

## Teratological Study of LBD-001, a Recombinant Human Interferon $\gamma$ , in Rabbits

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**ABSTRACT :** LBD-001, a recombinant human interferon  $\gamma$  produced by genetically engineered yeast as a host system, was intravenously administered to pregnant female rabbits (New Zealand White strain) from day 6 to 18 of gestation at dose levels of  $0.35 \times 10^6$ ,  $0.69 \times 10^6$ , and  $1.38 \times 10^6$  I.U./kg/day. Hydrocortisone sodium succinate (0.3 mg/kg/day) was also given in the same way. Teratological effects of the test agents on the organogenesis of fetuses and the development of offsprings (F1 rabbits) were investigated. The results were as followings : (1) No significant changes by the treatment of LBD-001 or hydrocortisone sodium succinate were observed in the body weights, the food and water consumption, the lactating or nursing behaviors, and the autopsy of the pregnant rabbits. (2) No significant changes in the resorption rate, the fetal organogenesis, and the normal development of offsprings (F1) by the treatment of LBD-001 or hydrocortisone sodium succinate were detected. The results show that LBD-001 at the dose of  $1.38 \times 10^6$  I.U./kg/day or less and hydrocortisone sodium succinate at the dose of 0.3 mg/kg/day are neither teratogenic in the organogenesis of the fetuses and the development of the offsprings (F1) nor toxic to the mother rabbits.

**Key Words :** LBD-001, Recombinant human interferon  $\gamma$ , Hydrocortisone sodium succinate, Teratological study, Rabbits, Intravenous injection

### I. INTRODUCTION

LBD-001 is a recombinant human interferon  $\gamma$  produced by fermentation of genetically engineered yeast containing the DNA which encodes for the human protein. The main action mechanism of naturally occurring interferon  $\gamma$ , a biological response modifier which is secreted from antigen-stimulated T-lymphocyte is related with enhancement of phagocytic function. LBD-001 was developed by Biotech Research Institute, Lucky Chemical Ltd. (84 Jang-Dong, Yousung-Ku, Daejeon, Korea). Previously, we reported on the fertility study (Lee and Cho, 1996) and the teratological study (Cho and Lee, 1996) of LBD-001 in rats. Here, as a part of toxicological tests of LBD-001, a teratogenic study was carried out in New Zealand White rabbits (National Institute of Safety Research of Korea, 1986). This study was performed to assess the po-

tential toxic effects of the agents on the visceral or skeletal organogenesis of fetuses and the normal development of offsprings (F1).

### II. MATERIALS AND METHODS

#### 1. Treatment of Test Substance

LBD-001 was supplied by Biotech Research Institute, Lucky Chemical Ltd. and serially diluted in 5% dextrose in phosphate buffered saline (PBS; pH 7.4) to make three doses of preparations. The dose of LBD-001 was  $0.35 \times 10^6$ ,  $0.69 \times 10^6$ , or  $1.38 \times 10^6$  I.U./kg/day. In vehicle-treated group, 5% dextrose in PBS (1 ml/kg/day) was treated. In nontreated group, none was treated. As a reference drug, hydrocortisone sodium succinate (Solu-Cortef, Upjohn Co.; shortly hydrocortisone) which was diluted by 5% dextrose in PBS shortly before the treatment was administered. Test substance, hydrocortisone, or the vehicle was administered in-

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travenously to the ear vein of pregnant rabbits from day 6 to 18 of gestation.

## 2. Animal Maintenances

Over 8-month-old female rabbits (New Zealand White strain) with the body weight of 2.5 to 3.5 kg bred in our Institute were kept at the temperature of 22~25°C and under the constant bright (6 *a.m.* to 7 *p.m.*) and dark (7 *p.m.* to 6 *a.m.*) cycle. Standard laboratory rabbit diet (Purina Korea Co.) and sterilized water were fed *ad libitum*.

