Letter

Changes in blood parameters in pregnant Japanese White rabbits

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ABSTRACT — Changes in blood parameters, especially those related to blood coagulations, were examined on gestational days (GDs) 6, 18 and 28 in Japanese White rabbits. As compared with the non-pregnant group, the following changes were observed in the pregnant group. For blood coagulation-related parameters, fibrinogen, platelets and antithrombin time III (ATIII) was significantly increased on GD 18 and/or GD 28, and activated partial thromboplastin time (APTT) was significantly prolonged on GDs 18 and 28. For the other hematological and blood chemical parameters, hemoglobin and hematocrit values were significantly decreased on GDs 18 and 28. Reticulocyte counts significantly and prominently increased on GD 18 and then decreased to significantly lower levels than those in the non-pregnant group on GD 28. Eosinophils and basophil showed significant decreases on GD 18 and/or GD 28. The serum progesterone concentration reached its highest level on GD 18. Total protein (TP), albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), cholesterol, calcium, glucose and blood urea nitrogen were significantly decreased on GD 18 and/or GD 28. These results were almost similar to those in the pregnant New Zealand White rabbits (Mizoguchi *et al.*, 2010).

Key words: Japanese White pregnant rabbits, Clinical pathology parameters, Blood coagulation

INTRODUCTION

The pregnant rabbits are frequently and widely used in embryo-fetal development toxicity (teratology) studies. Although the main purpose of the study is to evaluate the embryo-fetal toxicity, it is necessary to consider the secondary effects of maternal toxicity in order to assess precisely the fetotoxicity of the compound administered. However, since there are only a few detailed studies on the changes of hematology and biochemistry parameters in pregnant rabbits (Mariscal *et al.*, 1994; Wells *et al.*, 1999), it is difficult to refer to the secondary effects of maternal toxicity.

We previously examined the changes in maternal blood parameters along the progression of pregnancy in rabbits of the New Zealand White strain (Kbl:NZW) rabbits (Mizoguchi *et al.*, 2010). However, there is a paucity of data pertaining to hematology and blood biochemistry of pregnant Japanese White (JW) rabbits. Therefore, this study was carried out to examine the changes in blood

parameters especially those related to blood coagulation in JW rabbits on gestational days (GDs) 6, 18 and 28 in order to compare them with those in pregnant NZW rabbits.

MATERIALS AND METHODS

Eighteen female rabbits of the Japanese White strain (Kbl:JW, Kitayama Labes, Co., Ltd., Nagano, Japan) at 6 or 7 months of age were used in this study. The animals were maintained individually in aluminum cages in an animal room under controlled conditions (temperature; $22.5 \pm 3.5^{\circ}\text{C}$, relative humidity; $50 \pm 20\%$, air ventilation; 10 to 15 times per hour, artificial lighting; 12 hr per day), and were allowed free access to diet (RC4, Oriental Yeast Co., Ltd., Tokyo, Japan) and to water via an automatic water supply system (Fuji Water Union, Shizuoka, Japan). After a 1-week acclimation period, 10 females which were judged to be in estrus were housed together with male rabbits from the same colony in cir-

cles (650 mm in diameter × 500 mm in height) for mating on a 1:1 basis. The day when copulation was confirmed was regarded as GD 0. Ten pregnant animals were assigned to the pregnant (P) group and the remaining 8 animals which were not subjected to mating to the non-pregnant (NP) group.

All animals were checked daily for general conditions. Body weights and food consumptions were also recorded during the experimental period.

Blood sampling was done on all animals of the two groups via the auricular artery on GDs 6, 18 and 28. Hematology (red blood cells [RBC], haemoglobin [Hb], haematocrit [Hct], mean cell volume [MCV], mean cell haemoglobin [MCH], mean corpuscular haemoglobin concentration [MCHC], reticulocyte, white blood cells [WBC], differential white blood cell count), blood biochemistry parameters (blood urea N [BUN], creatinine, total protein [TP], albumin, alkaline phosphatase [ALP], total cholesterol, triglycerides, glucose, sodium [Na], potassium [K], calcium [Ca], phosphorus [P] and chloride [Cl], aspartate aminotransferase [AST], alanine aminotransferase [ALT], lactate dehydrogenase [LDH] and creatine kinase [CPK]), coagulation-related parameters (fibrinogen, platelets, activated partial thromboplastin time [APTT], prothrombin time [PT] and antithrombin time III [ATIII]) and progesterone concentrations were analyzed according to the methods described in our previous report on (NZW) rabbits (Mizoguchi *et al.*, 2010). All animals were euthanized on GD 28 by exsanguination from the abdominal aorta under pentobarbital anesthesia.

