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Changes in Blood Parameters and the Expression of Coagulation-Related Genes in Lactating Sprague–Dawley Rats

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This study measured blood parameters, particularly those related to coagulation, and alterations in the expression levels of blood-coagulation–related genes in lactating Sprague–Dawley rats. The day of delivery was designated as lactation day 0 (LD 0). On the day after delivery (LD 1), prothrombin time and overall activity of vitamin-K–dependent coagulation factors were decreased, whereas fibrinogen contents, platelet counts and antithrombin III concentrations were increased as compared with those in nonpregnant rats. In addition, hepatic expression of blood-coagulation–related genes in the liver was increased at LD 0 as compared with that in nonpregnant rats. These changes may be physiologic responses to prevent prolonged bleeding at delivery. Except for fibrinogen content, which remained elevated, the described changes returned to baseline on and after LD 7. Activities of AST, ALT, and ALP were increased on LD 7, 14, and 21 as compared with nonpregnant rats. In contrast, total protein, albumin, Cl, and Ca were consistently lower on LD 7, 14, or 21 as compared with levels in nonpregnant rats. These results provide background data for evaluation of nursing rats.

Abbreviations: GD, gestation day; LD, lactation day.

Pregnant animals are often used as models for pregnant women.[2,4,6] We previously evaluated maternal blood parameters, especially those related to blood coagulation, during the progression of pregnancy in Sprague–Dawley rats[21] and New Zealand White strain rabbits.[11]

Toxicity studies using infant Sprague–Dawley rats of the strain are common. Precise assessment of chemically induced toxicity in infants requires that consideration of maternal effects. However, few data regarding changes in maternal blood parameters during lactation have been reported. We performed the current study to clarify how blood parameters, especially those related to blood coagulation, change during the course of lactation in rats. In addition, we used DNA microarray analysis of the liver to examine changes in the expression of blood coagulation-related genes.

Materials and Methods

Animals. Male and female SPF Sprague–Dawley rats (age, 11 or 12 wk) were purchased from Charles River Laboratories Japan (Kanagawa, Japan). The animal room was maintained under controlled conditions (temperature, 21 to 26 °C; relative humidity, 30% to 64%; ventilation, 10 to 15 changes hourly; 12:12-h light:dark cycle). For mating, 1 male rat and 1 female rat were caged together for a maximum of 1 wk. Daily vaginal smears were obtained, and gestational day (GD) 0 was assigned when a sperm-positive vaginal smear was obtained. The day of delivery was designated as postpartum day 0 (that is, lactation day 0 [LD 0]). A total of 50 lactating rats were used as the experimental group for this study. In addition, 25 nonpregnant virgin female rats were included as a control group. The rats were housed individually in bracket-type stainless steel wire-mesh cages (254 mm × 350 mm × 170 mm) during the gestational period (until GD 17) and for 1 wk after weaning. During the last gestational stage and lactation, rats were housed with their litters in plastic cages (340 mm × 400 mm × 185 mm: CLEA Japan, Tokyo, Japan) containing bedding (White Flakes, Charles River Laboratories Japan, Yokohama, Japan). All rats received standard pelletted food (NMF, Oriental Yeast, Tokyo, Japan) and municipal tap water ad libitum throughout the acclimation and experimental periods. Experimental procedures were conducted according to the *Guide for the Care and Use of Laboratory Animals*.[9]

Experimental design. Rats were euthanized by exsanguination from the abdominal aorta under ether anesthesia. Ten lactating rats and 5 nonpregnant rats were euthanized on LD 1, 7, 4, and 21 (that is, the day of weaning) and at 1 wk after weaning. An additional 5 lactating rats and 5 nonpregnant rats were euthanized on LD 0 and 14 for DNA microarray analysis to detect blood-coagulation–related gene expression in the liver. Rats were checked daily for clinical signs and were weighed immediately before euthanasia.

Hematology. At euthanasia of each rat, blood (approximately 1 mL) was collected from the abdominal aorta into blood collection tubes (SB41, Sysmex, Hyogo, Japan) containing dipotassium EDTA. RBC count, hemoglobin, hematocrit, MCV, MCH, MCHC, reticulocyte count, platelet count, total WBC count, and differential leukocyte count were assayed by using an automated hematology system (Advia 120,Siemens Healthcare Diagnostics, Deerfield, IL). In addition, blood (approximately 0.9 mL) was collected into blood collection tubes containing 3.8% (w/v) sodium citrate (Wako Pure Chemical Industries, Tokyo, Japan) and centrifuged at 1600 × *g* for 10 min to separate plasma. The plasma sample obtained was examined

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for PT, aPTT, and fibrinogen content by using an automated coagulometer (ACL 100, Instrumentation Laboratory, Bedford, MA); for antithrombin III using an automatic clinical chemistry analyzer (TBA120FR, Toshiba Medical Systems, Tokyo, Japan); and for overall activity of vitamin-K–dependent coagulation factors by using a commercial assay system (Thrombo Test, Axis-shield, Oslo, Norway).