## 3. Observation of Mother Rabbits

The rabbits were mated twice and after that they were isolated into separate cages so that each group includes 16 animals. The copulated day was regarded as day 0 of gestation. The pregnancy was confirmed by observing the body weight gains and touching abdominal part of the rabbits.

The body weights were measured on day 0, 3, 7, 10, 14, 17, 21, 24, 26, and 28 of gestation. The body weight gains against day 0 of gestation were shown in Fig. 1. The contents of food consumption and water consumption were measured on day 3, 7, 10, 14, 17, 21, 24, 26, and 28 of gestation, respectively. The general behaviors and the lactating or nursing behaviors of the mother rabbits were observed every day. Two thirds of the animals confirmed to be pregnant were sacrificed by air injection into the ear vein on day 29 of gestation and caesarian-sectioned for fetal examination. About one third of the animals (4 animals per group) were naturally delivered and their offsprings were used for observation of the development upto week 4 of lactation period. The mother rabbits were also caesarian-sectioned and autopsied for observation of abnormalities on week 4 of lactation period.

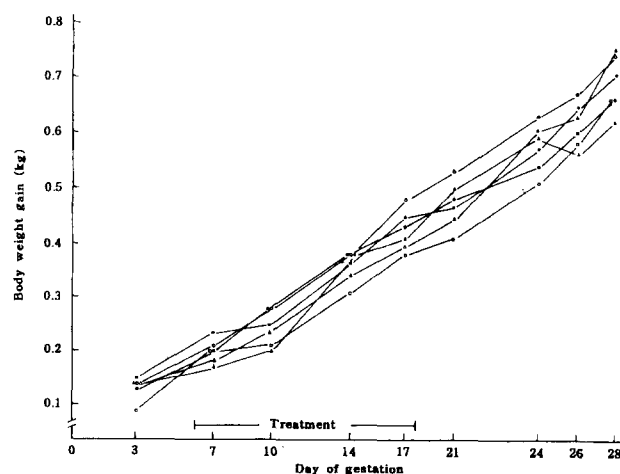
## 4. Observation of Fetuses

Eight to ten pregnant rabbits were sacrificed by air injection into the ear vein and their abdominal cavity and uterine horns were opened for observa-

tion of the number of corpora lutea, the number of implantation sites, the number of fetuses resorbed at early or late period of gestation, the number of live or dead fetuses, their external malformations, the number of live fetuses, the body weights of live fetuses, sex, and the placental weights of live fetuses. The implantation rate (=the number of implantation sites/the number of corpora lutea × 100), resorption rate, and sex ratio were also calculated. Fetuses delivered from about half number of the mother rabbits were used for observation of the skeletal abnormalities, variations, and ossification (Saito *et al.*, 1984) after staining the fetuses (Dawson, 1926). Fetuses delivered from another about half number of mother rabbits were used for visceral observation under the stereoscope (Wilson, 1965; Manson *et al.*, 1994; Shirasu and Matsuoka, 1981).

## 5. Observation of Delivered Offsprings (F1)

The mean of the gestation periods was calculated from 4 pregnant rabbits. After the delivery, the survival state (whether live or stillborn), sex, external malformations of the offsprings were checked and the offsprings were caesarian-sectioned to confirm the sex and calculate the sex ratio. The body weights of the male or female offsprings were measured on day 1 of lactation period

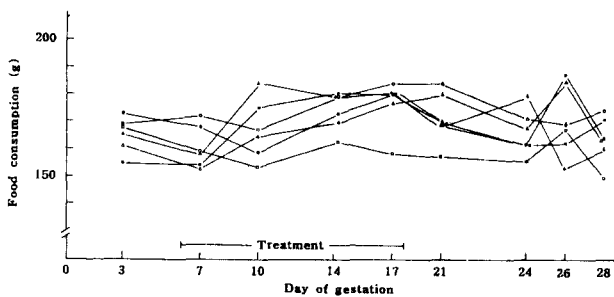


**Fig. 1.** Changes of body weight gains in rabbits. The treatments were done from day 6 to 18 of gestation. ○: Nontreated group, ●: Vehicle-treated group, ▲: LBD-001 ( $0.35 \times 10^6$  I.U./kg/day), □: LBD-001 ( $0.69 \times 10^6$  I.U./kg/day), △: LBD-001 ( $1.38 \times 10^6$  I.U./kg/day), ■: Hydrocortisone (0.3 mg/kg/day).

and on week 1, 2, 3, and 4 of lactation period, respectively. Birthdays of the offsprings were counted as day 1 of lactation period.