The experimental procedures were conducted according to the Animal Welfare Guidelines of Bozo Research Center Inc.

The means ± S.D. were calculated for the parameters examined in each group, and were first analyzed for homogeneity of variance by F test between the NP- and P-groups. Then, homogeneous data were analyzed by Student's t-test and heterogeneous data by Aspin-Welch's t-test, respectively. A two-way analysis variance was performed (Group × Day) and there were significant differences for the Group × Day interaction in almost all parameters except for neutrophils, monocytes, LDH, CPK, glucose, Na, K, P, creatinine and platelets so further analysis was performed on the data from each day.

RESULTS

Body weights and food consumptions

There were no abnormal clinical signs observed in any animal throughout the gestation period. The body weight was not significantly different between the two groups,

although the food consumption in the P-group was 36% lower than that in the NP-group on GD 28.

Changes in blood coagulation-related parameters

As to the blood coagulation-related parameters in the P-group, compared with those in the NP-group, fibrinogen, platelet and ATIII values were significantly increased on GD 18 and/or GD 28, and APTT was significantly prolonged on GDs 18 and 28. On the other hand, PT showed no significant changes (Table 1).

Serum progesterone concentration

The serum progesterone concentration was significantly higher in the P-group than in the NP-group on GDs 6, 18 and 28. The concentration in the P-group peaked on GD 18 (Table 2).

Hematological findings

As to RBC parameters, RBC, Hb and Hct were significantly lower in the P-group than in the NP-group on GDs 18 and 28. On the other hand, reticulocytes were significantly higher on GD 18 and significantly lower on GD 28 in the P-group than in the NP-group. There were no significant differences in MCV, MCH and MCHC values between the two groups (Table 3-1).

As to WBC parameters, eosinophils and basophils showed significantly lower values in the P-group than in the NP-group on GD 18 and/or GD 28. Except for a low value of WBC on GD 28 in the P-group, there were no significant changes in other parameters between the two groups (Table 3-2).

Blood biochemical findings

On GD 18 and/or GD 28, TP, albumin, ALP, ALT and cholesterol values were significantly lower in the P-group than in the NP-group. In the P-group, TP was significantly higher on GD 6, AST on GD 28 and LDH on GD 18, respectively, as compared with those in the NP-group. On the other hand, in the P-group, as compared with the NP-group, the value of triglycerides was significantly higher on GD 18 while it was significantly lower on GD 28 (Table 4-1). In addition, glucose, Na, Ca, Cl, urea N and creatinine values in the P-group were significantly lower on GD 18 and/or GD 28. On the other hand, Ca and urea N values on GD 6 were significantly higher in the P-group than in the NP-group. There were no significant differences in K and P values between the two groups (Table 4-2).

Blood parameters in pregnant JW rabbits

Table 1. Changes in blood coagulation-related parameters in pregnant and non-pregnant rabbits

	Day	n	Fibrinogen (mg/dl)	Platelets ($\times 10^4/\mu\text{l}$)	APTT (s)	PT (s)	ATIII (%)
NP	6	8	258 $\pm$ 25	40.2 $\pm$ 10.7	22.2 $\pm$ 3.0	6.4 $\pm$ 0.1	115 $\pm$ 9
	18	8	263 $\pm$ 30	41.6 $\pm$ 13.8	23.1 $\pm$ 3.0	6.5 $\pm$ 0.2	114 $\pm$ 11
	28	8	229 $\pm$ 27	38.3 $\pm$ 7.0	23.0 $\pm$ 2.9	6.2 $\pm$ 0.1	113 $\pm$ 8
P	6	10	249 $\pm$ 30	47.0 $\pm$ 13.3	23.6 $\pm$ 3.2	6.5 $\pm$ 0.2	106 $\pm$ 10
	18	10	324 $\pm$ 49**	49.9 $\pm$ 13.3	29.4 $\pm$ 5.0**	6.6 $\pm$ 0.1	127 $\pm$ 10*
	28	10	295 $\pm$ 56##	55.9 $\pm$ 14.5##	33.5 $\pm$ 5.0**	6.2 $\pm$ 0.1	146 $\pm$ 12**

P, Pregnant rabbits; NP, Non-pregnant rabbits; Day, Gestational day in pregnant rabbit or day after sampling point in non-pregnant rabbit; *: $P < 0.05$, **: $P < 0.01$, significantly different from NP group (Student's t-test); ##: $P < 0.01$, significantly different from NP group (Aspin-Welch t-test).

