.7)	33.7 (12.0)			
Alkaline phosphatase (IU/L)								
Normal	11311.0 (5158.8)	11902.6 (6048.0)	10351.6 (5590.1)	8994.6 (4430.4)	6477.7 (3161.1)	*	NS	NS
Dystocia	12582.7 (7574.4)	13212.5 (7884.0)	12300.0 (6899.9)	9990.0 (5308.8)	7470.0 (4279.7)			
Lactate dehydrogenase (IU/L)								
Normal	346.8 (83.5)	459.4 (111.2)	676.3 (157.2)	635.4 (105.3)	611.4 (156.1)	*	NS	NS
Dystocia	311.2 (66.6)	436.9 (79.3)	715.3 (214.9)	602.9 (95.7)	552.9 (118.7)			
Creatine kinase (IU/L)								
Normal	109.3 (58.1)	257.0 (129.7)	315.0 (249.4)	248.4 (218.6)	176.1 (126.3)	*	NS	**
Dystocia	119.9 (52.5)	386.5 (122.3)	510.2 (352.4)	319.8 (263.0)	152.2 (61.3)			
Calcium (mg/dL)								
Normal	13.0 (0.7)	11.7 (1.1)	11.8 (1.1)	11.4 (0.9)	11.9 (1.2)	*	NS	*
Dystocia	13.6 (2.1)	11.9 (1.7)	11.5 (1.5)	10.8 (1.0)	11.4 (1.4)			
Inorganic phosphate (mg/dL)								
Normal	4.9 (0.7)	4.0 (0.7)	4.2 (1.0)	5.0 (0.9)	5.7 (1.0)	*	NS	NS
Dystocia	5.4 (1.2)	4.1 (1.0)	4.4 (0.8)	4.7 (1.0)	5.2 (0.8)			
Magnesium (mg/dL)								
Normal	1.7 (0.1)	1.6 (0.2)	2.1 (0.4)	2.0 (0.3)	1.9 (0.2)	*	NS	NS
Dystocia	1.8 (0.3)	1.7 (0.3)	2.1 (0.4)	2.0 (0.3)	1.9 (0.3)			
Iron ($\mu\text{g/dL}$)								
Normal	469.4 (58.3)	463.2 (83.8)	338.0 (98.0)	192.3 (85.9)	108.0 (59.1)	*	NS	**
Dystocia	466.7 (81.1)	468.3 (84.1)	354.8 (69.7)	222.2 (83.8)	112.2 (52.9)			
Sodium (mEq/L)								
Normal	135.7 (29.2)	140.7 (7.3)	138.0 (8.3)	136.3 (6.9)	137.4 (9.0)	NS	NS	NS
Dystocia	137.0 (15.5)	137.3 (13.0)	134.7 (13.6)	134.0 (9.8)	133.5 (9.5)			
Potassium (mEq/L)								
Normal	4.8(1.1)	4.6 (0.4)	4.3 (0.4)	4.7 (0.3)	4.6 (0.4)	NS	NS	NS
Dystocia	4.9(0.5)	4.3 (0.5)	4.0 (0.5)	4.3 (0.5)	4.3 (0.3)			
Chlorine (mEq/L)								
Normal	95.1(20.3)	98.8 (5.0)	98.4 (5.7)	97.9 (5.1)	97.2 (5.9)	NS	NS	NS
Dystocia	95.5(10.3)	95.4 (8.5)	94.5 (9.2)	95.6 (6.6)	94.5 (6.6)			

150

151

152

153 **4. Discussion**

154 In the present study, we focused on heavy draft horses that have a higher incidence of dystocia and
155 examined the hematological and serum biochemical features of foals born after dystocia. Statistically
156 significant differences in some parameters were observed between the dystocia and normal birth group.
157 Some parameters of both groups changed with time during the experimental period. Because we obtained the
158 samples of 0 and 1 hr at night and the samples of 12 hr and 1 and 2 days during daytime in most cases, we
159 should take the circadian rhythm into consideration when discussing the change in blood profile with time.
160 Furthermore, we should also consider the influence by the dam because blood properties [12–13] and
161 milk components [14–15] of the dam dramatically change in peripartum period.

162 The APGAR scoring system is a useful tool for grading the health status of foals immediately after birth
163 [11]. A previous study found that APGAR score negatively correlates with plasma stress hormones such as
164 ACTH and cortisol in healthy and ill foals [16]. There was only a marginal (not significant) difference in
165 APGAR score and no significant difference in other physical findings between the groups in this study. This
166 may be because most dystocia cases examined in this study were moderate, and the mortality rate of foals
167 was very low (only one case). We should reconsider these results after more severe cases of dystocia are
168 examined in future studies.