Blood chemistry. Blood (approximately 4 mL) was collected from each rat as described earlier, put into test tubes containing coagulant (Venoject II-Autosep, Terumo, Tokyo, Japan), and centrifuged at 1600 × *g* for 10 min to separate serum. The serum samples obtained were evaluated for ALP, total cholesterol, triglycerides, phospholipids, total bilirubin, glucose, BUN, creatinine, Na, K, Cl, Ca, P, total protein, and albumin by using an automatic clinical chemistry analyzer (TBA120FR, Toshiba Medical Systems). Additional blood (approximately 2.0 mL) from each rat was collected into tubes containing heparin sodium (20 U heparin per 1 mL blood) and centrifuged at 1600 × *g* for 10 min to separate plasma. The plasma sample obtained was examined for AST, ALT, LDH, CPK, and GGT by using an automatic clinical chemistry analyzer (TBA120FR, Toshiba Medical Systems).

RNA extraction, microarray analysis, and microarray data analysis. Liver slices obtained at LD 0 and 14 were submerged immediately in RNA stabilization reagent (RNAlater, Qiagen, Valencia, CA). After being incubated at 4 °C overnight, samples were stored at –80 °C until total RNA was prepared. Total RNA was extracted and purified by using a commercial kit (RNeasy Mini Kit, Qiagen) according to the manufacturer's instructions. The integrity of the purified total RNA was verified by denaturing agarose gel electrophoresis.

Microarray analysis was performed according to the protocol provided by the manufacturer of the microarray chip (Affymetrix, Santa Clara, CA). Briefly, 10 µg total RNA from each rat was the template for cDNA synthesis by using the T7-$(dT)_{24}$ primer (5′ GGC CAG TGA ATT GTA ATA CGA CTC ACT ATA GGG AGG CGG $(dT)_{24}$ 3′).

The cDNA was resuspended in RNase-free water and used to synthesize biotinylated cRNA (3′-Amplification Reagents for IVL Labeling Kit, Affymetrix). After 16 h of incubation at 37 °C, the resultant biotin-labeled cRNA was fragmented and stored at –20 °C until hybridization. Hybridization solution containing biotinylated cRNA was prepared by using a commercial kit (GeneChip Eukaryotic Hybridization Control Kit, Affymetrix) and exposed to a microarray chip (Rat Expression Array 230A, Affymetrix) at 45 °C for 16 h in a hybridization oven (GeneChip Hybridization Oven 640, Affymetrix). The chips were washed and stained automatically (Fluidics Station, Affymetrix) and then scanned (Gene Array Scanner, Affymetrix).

Images from the scanned chips were processed and analyzed by using Affymetrix Microarray Analysis Suite 5.0 and Excel (Microsoft, Redmond, WA). For analysis of the different target RNA samples (LD 0 and 14), values for gene expression from samples from nonpregnant virgin controls were set as baseline. After global normalization of each experimental datum, the fold change was derived as the ratio of average differences from an experimental array compared with a control array. Statistical analysis was performed by using Student *t* and Welch *t* tests (Excel, Microsoft) as appropriate. Genes with low reliability (detection *P* value greater than 0.05) were excluded from the analysis.

Statistical analysis. The mean (± 1 SD) was calculated for each of the parameters examined for each rat, and these results were analyzed for differences between the lactating groups by using an F test (Excel, Microsoft). Further analysis was performed by using Student or Aspin–Welch *t* tests (Excel, Microsoft). A *P* value of less than 0.05 indicated statistically significant difference.

Results

Clinical signs and pathologic findings. No abnormal clinical signs or pathologic findings were observed in any animals.

Blood coagulation-related parameters. Fibrinogen content, platelet count, and antithrombin III concentration were significantly ($P < 0.01$) higher in rats at LD 1 as compared with nonpregnant rats. Platelet count gradually returned to baseline levels, whereas fibrinogen content remained elevated. The antithrombin III concentration was significantly ($P < 0.01$ or $P < 0.05$) lower on and after LD 7 as compared with that in nonpregnant rats.

PT and overall activities of vitamin-K-dependent coagulation factors were significantly ($P < 0.01$) decreased in rats at LD 1 as compared with nonpregnant rats. Thereafter no significant differences in vitamin-K–dependent coagulation factors were observed between lactating rats and nonpregnant rats, except for significantly prolonger values of overall activities of vitamin-K-dependent coagulation factors in rats at LD 14 and aPTT in rats at LD 21 than in the nonpregnant group.

Compared with nonpregnant rats, PT and the overall activity of vitamin-K–dependent coagulation factors were significantly ($P < 0.01$ or 0.05) increased in lactating rats at 1 wk after weaning. However, compared with that in nonpregnant rats, antithrombin III concentration was significantly ($P < 0.05$) lower in lactating rats at 1 wk after weaning. The other parameters showed no significant differences between the 2 groups (Table 1).