Table 2. Changes in serum progesterone concentrations in pregnant and non-pregnant rabbits

	Day	n	Concentration (ng/ml)
NP	6	5	0.1 $\pm$ 0.1
	18	5	0.1 $\pm$ 0.2
	28	5	0.1 $\pm$ 0.1
P	6	5	5.9 $\pm$ 0.5##
	18	5	8.8 $\pm$ 1.0##
	28	5	7.7 $\pm$ 2.3##

P, Pregnant rabbits; NP, Non-pregnant rabbits; Day, Gestational day (P) ##: $P < 0.01$, significantly different from NP group (Aspin-Welch t-test).

DISCUSSION

In the present study, changes in blood parameters, especially those related to blood coagulation, were examined on GDs 6, 18 and 28 in JW rabbits and compared them with those in NZW ones (Mizoguchi *et al.*, 2010).

As a result, significant increases in fibrinogen, platelet and ATIII values and significant prolongation of APTT were detected on GD 18 and/or GD 28 as previously reported in NZW rabbits (Mizoguchi *et al.*, 2010). An increase in fibrinogens on GD 28 was more prominent in JW rabbits than in NZW rabbits (Mizoguchi *et al.*, 2010). PT values showed no significant changes like in the case of NZW rabbits (Mizoguchi *et al.*, 2010).

In JW rabbits, main RBC and WBC parameters signif-

icantly decreased on GDs 18 and 28, and this was considered to be secondary to hemodilution as reported in pregnant women (Burtis and Ashwood, 1994; DeRijk *et al.*, 2002) and pregnant NZW rabbits (Wells *et al.*, 1999; Mizoguchi *et al.*, 2010).

Reticulocytes drastically increased on GD18 when the serum progesterone concentration reached its peak level, suggesting that the increase of serum progesterone concentration might initiate the enhancement of erythropoiesis (Beischer and MacKay, 1978; DeRijk *et al.*, 2002). There are, however, conflicting reports of reticulocyte changes in pregnant women (Howells *et al.*, 1986; Mercelina-Roumans *et al.*, 1995). TP and albumin in pregnant JW rabbits decreased to the same level as previously reported in pregnant NZW rabbits (Wells *et al.*, 1999; Mizoguchi *et al.*, 2010) and women (Lind, 1980), and this is thought to reflect the increased blood volume. In addition, glucose and cholesterol values decreased on GD 18 and/or GD 28 as previously reported (Wells *et al.*, 1999; Mizoguchi *et al.*, 2010). Well *et al.* (1999) suggested that the decrease in serum concentration of glucose with the progression of pregnancy might be due to the increased demand for fetal growth. Triglycerides significantly increased on GD 18 and then decreased on GD 28 in JW rabbits as reported in NZW rabbits (Mizoguchi *et al.*, 2009). This suggests that the decreases of cholesterol, ALP and triglycerides values on GD 28 may reflect the decreased food consumption (36% lower compared with the NP-group).

Serum Ca and urea N significantly decreased on GD 18 and/or GD 28. In this connection, it was reported that women exhibited steady decreases in electrolytes because of the demand by the fetus during the fetal growth stage