169 The RBC count, Hb level, and PCV were significantly lower in foals in the dystocia group than in those
170 in the normal birth group. The cause of relative anemia in the dystocia group was suspected to be blood loss
171 because there were no significant differences in MCV and MCHC between the two groups [17]. The cause of
172 blood loss may have been continuous hemorrhage from the umbilical artery after premature rupture of the
173 umbilical cord. However, we did not observe hemorrhage from the umbilicus during the study period. A
174 recent study reported that lower red blood cell count is associated with increased risk of infectious diseases
175 in the first 30 days in neonatal foals [18]. However, a relationship between low red blood cell counts and
176 dystocia has not been revealed. Although we revealed that the RBC count was lower in dystocia foals,
177 additional studies about the causes and outcomes of anemia are needed.

178 In general, infectious disease or physical stress causes increases in WBC counts in the peripheral blood
179 [19–20]. The blood level of SAA, a major acute-phase protein, increases when there is inflammation caused

180 by an infectious disease. SAA is often measured in equine medical practice and quickly responds to
181 infectious disease and inflammation [21–24]. Blood SAA levels rapidly increased within 1 day after birth in
182 the normal birth group in the present study (Table 3). A similar result has been reported in a previous study
183 [25]. There was no significant difference between normal birth foals and dystocia foals, suggesting that
184 foaling difficulty would not affect blood SAA levels. Higher cortisol levels may have increased WBC counts
185 of the dystocia group 1 day after birth. Neutrophilia is induced by the anti-inflammatory action of cortisol,
186 but the migration of neutrophils to a specific site is suppressed [20]. More studies that investigate whether
187 foaling stress during dystocia suppresses the immune reaction and is associated with susceptibility to
188 infection are needed.

189 When animals are under stress, the secretion of corticotropin hormone from the neurohypophysis
190 stimulates adrenocorticotropin (ACTH) release from the adenohypophysis. ACTH travels through the blood
191 to the adrenal cortex to stimulate the production and release of cortisol [26]. Cortisol has anti-inflammatory
192 effects and increases protein catabolism and decomposition of body fat [27]. Previous studies have reported
193 that the cortisol concentration in the saliva [9] and blood [10] is higher in dystocia calves and causes
194 metabolic changes and increases in Glu and cholesterol levels [10]. The cortisol concentration in normal
195 neonatal foals is high immediately after birth and returns to the normal range by 24–48 hr after birth [28].
196 We observed similar changes in the present study, but the cortisol concentration in dystocia foals was higher
197 than that in normal birth foals at 1 hr after birth. This suggests that dystocia foals are under more stressful
198 conditions. Although, we assumed that strong traction at birth causes physical pain and stress, more
199 investigation is needed to clarify the cause of high cortisol levels among dystocia foals. It has been reported
200 that the cortisol concentration is associated with prognosis in sepsis foals [29]. We would like to examine the
201 relationship between cortisol levels and prognosis in foals born after dystocia in future research.

202 The blood UN level is dependent on excretory function of the kidneys and protein catabolism [30]. Blood
203 UN and Cre levels are widely used as indicators of kidney function. The blood Cre level did not differ
204 significantly between the groups in the present study. We therefore assumed that the high UN level in
205 dystocia foals was not the result of reduced kidney function but was caused by acceleration of protein
206 catabolism by cortisol.

207 Blood FFA is produced by the degradation of TG mobilized from body fat and is used as an index of
208 body fat mobilization [27]. The causes of higher FFA levels in dystocia foals may be mobilization of body
209 fat by a negative energy balance or increased degradation of body fat by cortisol action. Hyperlipidemia is
210 associated with excess circulating lipids that are mobilized in periods of negative energy balance and is
211 diagnosed by serum TG levels [31]. Hyperlipidemia is a disease with high mortality and requires emergency
212 treatment in equine medicine [32]. Although there was no significant difference in the mean values between
213 the groups, a foal that died 1–2 days after birth had hyperlipidemia 1 day after birth (TG 565 mg/dL; normal
214 range: 4–44 mg/dL) [33]. The possibility that hyperlipidemia causes neonatal death in dystocia foals should
215 be examined in future research.