## 6. Statistical Analyses

To analyze the statistical significance, Student's *t*-test or  $\chi^2$ -test was used. Experimental data in a group were compared with those of vehicle-treated group. When the difference shows  $p < 0.05$ , the data were considered significantly different.



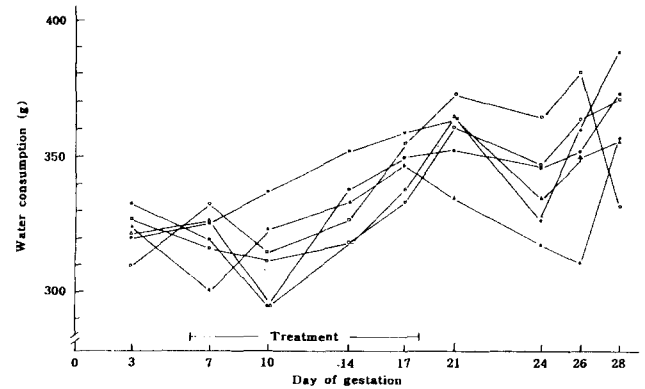
**Fig. 2.** Changes of food consumption in rabbits. The treatments were done from day 6 to 18 of gestation. ○: Nontreated group, ●: Vehicle-treated group, ▲: LBD-001 ( $0.35 \times 10^6$  I.U./kg/day), □: LBD-001 ( $0.69 \times 10^6$  I.U./kg/day), △: LBD-001 ( $1.38 \times 10^6$  I.U./kg/day), ■: Hydrocortisone (0.3 mg/kg/day).

## III. RESULTS AND DISCUSSION

### 1. Influences on Mother Rabbits

Any abnormal symptoms in the general behaviors by the treatment of LBD-001 was not detected.

Since 2 to 4 mother rabbits were not fertilized among 16 animals per group even after two times



**Fig. 3.** Changes of water consumption in rabbits. The treatments were done from day 6 to 18 of gestation. ○: Nontreated group, ●: Vehicle-treated group, ▲: LBD-001 ( $0.35 \times 10^6$  I.U./kg/day), □: LBD-001 ( $0.69 \times 10^6$  I.U./kg/day), △: LBD-001 ( $1.38 \times 10^6$  I.U./kg/day), ■: Hydrocortisone (0.3 mg/kg/day).