Table 3-1. Changes in red blood parameters in pregnant and non-pregnant rabbits

	Day	n	RBC ($\times 10^4/\text{l}$)	Hb (g/dl)	Hct (%)	MCV (fl)	MCH (pg)	MCHC (%)	Reticulocytes ($10\text{E}^4/\mu\text{l}$)
NP	6	8	674 ± 33	14.9 ± 0.6	43.2 ± 1.7	64.2 ± 2.2	22.1 ± 1.0	34.5 ± 0.8	3.0 ± 0.4
	18	8	650 ± 37	14.0 ± 0.3	40.2 ± 1.4	62.0 ± 1.8	21.7 ± 1.1	34.9 ± 1.0	3.7 ± 0.7
	28	8	645 ± 36	13.9 ± 0.5	39.9 ± 1.5	61.9 ± 2.3	21.6 ± 1.1	34.9 ± 0.9	3.6 ± 0.4
P	6	10	656 ± 24	14.6 ± 0.4	43.3 ± 1.6	66.0 ± 1.8	22.3 ± 0.6	33.8 ± 0.8	2.7 ± 0.5
	18	10	$607 \pm 17^{\#}$	$13.4 \pm 0.5^{**}$	$37.7 \pm 1.5^{**}$	62.1 ± 2.2	22.1 ± 0.6	35.6 ± 0.6	$6.0 \pm 1.1^{**}$
	28	10	$560 \pm 25^{**}$	$12.0 \pm 0.7^{**}$	$34.5 \pm 1.8^{**}$	61.7 ± 2.7	21.4 ± 0.8	34.7 ± 1.0	$1.5 \pm 0.4^{**}$

P, Pregnant rabbits; NP, Non-pregnant rabbits; Day, Gestational day in pregnant rabbit or day after sampling point in non-pregnant rabbit; **: $P < 0.01$, significantly different from NP group (Student's t-test); #: $P < 0.05$, significantly different from NP group (Aspin-Welch t-test).

Table 3-2. Changes in white blood cell parameters in pregnant and non-pregnant rabbits

	Day	n	WBC ($\times 10^2/\mu\text{l}$)	Neutrophils ($\times 10^2/\mu\text{l}$)	Lymphocytes ($\times 10^2/\mu\text{l}$)	Eosinophils ($\times 10^2/\mu\text{l}$)	Basophils ($\times 10^2/\mu\text{l}$)	Monocytes ($\times 10^2/\mu\text{l}$)
NP	6	8	68.6 ± 13.6	14.4 ± 4.6	45.2 ± 12.6	1.0 ± 0.3	3.5 ± 0.7	3.1 ± 1.4
	18	8	68.0 ± 9.2	15.5 ± 4.6	42.5 ± 10.0	1.2 ± 0.4	3.6 ± 0.8	3.0 ± 1.2
	28	8	63.1 ± 10.9	14.3 ± 5.0	39.1 ± 9.4	1.2 ± 0.5	3.1 ± 0.8	3.1 ± 1.6
P	6	10	76.7 ± 11.4	16.2 ± 3.8	51.6 ± 8.9	1.0 ± 0.2	3.2 ± 0.5	3.5 ± 1.3
	18	10	72.4 ± 15.1	14.5 ± 2.3	49.0 ± 12.5	0.9 ± 0.3	$2.6 \pm 0.6^{**}$	3.5 ± 2.1
	28	10	53.8 ± 10.5	15.4 ± 3.7	33.0 ± 10.1	$0.4 \pm 0.2^{##}$	$1.3 \pm 0.5^{**}$	3.1 ± 1.3

P, Pregnant rabbits; NP, Non-pregnant rabbits; Day, Gestational day in pregnant rabbit or day after sampling point in non-pregnant rabbit; **: $P < 0.01$, significantly different from NP group (Student's t-test); #: $P < 0.01$, significantly different from NP group (Aspin-Welch t-test).

Table 4-1. Changes in blood biochemical parameters in pregnant and non-pregnant rabbits

	Day	n	TP (g/dl)	Albumin (g/dl)	ALP (IU/l)	AST (IU/l)	ALT (IU/l)	LDH (IU/l)	CPK (IU/l)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
NP	6	8	5.8 ± 0.3	4.7 ± 0.3	156 ± 23	16 ± 5	43 ± 14	57 ± 18	303 ± 88	44 ± 10	63 ± 20
	18	8	5.8 ± 0.3	4.6 ± 0.3	159 ± 23	17 ± 6	38 ± 17	53 ± 5	225 ± 71	41 ± 11	63 ± 18
	28	8	5.7 ± 0.3	4.5 ± 0.3	150 ± 30	17 ± 7	42 ± 14	66 ± 9	364 ± 197	38 ± 10	61 ± 19
P	6	10	$6.1 \pm 0.2^*$	4.9 ± 0.2	152 ± 36	16 ± 4	36 ± 14	60 ± 14	288 ± 93	37 ± 7	78 ± 44
	18	10	5.9 ± 0.3	4.6 ± 0.3	$100 \pm 21^{**}$	20 ± 6	37 ± 15	$61 \pm 7^*$	227 ± 81	$20 \pm 5^{##}$	$198 \pm 105^{##}$
	28	10	$5.0 \pm 0.3^{**}$	$3.7 \pm 0.3^{**}$	$75 \pm 24^{**}$	$35 \pm 24^{\#}$	$27 \pm 10^*$	71 ± 8	279 ± 140	$11 \pm 4^{##}$	$29 \pm 15^{**}$