216 CK is present in heart and skeletal muscles and the brain [27]. It has been reported that the serum CK level
217 increases when muscle fibers are damaged by vigorous exercise in horses [34]. Increased CK levels were
218 revealed in both groups in the present study, which demonstrates that an elevation in the CK level is natural
219 in neonatal foals. Possible causes of this elevated CK level in neonatal foals are pressure in the birth canal,
220 muscle damage by falling when foals try to stand up after birth, and muscle damage by reactive oxygen [35].
221 Reactive oxygen, which is produced when cortisol is released [36], may be the cause of muscle damage.

222 The present study revealed that dystocia foals have relative anemia and more physical stress and muscle
223 damage than normal birth foals. In future research, we plan to examine whether the administration of
224 analgesic agents such as flunixin meglumine for physical stress and muscle damage and whole blood
225 transfusion from dam to foal for anemia will reduce neonatal morbidity and mortality in dystocia foals.

226

227

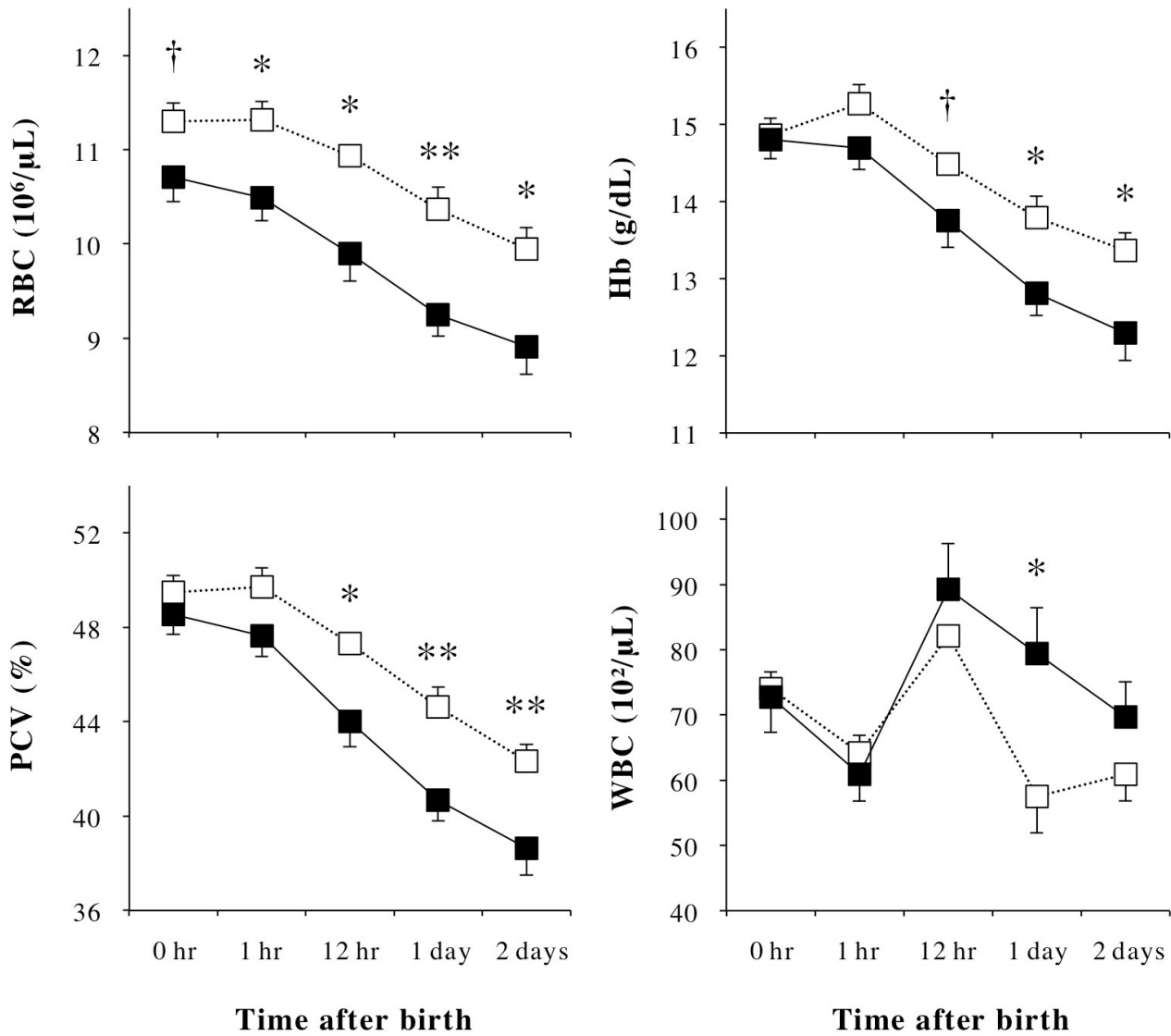
228

229 **References**

- 230 [1] Ginther OJ, Williams D. On-the-farm incidence and nature of equine dystocias. *J Equine Vet Sci*
231 1996;16:159–64.
- 232 [2] Vandeplassche M. Dystocia. In: Mckinnon A, Voss J, editors. *Equine reproduction*, Philadelphia: Lea
233 and Febiger; 1993, p. 578–87.
- 234 [3] Palmer JE. Rescuing foals during dystocia. In: Robinson NE, Kim AS, editors. *Current therapy in equine*
235 *medicine*. 6th ed. Philadelphia: Saunders Elsevier; 2009, p. 848–50.
- 236 [4] Lu KG, Barr BS, Embertson R, Schaer BD. Dystocia-a true equine emergency. *Clin Tech Equine Pract*