**Table 1.** Prenatal effects of LBD-001 on rabbit fetuses

Parameters	Nontreated group	Vehicle group	LBD-001 (I.U./kg/day)			Hydrocortisone (mg/kg/day)
			$0.35 \times 10^6$	$0.69 \times 10^6$	$1.38 \times 10^6$	0.3
No. of dams	12	12	12	12	12	12
No. of infertilized or aborted dams	3	4	2	3	4	4
Total number of corpora lutea (mean ± S.D.)	77 (8.5 ± 2.4)	65 (8.1 ± 2.7)	81 (8.1 ± 1.9)	75 (8.3 ± 2.1)	66 (8.3 ± 2.0)	64 (8.0 ± 2.5)
Total number of implantation sites (mean ± S.D.)	73 (8.1 ± 2.33)	58 (7.3 ± 1.6)	74 (7.4 ± 2.2)	69 (7.7 ± 2.4)	62 (7.8 ± 1.6)	59 (7.4 ± 2.2)
Implantation rate (%) <sup>a</sup>	94.8	89.2	91.4	92.0	93.9	92.2
Total number of resorptions at early stage	4	3	4	3	4	4
at late stage	4	3	4	2	4	3
Resorption rate (%) <sup>b</sup>	5.5	5.2	5.4	4.3	6.5	6.8
Total number of live fetuses (mean ± S.D.)	69 (7.7 ± 2.2)	55 (6.9 ± 1.9)	70 (7.0 ± 2.1)	66 (7.3 ± 2.2)	58 (7.3 ± 1.4)	55 (6.9 ± 2.0)
Sex ratio (no. of male / no. of female)	0.97 (34/35)	1.12 (29/26)	0.71 (29/41)	0.94 (32/34)	1.15 (31/27)	1.04 (28/27)
Body weight of live fetuses (g, mean ± S.D.)						
Male	41.40 ± 6.44	41.10 ± 7.38	42.70 ± 6.53	44.43 ± 5.43	42.54 ± 6.38	41.75 ± 8.59
Female	41.00 ± 7.13	43.00 ± 5.06	41.50 ± 6.12	43.60 ± 4.29	42.50 ± 6.82	42.50 ± 7.30
Placental weight of live fetuses (g, mean ± S.D.)	5.59 ± 0.82	5.65 ± 1.02	5.78 ± 0.91	5.57 ± 0.89	5.62 ± 0.52	5.42 ± 0.72

<sup>a</sup>No. of corpora lutea / No. of implantation sites × 100. <sup>b</sup>No. of resorptions / (No. of live fetuses + No. of resorbed fetuses) × 100.

**Table 2.** External, skeletal, and visceral findings in rabbit fetuses treated with LBD-001

Parameters	Nontreated group	Vehicle group	LBD-001 (I.U./kg/day)			Hydrocortisone (mg/kg/day)
			$0.35 \times 10^6$	$0.69 \times 10^6$	$1.38 \times 10^6$	0.3
External malformation/Examined (%)	0/69 (0)	0/55 (0)	0/70 (0)	0/66 (0)	0/58 (0)	0/55 (0)
Skeletal abnormalities/Examined (%)	0/34 (0)	0/28 (0)	0/35 (0)	0/34 (0)	0/29 (0)	1/28 (3.6)
Nodulated ribs	0	0	0	0	0	1
Visceral abnormalities/Examined (%)	0/35 (0)	0/27 (0)	0/35 (0)	0/32 (0)	0/29 (0)	0/27 (0)
Skeletal variations/Examined (%)	23/34 (67.6)	23/28 (82.1)	24/35 (68.6)	24/34 (70.6)	19/29 (65.5)	17/28 (60.7)
Unilateral 13th rib	6	9	4	5	6	5
Bilateral 13th ribs	17	13	20	19	13	12
8th lumbar vertebrae	9	10	12	13	8	9
Split of caudal vertebrae	0	1	0	0	0	0

Values represent the total number or fetuses of percent in case of the figures in the parentheses.