Hematologic parameters. During most of the lactation period, RBC count, hemoglobin, hematocrit, and MCHC were significantly ($P < 0.01$ or $P < 0.05$) lower in the lactating group than in the nonpregnant group. In contrast, MCV, MCH, and reticulocyte counts were significantly ($P < 0.01$) higher in the lactating group than in the nonpregnant group on LD 7, 14, and/or 21. Neutrophil counts were significantly ($P < 0.01$) higher in the lactating group than in the nonpregnant group on LD 1, 7, and 14. However, there were no significant differences in total WBC counts between the 2 groups throughout the lactation period.

At 1 wk after weaning, almost all of the RBC-related parameters were significantly ($P < 0.01$) higher in the lactating group than in the nonpregnant group, except for significantly lower values of MCHC and reticulocyte counts in the lactating group than in the nonpregnant group. Lymphocyte, eosinophil, and monocyte counts were significantly ($P < 0.01$ or $P < 0.05$) higher in lactating rats than nonpregnant rats, but there were no significant differences in total WBC counts between the 2 groups throughout the lactation period (Tables 2 and 3).

Blood chemistry parameters. There were no significant changes in any enzyme activity parameters at LD 1 as compared with data from nonpregnant rats. From LD 7 to 21, activities of AST, ALT, ALP, and CPK were significantly ($P < 0.01$ or $P < 0.05$) higher in lactating rats than in nonpregnant rats, except for AST at LD 21. Except for LDH activity at LD 14, which was significantly ($P < 0.05$) lower in the lactating rats than in nonpregnant rats, there were no significant differences in LDH and GGT between the 2 groups throughout the lactation period. On LD 1, lactating and nonpregnant rats showed no significant differences in total cholesterol, phospholipids, total bilirubin, BUN, Na, K, Cl, Ca, P, total protein, and albumin values, whereas values for triglycerides, glucose, and albumin:globulin ratio were significantly ($P < 0.01$ or $P < 0.05$) lower and creatinine values

Table 1. Blood coagulation-related parameters (mean ± 1 SD) in nonpregnant and lactating rats

Sampling day		Fibrinogen (mg/dL)	Platelets ($\times 10^4$/μL)	PT (s)	Overall activity of vitamin-K–dependent coagulation factors (s)	aPTT (s)	Antithrombin III (%)
LD 1	Nonpregnant	213 ± 16	103.3 ± 9.7	14.2 ± 0.6	29.31 ± 1.81	17.0 ± 1.6	152 ± 10
	Lactating	332 ± 34[a]	192.2 ± 19.3[a]	12.6 ± 0.9[a]	25.00 ± 1.70[a]	18.6 ± 2.1	179 ± 18[a]
LD 7	Nonpregnant	203 ± 15	111.3 ± 9.6	13.7 ± 1.3	28.36 ± 2.65	16.3 ± 2.3	146 ± 8
	Lactating	250 ± 26[a]	103.8 ± 11.5	13.4 ± 1.5	30.37 ± 2.95	16.7 ± 3.8	134 ± 11[b]
LD 14	Nonpregnant	211 ± 7	116.7 ± 12.8	12.9 ± 1.3	26.52 ± 1.93	16.9 ± 1.4	149 ± 7
	Lactating	244 ± 32[c]	109.3 ± 9.6	13.8 ± 0.7	29.53 ± 1.91[b]	17.8 ± 2.2	130 ± 9[a]
LD 21	Nonpregnant	216 ± 17	120.6 ± 11.4	13.0 ± 0.8	28.58 ± 2.22	15.7 ± 0.9	144 ± 6
	Lactating	240 ± 16[b]	125.2 ± 14.2	13.6 ± 0.8	30.16 ± 1.51	18.5 ± 2.8[d]	130 ± 5[a]
1 wk after weaning	Nonpregnant	212 ± 11	114.7 ± 10.5	12.4 ± 0.3	25.53 ± 0.86	17.1 ± 1.3	161 ± 4
	Lactating	225 ± 16	104.3 ± 18.6	13.3 ± 0.4[a]	27.19 ± 1.43[b]	18.9 ± 2.1	152 ± 7[b]

[a]$P < 0.01$ compared with value from nonpregnant animals (Student t test; $n = 5$ nonpregnant group, 10 lactating group)
[b]$P < 0.05$ compared with value from nonpregnant animals (Student t test; $n = 5$ nonpregnant group, 10 lactating group)
[c]$P < 0.01$ compared with value from nonpregnant animals (Aspin–Welch t test; $n = 5$ nonpregnant group, 10 lactating group)
[d]$P < 0.05$ compared with value from nonpregnant animals (Aspin–Welch t test; $n = 5$ nonpregnant group, 10 lactating group)