237 2006;5:145–53.
- 238 [5] Frazer G. Dystocia management. In: McKinnon AO, Squires EL, Vaala WE, Varner DD, editors. *Equine*
239 *reproduction* 2nd ed. New Jersey: Wiley-Backwell; 2011, p. 2479–96.
- 240 [6] Norton JL, Dallap BL, Johnston JK, Palmer JE, Sertich PL, Boston R, et al. Retrospective study of
241 dystocia in mares at a referral hospital. *Equine Vet J* 2007;39:37–41.
- 242 [7] Haas SD, Bristol F, Card CE. Risk factors associated with the incidence of foal mortality in an
243 extensively managed mare herd. *Can Vet J* 1996;37:91–5.
- 244 [8] Morley PS, Townsend HGG. A survey of reproductive performance in Thoroughbred mares and
245 morbidity, mortality and athletic potential of their foals. *Equine Vet J* 1997;29:290–7.
- 246 [9] Barrier AC, Haskell MJ, Birch S, Bagnall A, Bell DJ, Dickinson J, et al. The impact of dystocia on dairy
247 calf health, welfare, performance and survival. *Vet J* 2013;195:86–90.
- 248 [10] Civelek T, Celik HA, Avci G, Cingi CC. Effects of dystocia on plasma cortisol and cholesterol levels in
249 Holstein heifers and their newborn calves. *Bull Vet Inst Pulawy* 2008;52:649–54.
- 250 [11] Knottendelt D, Holdstock N, Madigan J. *Equine neonatology*. Philadelphia: Saunders; 2004.
- 251 [12] Aoki T, Ishii M. Hematological and Biochemical Profiles in Peripartum Mares and Neonatal
252 Foals (Heavy Draft Horse). *J Equine Vet Sci* 2012;32:170–6.
- 253 [13] Bazzano M, Giannetto C, Fazio F, Rizzo M, Giudice E, Piccione G. Physiological adjustments of
254 haematological profile during the last trimester of pregnancy and the early post partum period in mares.
255 *Anim Reprod Sci*. 2014;149:199–203.