**Table 3.** Skeletal ossification in rabbit fetuses treated with LBD-001

Parameters	Nontreated group	Vehicle group	LBD-001 (I.U./kg/day)			Hydrocortisone (mg/kg/day)
			$0.35 \times 10^6$	$0.69 \times 10^6$	$1.38 \times 10^6$	0.3
Examined fetuses <sup>a</sup>	34	28	35	34	29	28
Fetuses with poorly ossified sternebrae <sup>a</sup>	3	4	3	6	5	4
Sternebrae <sup>b</sup>	$5.8 \pm 0.62$	$5.8 \pm 0.57$	$5.9 \pm 0.38$	$5.8 \pm 0.45$	$5.8 \pm 0.48$	$5.8 \pm 0.63$
Caudal vertebrae <sup>b</sup>	$16.2 \pm 0.90$	$16.1 \pm 0.98$	$16.3 \pm 0.46$	$16.2 \pm 0.68$	$16.0 \pm 0.76$	$15.9 \pm 0.84$
Distal phalanges <sup>b</sup>	$9.9 \pm 0.23$	$10.0 \pm 0.00$	$10.0 \pm 0.00$	$10.0 \pm 0.17$	$10.0 \pm 0.00$	$10.0 \pm 0.00$
Middle phalanges <sup>b</sup>	$8.0 \pm 0.00$	$7.9 \pm 0.31$	$8.0 \pm 0.00$	$8.0 \pm 0.00$	$7.9 \pm 0.40$	$8.0 \pm 0.00$
Proximal phalanges <sup>b</sup>	$10.0 \pm 0.00$	$10.0 \pm 0.00$	$10.0 \pm 0.00$	$10.0 \pm 0.00$	$10.0 \pm 0.18$	$9.9 \pm 0.26$
Metacarpus <sup>b</sup>	$10.0 \pm 0.00$	$10.0 \pm 0.00$	$10.0 \pm 0.00$	$10.0 \pm 0.00$	$10.0 \pm 0.00$	$10.0 \pm 0.00$
Metatarsus <sup>b</sup>	$8.0 \pm 0.00$	$8.0 \pm 0.00$	$8.0 \pm 0.00$	$8.0 \pm 0.00$	$8.0 \pm 0.00$	$8.0 \pm 0.00$

<sup>a</sup>The total number. <sup>b</sup>Mean  $\pm$  S.D. of the number of the corresponding bones per fetus.

of the mating, the animals were excluded in examining the prenatal effects of the test substance.

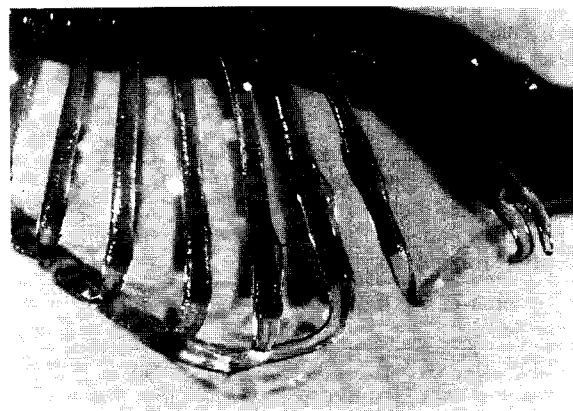
The body weight gains during gestation were shown in Fig. 1. Consumption of food and water was shown in Fig. 2 and Fig. 3. Neither the body weight gains nor the consumptions of food and water were significantly different among all the groups.

No external abnormalities were found in the organs autopsied on day 29 of gestation.

## 2. Influences on Fetuses

### 1) Intrauterine Observation

The results of intrauterine observation of the mother rabbits were shown in Table 1. In vehicle-treated group, the mean number of corpora lutea,



**Photo. 1.** Nodulated ribs found in hydrocortisone (0.3 mg/kg/day)-treated group.

the number of implantation sites, and the number of live fetuses was 8.1, 7.3, and 6.9, respectively

which were not significantly different among the groups. In the mean body weights of fetuses and the mean placental weights were not much different among the groups.

## 2) External, Skeletal, and Visceral Observation

The results were shown in Table 2, Table 3, and Photo. 1. Any external malformations was not detected. In skeletal observation, only one case of nodulated ribs was observed in hydrocortisone (0.3 mg/kg)-treated group (Photo 1). This case contributed to higher skeletal abnormality rate (3.6%) in hydrocortisone-treated group as compared with those of the other groups (0%), even though it was not significantly high (Table 2).

Skeletal variations were also observed using the criteria of Satoh *et al.* (1984). The presence of the 13th ribs, the presence of 8th lumbar ribs, the split of caudal vertebrae, the asymmetry of cervical vertebrae, and the fusion or hyperplasia of cervical vertebrae were checked. The skeletal variation rate (the number of fetuses with skeletal variations/the number of fetuses examined  $\times$  100) ranged from 60.7 to 82.1%, but the rates were not significantly different among the groups (Table 2). Even though higher skeletal variation rate (82.1%) was observed in vehicle-treated group as compared with lower rates in LBD-001-treated groups (65.5 to 70.6%) and hydrocortisone (0.3 mg/kg)-treated group (60.