Table 2. Hematology values (mean ± 1 SD) in nonpregnant and lactating rats

Sampling day		RBC count ($\times 10^4$/μL)	Hemoglobin (g/dL)	Hematocrit (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Reticulocytes (%)
LD 1	Nonpregnant	791 ± 44	15.7 ± 0.3	40.6 ± 0.8	51.4 ± 1.4	19.9 ± 1.3	38.6 ± 0.5	1.6 ± 0.2
	Lactating	660 ± 33[a]	12.6 ± 0.5[a]	33.6 ± 1.4[a]	50.9 ± 2.1	19.0 ± 0.7	37.4 ± 0.4[a]	2.0 ± 1.4
LD 7	Nonpregnant	821 ± 25	15.9 ± 0.4	41.6 ± 1.6	50.7 ± 2.6	19.4 ± 0.7	38.3 ± 0.4	1.8 ± 0.7
	Lactating	688 ± 26[a]	13.9 ± 0.6[a]	37.6 ± 1.3[a]	54.7 ± 2.1[a]	20.1 ± 0.5[c]	36.8 ± 0.5[a]	5.5 ± 0.8[a]
LD 14	Nonpregnant	790 ± 28	15.4 ± 0.5	40.2 ± 1.2	50.9 ± 2.2	19.5 ± 0.3	38.3 ± 0.5	2.1 ± 0.2
	Lactating	740 ± 34[b]	14.7 ± 0.6	39.4 ± 1.5	53.2 ± 1.5[c]	19.9 ± 0.5	37.4 ± 0.5[a]	2.0 ± 0.4
LD 21	Nonpregnant	817 ± 29	15.5 ± 0.3	40.5 ± 1.1	49.5 ± 0.6	19.0 ± 0.5	38.5 ± 0.4	1.4 ± 0.4
	Lactating	771 ± 30[b]	15.5 ± 0.4	41.4 ± 1.0	53.8 ± 1.7[a]	20.2 ± 0.9[c]	37.5 ± 0.6[a]	1.8 ± 0.6
1 wk after weaning	Nonpregnant	803 ± 27	15.3 ± 0.3	41.0 ± 0.6	51.1 ± 1.3	19.1 ± 0.5	37.4 ± 0.2	2.2 ± 0.3
	Lactating	848 ± 23[a]	17.1 ± 0.5[a]	46.5 ± 1.3[a]	54.9 ± 1.8[a]	20.2 ± 0.6[a]	36.8 ± 0.4[a]	0.5 ± 0.2[a]

[a]$P < 0.01$ compared with value from nonpregnant animals (Student t test; $n = 5$ nonpregnant group, 10 lactating group)
[b]$P < 0.05$ compared with value from nonpregnant animals (Student t test; $n = 5$ nonpregnant group, 10 lactating group)
[c]$P < 0.01$ compared with value from nonpregnant animals (Aspin–Welch t test; $n = 5$ nonpregnant group, 10 lactating group)

were significantly ($P < 0.05$) higher in lactating compared with nonpregnant rats. On and after LD 7, total cholesterol , glucose, and BUN were significantly ($P < 0.01$ or $P < 0.05$) higher in lactating than nonpregnant rats. In contrast, total bilirubin, Cl, Ca, P, total protein, and albumin were significantly ($P < 0.01$) lower in lactating than nonpregnant rats at LD 7, 14, and/or 21. During the same period, the 2 groups showed no significant differences in triglycerides, phospholipids, creatinine, Na, K, and albumin:globulin ratio.

At 1 wk after weaning, the 2 groups no longer showed significant ($P < 0.05$) differences in blood chemistry, except for significantly lower values in AST, ALT, Cl, and Ca and a significantly higher values in CPK value in lactating compared with nonpregnant rats (Tables 4 through 6).

Changes in blood coagulation-related genes expression. The expression of blood coagulation-related genes in the liver, fibrinogen, coagulation factors, and vitronectin was higher in lactating rats than in nonpregnant rats on LD 0 but did not differ between the groups on LD 14 (Table 7).

Discussion

Compared with those in nonpregnant rats, PT and overall activities of vitamin-K-dependent coagulation factors were decreased and fibrinogen contents and platelet counts were increased in lactating rats on LD 1. In addition, the hepatic expression of blood coagulation-related genes such as fibrinogen, coagulation factors, and vitronectin was higher at LD 0 in lactating rats than in nonpregnent rats. These changes were