- 256 [14] Ullrey DE, Struthers RD, Hendricks DG, Brent BE. Composition of mare's milk. *J Anim Sci.*
257 1966;25:217-22.
- 258 [15] Doreau M, Boulot S. Recent knowledge on mare milk production: a review. *Livest Prod Sci*
259 1989;22:213-35.
- 260 [16] Castagnetti C, Rametta M, Tudor Popeia R, Govoni N, Mariella J. Plasma levels of ACTH and cortisol
261 in normal and critically-ill neonatal foals. *Vet Res Commun* 2008;32(Suppl 1):127–9.
- 262 [17] Sellon DC, Wise LN. Disorder of the hematopoietic system. In Reed SM, Bayly WM, Sellon DC,
263 editors. *Equine Internal Medicine* 3rd ed. Philadelphia: Saunders; 2010, p. 730–76
- 264 [18] Wohlfender FD, Barrelet FE, Doherr MG, Straub R, Meier HP. Disease in neonatal foals. Part 2:
265 Potntial risk factors for a higher incidence of infectious diseases during the first 30 days postpartum.
266 *Equine Vet J* 2009;41:186–91.
- 267 [19] Sanchez LC. Sepsis. In Bradford PM, editors. *Large animal internal medicine* 5th ed. Missouri: Elsevier
268 mosby; 2015.
- 269 [20] Jain Nemi C. *Schalm's veterinary hematology* 4th ed. Philadelphia: Lea & Febiger; 1986.
- 270 [21] Pepys MB, Baltz ML, Tennent GA, Kent J, Ousey J, Rossdale PD. Serum amyloid A protein (SAA) in
271 horses: objective measurement of the acute phase response. *Equine Vet J* 1989;21:106–10.
- 272 [22] Chavatte PM, Pepys MB, Roberts B, Ousey JC, McGladdery AM, Rossdale PD. Measurement of serum
273 amyloid A protein (SAA) as an aid to differential diagnosis of infection in new-born foals. *Equine*
274 *infectious diseases* 4, 1992:33–8.
- 275 [23] Hultén C, Tulamo RM, Suominen MM, Burvall K, Marhaug G, Forsberg M. A non-competitive
276 chemiluminescence enzyme immunoassay for the acute phase protein serum amyloid A (SAA) - a
277 clinically useful inflammatory marker in the horse. *Vet Immunol Immunopathol* 1999;68:267–81.
- 278 [24] Jacobsen S, Andersen PH. The acute phase protein serum amyloid A (SAA) as a marker of
279 inflammation in horses. *Equine Vet Educ* 2007;19:38–46.
- 280 [25] Stoneham SJ, Palmer L, Cash R, Rossdale PD. Measurement of serum amyloid A in the neonatal foal
281 using a latex agglutination immunoturbidimetric assay: determination of the normal range, variation
282 with age and response to disease. *Equine Vet J* 2001;33:599–603.

- 283 [26] Gold JR, Divers TJ, Barton MH, Lamb SV, Place NJ, Mohammed HO, et al. Plasma
284 adrenocorticotropin, cortisol, and adrenocorticotropin/cortisol ratios in septic and normal-term foals. J
285 Vet Intern Med 2007;21:791–6.
- 286 [27] Engelking LR. Textbook of veterinary physiological chemistry 2nd ed. Massachusetts: Academic Press;
287 2010.
- 288 [28] Hart KA, Barton MH, Ferguson DC, Berghaus R, Slovis NM, Heusner GL, et al. Serum free cortisol
289 fraction in healthy and septic neonatal foals. J Vet Intern Med 2011;25:345–55.
- 290 [29] Hurcombe SDA, Toribio RE, Slovis NM, Kohn CW, Refsal K, Saville W, et al. Blood arginine
291 vasopressin, adrenocorticotropin hormone, and cortisol concentrations at admission in septic and
292 critically ill foals and their association with survival. J Vet intern Med 2008;22:639–47.
- 293 [30] Kohn RA, Dinneen MM, Russek-Cohen E. Using blood urea nitrogen to predict nitrogen excretion and
294 efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats. J Animal Sci
295 2005;83:879–89.
- 296 [31] McKenzie HC 3rd. Equine hyperlipidemias. Vet Clin North Am Equine Pract 2011;27:59–72.
- 297 [32] Watson TD, Murphy D, Love S. Equine hyperlipaemia in the United Kingdom: clinical features and
298 blood biochemistry of 18 cases. Vet Rec 1992;131:48–51.
- 299 [33] Kaneko JJ, Harvey JW, Bruss ML. Clinical biochemistry of domestic animals 6th ed. Massachusetts:
300 Academic Press; 2008.
- 301 [34] Valberg S, Jönsson L, Lindholm A, Holmegren N. Muscle histopathology and plasma aspartate
302 aminotransferase, creatine kinase and myoglobin changes with exercise in horses with recurrent
303 exertional rhabdomyolysis. Equine Vet J 1993;1:11–6.
- 304 [35] Ji LL. Oxidative stress during exercise: implication of antioxidant nutrients. Free Radic Biol Med
305 1995;18:1079–86.
- 306 [36] McIntosh LJ, Sapolsky RM. Glucocorticoids increase the accumulation of reactive oxygen species and
307 enhance adriamycin-induced toxicity in neuronal culture. Exp Neurol 1996;141:201–6.
- 308