P, Pregnant rabbits; NP, Non-pregnant rabbits Day, Gestational day in pregnant rabbit or day after sampling point in non-pregnant rabbit; *: $P < 0.05$, **: $P < 0.01$, significantly different from NP group (Student's t-test); #: $P < 0.05$, #: $P < 0.01$, significantly different from NP group (Aspin-Welch t-test).

Blood parameters in pregnant JW rabbits

Table 4-2. Changes in blood biochemistry parameters in pregnant and non-pregnant rabbits

	Day	n	Glucose (mg/dl)	Na (mmol/l)	K (mmol/l)	Ca (mg/dl)	P (mg/dl)	Cl (mmol/l)	Urea N (mg/dl)	Creatinine (mg/dl)
NP	6	8	131 ± 14	147 ± 2	4.1 ± 0.3	14.5 ± 0.3	3.9 ± 0.7	107 ± 3	24 ± 2	0.98 ± 0.23
	18	8	132 ± 18	146 ± 2	4.3 ± 0.5	14.2 ± 0.3	4.0 ± 0.5	110 ± 3	26 ± 2	0.94 ± 0.17
	28	8	127 ± 10	145 ± 2	4.0 ± 0.5	14.0 ± 0.5	3.8 ± 0.4	108 ± 3	26 ± 2	0.93 ± 0.14
P	6	10	142 ± 35	147 ± 2	4.1 ± 0.4	15.0 ± 0.5*	4.0 ± 0.4	107 ± 2	27 ± 3*	0.89 ± 0.10
	18	10	127 ± 19	144 ± 2*	4.0 ± 0.7	14.3 ± 0.8	4.1 ± 0.6	105 ± 2 ^{##}	21 ± 4 ^{##}	0.78 ± 0.11*
	28	10	114 ± 11*	144 ± 2	3.9 ± 0.5	12.8 ± 0.7**	3.5 ± 0.5	106 ± 3	19 ± 4**	0.87 ± 0.13

P, Pregnant rabbits; NP, Non-pregnant rabbits Day, Gestational day in pregnant rabbit or day after sampling point in non-pregnant rabbit; *, P < 0.05, **: P < 0.01, significantly different from NP group (Student's t-test); ##: P < 0.01, significantly different from NP group (Aspin-Welch t-test).

(Watney and Rudd, 1974).

In the present study, ALP activity, one of the indicators of liver function, significantly decreased on GDs 18 and 28. Wells *et al.* (1999) reported that a decrease in ALP activity might reflect increased estrogen levels or an increase in the ratio of serum estrogen to progesterone. It has been reported that, in pregnant rabbits, estrogen concentration increased from GD 7 and thereafter it remained relatively stable until the last 2 or 3 days before parturition (Challis *et al.*, 1973; Mariscal *et al.*, 1994), and that progesterone concentration decreased from GD 15 to the last stage of gestation (Challis *et al.*, 1973).

Increased AST activity and decreased ALT activity observed in the present study on GD 28 were consistent with those of NZWs (Mizoguchi *et al.*, 2010). As reported in rats subjected to fasting or restricted feeding (Toropila *et al.*, 2005; Moriyama *et al.*, 2008), the former was considered to be brought about by the hepatic hyperfunction. However, the cause of the latter remains obscure.

In the present study, as mentioned before, serum progesterone concentration reached its highest level on GD 18, and then decreased until GD 28, although plasma estrogen concentration was not examined.

In conclusion, pregnant rabbits of the Kbl:JW strain showed almost the similar changes in blood parameters with those in pregnant rabbits of Kbl:NZW strain, although an increase in fibrinogens was more prominent in JW rabbits than in NZW rabbits.

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