Table 3. WBC parameters (mean ± 1 SD) in nonpregnant and lactating rats

			Differential leukocyte counts ($\times 10^2/\mu L$)					
Sampling day		Total WBC	Lymphocytes	Neutrophils	Eosinophils	Basophils	Monocytes	Large unstained cells
LD 1	Nonpregnant	113.7 ± 43.5	95.0 ± 36.1	13.1 ± 5.0	1.5 ± 0.7	0.5 ± 0.3	2.3 ± 1.6	1.2 ± 0.9
	Lactating	93.7 ± 18.5	61.0 ± 15.3	28.9 ± 5.4[a]	1.0 ± 0.6	0.2 ± 0.1	2.0 ± 0.7	0.7 ± 0.2
LD 7	Nonpregnant	84.9 ± 23.6	71.2 ± 26.6	10.0 ± 5.7	1.0 ± 0.7	0.3 ± 0.2	1.5 ± 0.7	0.9 ± 0.6
	Lactating	86.9 ± 21.1	54.8 ± 14.5	28.6 ± 8.2[a]	0.9 ± 0.2	0.2 ± 0.1	1.8 ± 0.6	0.6 ± 0.2
LD 14	Nonpregnant	75.5 ± 23.0	58.2 ± 22.5	13.5 ± 2.4	0.9 ± 0.3	0.2 ± 0.1	1.8 ± 0.8	0.9 ± 0.5
	Lactating	73.1 ± 14.2	48.2 ± 10.1	22.1 ± 7.7[c]	0.9 ± 0.4	0.2 ± 0.0	1.3 ± 0.5	0.6 ± 0.2
LD 21	Nonpregnant	84.4 ± 27.4	66.6 ± 20.5	13.2 ± 5.5	1.3 ± 0.6	0.3 ± 0.2	1.9 ± 0.6	1.2 ± 1.0
	Lactating	76.4 ± 16.5	54.0 ± 11.7	18.9 ± 7.9	0.7 ± 0.3	0.2 ± 0.1	1.8 ± 0.7	0.8 ± 0.3
1 wk after weaning	Nonpregnant	48.4 ± 10.1	37.1 ± 5.9	8.8 ± 4.6	0.9 ± 0.6	0.1 ± 0.1	1.0 ± 0.3	0.5 ± 0.3
	Lactating	63.5 ± 14.2	48.5 ± 10.5[b]	9.8 ± 3.5	2.4 ± 0.9[a]	0.1 ± 0.1	1.7 ± 0.6[b]	0.9 ± 0.2

[a]$P < 0.01$ compared with value from nonpregnant animals (Student t test; $n = 5$ nonpregnant group, 10 lactating group)
[b]$P < 0.05$ compared with value from nonpregnant animals (Student t test; $n = 5$ nonpregnant group, 10 lactating group)
[c]$P < 0.01$ compared with value from nonpregnant animals (Aspin–Welch t test; $n = 5$ nonpregnant group, 10 lactating group)

Table 4. Blood chemistry parameters (IU/L; mean ± 1 SD) in nonpregnant and lactating rats

Sampling day		AST	ALT	LDH	GGT	ALP	CPK
LD 1	Nonpregnant	58 ± 8	37 ± 6	49 ± 9	1 ± 0	446 ± 148	54 ± 8
	Lactating	61 ± 14	32 ± 8	54 ± 12	1 ± 0	313 ± 228	58 ± 10
LD 7	Nonpregnant	52 ± 7	35 ± 6	46 ± 11	1 ± 0	398 ± 70	51 ± 5
	Lactating	64 ± 6[a]	60 ± 8[a]	40 ± 6	1 ± 0	956 ± 418[c]	84 ± 42[d]
LD 14	Nonpregnant	51 ± 4	31 ± 8	48 ± 10	1 ± 0	379 ± 202	52 ± 3
	Lactating	67 ± 9[a]	62 ± 9[a]	35 ± 9[b]	1 ± 0	720 ± 219[b]	96 ± 20[a]
LD 21	Nonpregnant	63 ± 18	44 ± 13	56 ± 17	1 ± 0	419 ± 174	62 ± 16
	Lactating	78 ± 12	76 ± 11[a]	42 ± 7	1 ± 0	902 ± 404[a]	128 ± 30[c]
1 wk after weaning	Nonpregnant	63 ± 6	39 ± 5	42 ± 9	1 ± 1	409 ± 105	53 ± 9
	Lactating	53 ± 5[a]	28 ± 4[a]	41 ± 4	2 ± 1	364 ± 67	67 ± 11[b]

[a]$P < 0.01$ compared with value from nonpregnant animals (Student t test; $n = 5$ nonpregnant group, 10 lactating group)
[b]$P < 0.05$ compared with value from nonpregnant animals (Student t test; $n = 5$ nonpregnant group, 10 lactating group)
[c]$P < 0.01$ compared with value from nonpregnant animals (Aspin–Welch t test; $n = 5$ nonpregnant group, 10 lactating group)
[d]$P < 0.05$ compared with value from nonpregnant animals (Aspin–Welch t test; $n = 5$ nonpregnant group, 10 lactating group)

similar to those observed during the late period of pregnancy in rats,[21] and to those reported in women during gestation.[18] These changes may be physiologic responses to prevent prolonged bleeding at delivery. Moreover, antithrombin III concentration was increased at the same time, suggesting prevention of the development of deep-tissue thrombosis, as reported for pregnant women.[2,3,4,6,8]