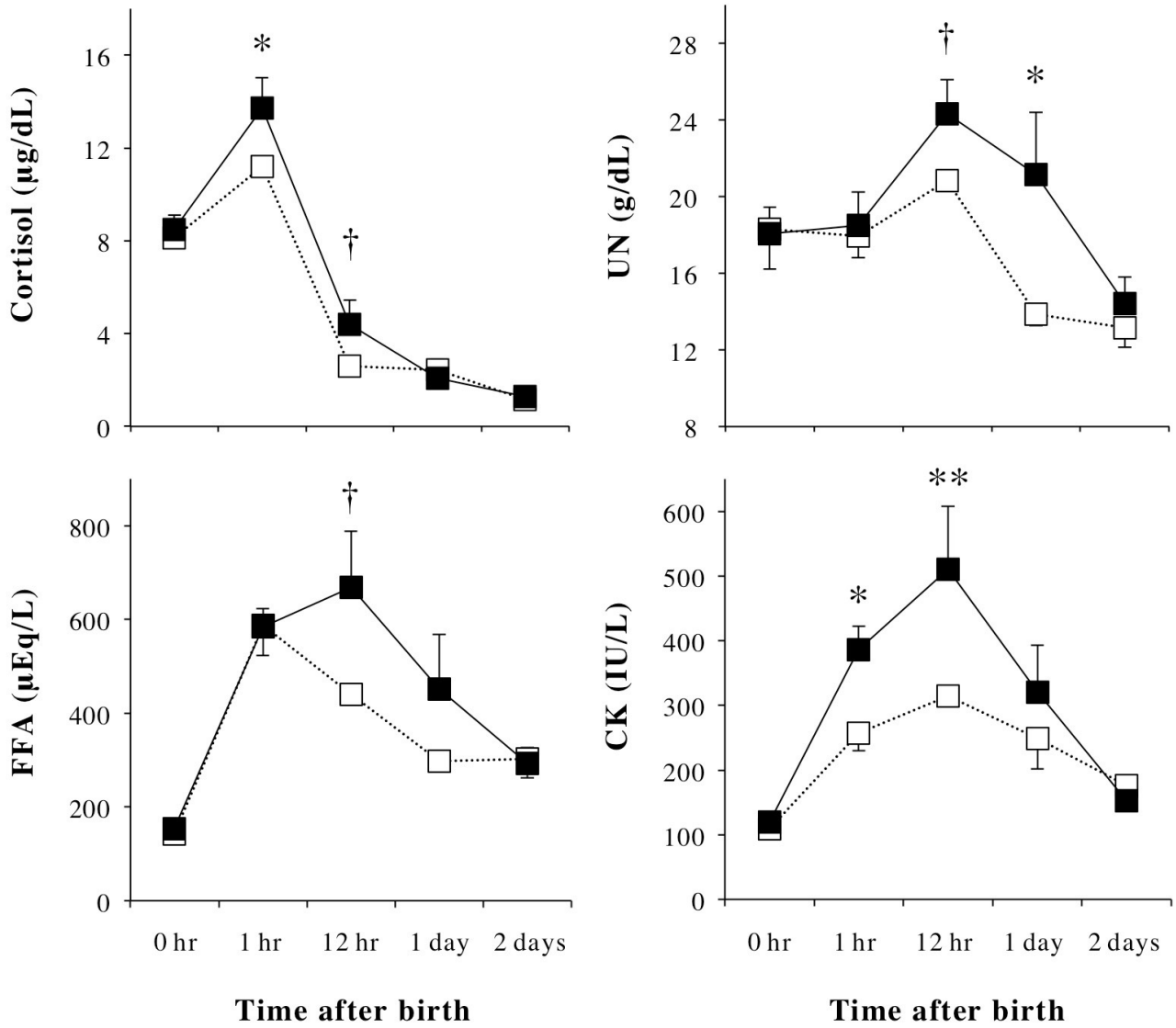


311

312

313 Fluctuations in red blood cell (RBC) and white blood cell (WBC) counts, hemoglobin (Hb) concentration,
 314 and packed cell volume (PCV) in newborn heavy draft foals within 2 days after birth. □, normal birth group
 315 ($n = 23$); ■, dystocia group ($n = 13$). Mean \pm standard error is shown. Significant differences between
 316 groups are denoted by * ($P < .05$) or ** ($P < .01$).

317



320

321

322 Fluctuations in serum cortisol, urea nitrogen (UN), free fatty acid (FFA), and creatine kinase (CK) levels
 323 in newborn heavy draft foals within 2 days after birth. □, normal birth group ($n = 23$); ■, dystocia group
 324 ($n = 13$). Mean \pm standard error is shown. Significant differences between groups are denoted by * (P
 325 $< .05$) or ** ($P < .01$).

326