Except for fibrinogen values (which remained increased throughout lactation), the observed changes blood coagulation-related parameters were no longer present on LD 7 and later. Reduced antithrombin III concentrations around the midstage of lactation has been reported in rats.[13,14] A previous study[7] found reduced levels cytochrome P isozymes in the liver during lactation; this response may be related to the hormonal demands of milk production during lactation. Given that antithrombin III is synthesized in the liver, decreased antithrombin III concentration may occur by a similar mechanism.[16] By this same rationale, because vitamin-K-dependent coagulation factors are produced in the liver,[1] their prolonged overall activity at LD 14 similarly seems to be due to the demand for milk production.

Our lactating rats demonstrated decreases in RBC count, hemoglobin, hematocrit, and MCHC on LD 1. These changes suggest an occurrence of bleeding at delivery. The subsequent increases in reticulocyte counts on LD 7 and MCV and MCHC on and after LD 7 suggested the presence of anemia, but these parameters gradually returned to baseline levels. At 1 wk after weaning, RBC count, hemoglobin, hematocrit, MCV, and MCH were increased, whereas MCHC remained constant and reticulocyte counts were decreased. In the lactating group, neutrophil counts were increased from LD1 to 14 and lymphocytes, eosi-

Table 5. Blood chemistry parameters (mg/dL; mean ± 1 SD) in lactating rats

Sampling day		Total cholesterol	Triglycerides	Phospholipids	Total bilirubin	Glucose	BUN	Creatinine
LD 1	Nonpregnant	61 ± 7	62 ± 7	128 ± 10	0.0 ± 0.0	165 ± 12	19 ± 2	0.27 ± 0.01
	Lactating	72 ± 11	27 ± 8[a]	147 ± 22	0.1 ± 0.1	132 ± 25[b]	16 ± 4	0.30 ± 0.03[b]
LD 7	Nonpregnant	61 ± 6	82 ± 27	138 ± 12	0.1 ± 0.1	171 ± 17	19 ± 2	0.26 ± 0.03
	Lactating	74 ± 6[a]	58 ± 12	151 ± 12	0.0 ± 0.0	153 ± 8	23 ± 3[b]	0.30 ± 0.03
LD 14	Nonpregnant	69 ± 11	55 ± 32	144 ± 28	0.1 ± 0.0	167 ± 22	18 ± 3	0.29 ± 0.03
	Lactating	71 ± 7	46 ± 7	136 ± 12	0.0 ± 0.0[c]	163 ± 10	23 ± 2[a]	0.27 ± 0.02
LD 21	Nonpregnant	64 ± 8	144 ± 110	148 ± 23	0.0 ± 0.0	156 ± 14	19 ± 2	0.30 ± 0.03
	Lactating	66 ± 5	66 ± 5	120 ± 12	0.0 ± 0.0	170 ± 11[b]	24 ± 2[a]	0.29 ± 0.03
1 wk after weaning	Nonpregnant	69 ± 6	45 ± 15	137 ± 13	0.1 ± 0.1	156 ± 12	20 ± 2	0.26 ± 0.02
	Lactating	69 ± 10	58 ± 15	132 ± 16	0.1 ± 0.0	152 ± 10	20 ± 1	0.26 ± 0.04

[a]$P < 0.01$ compared with value from nonpregnant animals (Student t test; $n = 5$ nonpregnant group, 10 lactating group)
[b]$P < 0.05$ compared with value from nonpregnant animals (Student t test; $n = 5$ nonpregnant group, 10 lactating group)
[c]$P < 0.01$ compared with value from nonpregnant animals (Aspin–Welch t test; $n = 5$ nonpregnant group, 10 lactating group)

Table 6. Clinical chemistry parameters (mean ± 1 SD) in nonpregnant and lactating rats

Sampling day		Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	Ca (mg/dL)	P (mg/dL)	Total Protein (g/dL)	Albumin (g/dL)	Albumin: globulin ratio
LD 1	Nonpregnant	143 ± 1	4.1 ± 0.4	109 ± 2	10.2 ± 0.2	6.1 ± 0.8	6.5 ± 0.4	3.1 ± 0.2	0.93 ± 0.05
	Lactating	144 ± 1	4.3 ± 0.3	108 ± 2	9.8 ± 0.5	5.4 ± 0.9	6.6 ± 0.4	3.0 ± 0.2	0.69 ± 0.06[a]
LD 7	Nonpregnant	142 ± 2	4.0 ± 0.1	110 ± 2	10.0 ± 0.2	5.9 ± 0.7	6.4 ± 0.3	3.0 ± 0.1	0.91 ± 0.12
	Lactating	142 ± 2	4.1 ± 0.3	108 ± 2	9.8 ± 0.4	5.1 ± 1.2	6.0 ± 0.2[a]	2.7 ± 0.1[a]	0.80 ± 0.07
LD 14	Nonpregnant	142 ± 1	4.1 ± 0.4	109 ± 1	10.3 ± 0.5	5.9 ± 0.6	6.8 ± 0.3	3.2 ± 0.2	0.83 ± 0.08
	Lactating	142 ± 1	4.1 ± 0.3	107 ± 1[a]	9.4 ± 0.4[a]	4.8 ± 0.6[a]	5.7 ± 0.3[a]	2.6 ± 0.1[c]	0.81 ± 0.06
LD 21	Nonpregnant	142 ± 1	4.0 ± 0.2	109 ± 2	10.0 ± 0.4	5.3 ± 0.1	6.8 ± 0.4	3.2 ± 0.2	0.89 ± 0.08
	Lactating	142 ± 1	4.2 ± 0.4	107 ± 2[a]	9.2 ± 0.2[a]	5.5 ± 1.1	5.6 ± 0.3[a]	2.7 ± 0.1[a]	0.88 ± 0.09
1 wk after weaning	Nonpregnant	142 ± 1	4.3 ± 0.3	110 ± 0	10.4 ± 0.3	5.3 ± 0.9	6.8 ± 0.4	3.3 ± 0.3	0.93 ± 0.06
	Lactating	141 ± 1	4.2 ± 0.3	109 ± 1[d]	10.1 ± 0.2[b]	5.6 ± 0.4	6.6 ± 0.3	3.2 ± 0.2	0.91 ± 0.03

[a]$P < 0.01$ compared with value from nonpregnant animals (Student t test; $n = 5$ nonpregnant group, 10 lactating group)
[b]$P < 0.05$ compared with value from nonpregnant animals (Student t test; $n = 5$ nonpregnant group, 10 lactating group)
[c]$P < 0.01$ compared with value from nonpregnant animals (Aspin–Welch t test; $n = 5$ nonpregnant group, 10 lactating group)
[d]$P < 0.05$ compared with value from nonpregnant animals (Aspin–Welch t test; $n = 5$ nonpregnant group, 10 lactating group)

Table 7. Changes in blood coagulation-related genes expression.

Accession number	GeneBank definition and comment	Fold change[a] on LD 0
NM_012559.1	Fibrinogen B beta chain mRNA	1.57
05675.1	Fibrinogen B beta chain mRNA	1.52
AI072045	Fibrinogen, A alpha polypeptide	1.52
NM_022924.1	Coagulation factor II (F2)	1.62
NM_019156.1	vitronectin (Vtn)	1.36

LD, Lactation day,
[a]: Compared with value in nonpregnant animals; no change on LD 14.

nophils, and monocytes were increased at 1 wk after weaning. However, total WBC counts did not differ at any point between the 2 groups.

Decreases in values for triglycerides and glucose were present on LD 1 in lactating rats. Decreased glucose was seen during the last stage of gestation,[21] which may be due, at least in part, to an increased demand for glucose by developing fetuses and

because of energy consumption during delivery. Activities of AST, ALT, and ALP were increased in our lactating rats on LD 7, 14, and/or 21. Previous studies[12,15] reported that these activities increased during the lactation period of rats, and the authors suggested that such changes might reflect an increase in hepatic metabolism of dams as they adapt to increased food consumption and milk production. In addition, a study in ewes[5] showed that the increases in AST and ALT activities reflected a state of hyperfunction during the lactation period, and another study in goats[17] showed that AST activity was high postpartum and positively correlated with milk yield. Moreover, a previous study[20] reported that ALT and AST activities increased in the hepatocytes of fasting rats because of endogenous energy synthesis. Increases in AST and ALT activities of mitochondrial origin were considered to indicate increased demand in the livers of rats that received hypolipidemic agents.[9] Therefore, the increases in AST, ALT, and ALP activities that we noted in the current study may similarly reflect increased food consumption and milk production, although we did not measure these parameters in the present study. Total protein, albumin, and several electrolytes were decreased consistently at LD 7, 14, and/or 21. These changes may preferentially reflect the increased demand of milk production because the albumin in a mother's milk originates from plasma,[19] because calcium and phosphorus are necessary for bone formation in offspring, and because milk production peaks at mid-lactation.[22,23] In addition, significant increases in total cholesterol and glucose values were detected at LD 7 or 21, and these changes might likewise indicate hepatic energy synthesis due to milk production. We also noted increased creatinine, BUN, and CPK values and decreased LDH and total bilirubin levels in our lactating rats, but the mechanisms underlying these results are unclear.

In conclusion, the present study revealed various changes in blood parameters throughout lactation in rats and provides background data for evaluation of nursing rats.

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References

1. **Bloom JC, Brandt JT.** 2001. Toxic responses of the blood, p 405–408. In: Klaassen CD, editor. Casarett and Doull's toxicology: the basic science of poisons, 6th ed. New York (NY): McGraw-Hill.
2. **Bremme KA.** 2003. Haemostatic changes in pregnancy. Best Pract Res Clin Haematol **16:**153–168.
3. **Brenner B.** 2004. Haemostatic changes in pregnancy. Thromb Res **114:**409–414.
4. **Clark P.** 2003. Changes of hemostasis variables during pregnancy. Semin Vasc Med **3:**13–24.
5. **El-Sherif MM, Assad F.** 2001. Changes in some blood constituents of Barki ewes during pregnancy and lactation under semiarid conditions. Small Rumin Res **40:**269–277.
6. **Franchini M.** 2006. Haemostasis and pregnancy. Thromb Haemost **95:**401–413.
7. **He XJ, Ejiri N, Nakayama H, Doi K.** 2005. Changes in cytochrome P450 isozymes (CYPs) protein levels during lactation in rat liver. Exp Mol Pathol **79:**224–228.
8. **Holmes VA, Wallace JM.** 2005. Haemostasis in normal pregnancy: a balancing act? Biochem Soc Trans **33:**428–432.
9. **Institute for Laboratory Animal Research.** 1996. Guide for the care and use of laboratory animals. Washington (DC): National Academies Press.
10. **Kobayashi A, Suzuk Y, Kuno H, Sugai S, Sakakibara H, Shimoi K.** 2009. Effects of fenofibrate on plasma and hepatic transaminase activities and hepatic transaminase gene expression in rats. J Toxicol Sci **34:**377–387.
11. **Mizoguchi Y, Matsuoka T, Mizuguchi H, Endoh T, Kamata R, Fukuda K, Ishikawa T, Asano Y.** 2010. Changes in blood parameters in New Zealand white rabbits during pregnancy. Lab Anim **44:**33–39.
12. **Mochizuki M, Shimizu S, Kidokoro Y, Kamata T, Kitazawa T, Kishi D, Okazaki E, Nishihara Y, Oihishi T.** 2009b. Phenobarbital (PB)-induced changes in blood-coagulation–related parameters in pregnant rats, lactating rats, and pups. J Toxicol Sci **34:**603–610.
13. **Mochizuki M, Shimizu S, Urasoko Y, Umeshita K, Kamata T, Kitazawa T, Nakamura D, Nishihara Y, Oihishi T, Edamoto H.** 2009a. Carbon tetrachloride-induced hepatotoxicity in pregnant and lactating rats. J Toxicol Sci **34:**175–181.
14. **Ohishi T, Kitazawa T, Suzuki S, Nakamura D, Nishihara Y, Mochizuki M.** 2010. Effects of pregnancy and lactation on warfarin-induced changes in blood-coagulation-related parameters in rats. J Toxicol Sci **35:**189–196.
15. **Papworth TA, Clubb SK.** 1995. Clinical pathology in the female rat during the pre- and postnatal period. Comp Haematol Int **5:**13–24.
16. **Sakurakawa N.** 1985. Acquired clotting abnormality, p 1204–1207. In: Miwa S, editor. Hematology. Tokyo (Japan): Bunkodo.
17. **Sarma PV, Ray TK.** 1985. Effect of physiological status on some blood enzyme levels and its relation to milk production. Indian J Dairy Sci **38:**237–239.
18. **Stirling Y, Woolf L, North WR, Seghatchian MJ, Meade TW.** 1984. Haemostasis in normal pregnancy. Thromb Haemost **52:**176–182.
19. **Susan MJ, Morgan EH.** 1967. Albumin, transferring, and γ-globulin metabolism during lactation in the rat. Q J Exp Physiol Cogn Med Sci **52:**422–429.
20. **Toropila M, Novakova J, Danova D, Paluchova K, Kafka I, Laktikova K.** 2005. Changes in the activity of selected adaptive enzymes in laboratory rats in relation to sex during prolonged fasting. Folia Vet **49 Suppl 3:**48–50.
21. **Urasoko Y, He XJ, Ebata T, Kinoshita Y, Kobayashi J, Mochizuki M, Ikeya M.** 2009. Chenges in blood parameters and coagulation-related gene expression in pregnant rats. J Am Assoc Lab Anim Sci **48:**272–278.
22. **Zaragozá R, García C, Diana RA, Pallardó FV, Barber T, Torres L, Miralles VJ, Viña JR.** 2003. Inhibition of liver trans-sulphuration pathway by propargylglycine mimics gene expression changes found in the mammary gland of weaned lactating rats: role of glutathione. Biochem J **373:**825–834.
23. **Zaragozá R, Miralles VJ, Diana RA, García C, Carmena R, García-Trevijano ER, Barber T, Pallardó FV, Torres L, Viña JR.** 2005. Weaning induces NOS2 expression through NFκB modulation in the lactating mammary gland: importance of GSH. Biochem J **391:**581–588.