7%), the differences might be accidental since the rate was also relatively low in nontreated group (67.6%). Asymmetry and fusion, or hyperplasia of cervical vertebrae did not appear. No visceral abnormality was observed (Table 2).

Skeletal ossifications which was evaluated by counting the number of sternbrae, caudal vertebrae, distal phalanges, middle phalanges, proximal phalanges, metacarpus, and metatarsus were not significantly different among the groups (Table 3).

Altogether, the external malformation, the skeletal abnormalities, and the skeletal or visceral abnormalities in LBD-001-treated groups and hydrocortisone (0.3 mg/kg)-treated group might be negligible. It is reported that cleft palates can be induced by the treatment of several glucocorticosteroids (Ishimura *et al.*, 1975; Koga *et al.*, 1975; Walker *et al.*, 1967). But in our test, neither occurrence of the cleft palates nor the significant retardation of fetal or postnatal growth was observed. It might result from the fact that we used lower dosage than that used in other reports, otherwise that hydrocortisone sodium succinate has milder potency than other synthetic derivatives of glucocorticosteroids.

## 3) Influences on Offsprings

Records in delivery and body weight changes of

**Table 4.** Postnatal development of offsprings (F1) born from rabbits treated with LBD-001 from day 6 to 18 of gestation

Parameters		Nontreated group		Vehicle group		LBD-001 (I.U./kg/day)	
						0.35 $\times$ 10 <sup>6</sup>	
No. of dams		4		4		4	
Live offsprings	Total no.	26		28		23	
	Mean $\pm$ S.D.	6.5 $\pm$ 0.9		7.0 $\pm$ 1.2		5.8 $\pm$ 1.9	
Sex ratio (no. of male/no. of female)		1.17 (14/12)		0.87 (13/15)		1/3 (13/10)	
Sex of offsprings		male	female	male	female	male	female
Body weight (g, Mean $\pm$ S.D.)	Day 1 <sup>a</sup>	54.9 $\pm$ 8.4 (14) <sup>c</sup>	55.5 $\pm$ 7.5 (12)	55.2 $\pm$ 7.7 (13)	55.0 $\pm$ 6.8 (15)	56.0 $\pm$ 6.7 (13)	54.6 $\pm$ 6.29 (10)
	Week 1 <sup>b</sup>	132.4 $\pm$ 19.6 (14)	130.8 $\pm$ 18.4 (11)	128.6 $\pm$ 15.0 (12)	120.3 $\pm$ 12.7 (13)	135.2 $\pm$ 16.6 (11)	127.9 $\pm$ 14.6 (10)
	Week 2	256.5 $\pm$ 41.2 (14)	250.5 $\pm$ 29.1 (11)	252.6 $\pm$ 32.5 (12)	233.9 $\pm$ 26.0 (13)	255.9 $\pm$ 30.0 (11)	238.9 $\pm$ 5.4 (9)
	Week 3	395.0 $\pm$ 46.2 (14)	373.1 $\pm$ 24.6 (11)	391.2 $\pm$ 44.2 (12)	374.9 $\pm$ 29.1 (13)	404.4 $\pm$ 20.2 (11)	399.4 $\pm$ 24.7 (9)
	Week 4	319.1 $\pm$ 25.4 (14)	523.4 $\pm$ 19.7 (11)	523.4 $\pm$ 20.0 (12)	519.7 $\pm$ 18.3 (11)	530.8 $\pm$ 27.6 (11)	520.0 $\pm$ 33.1 (9)

Table 4. Continued.

Parameters		LBD-001 (I.U./kg/day)		Hydrocortisone (mg/kg/day)			
		$0.69 \times 10^6$	$1.38 \times 10^6$	0.3			
No. of dams		4	4	4			
Live offsprings	Total no.	30	29	25			
	Mean $\pm$ S.D.	$7.5 \pm 0.9$	$7.3 \pm 0.8$	$6.3 \pm 1.3$			
Sex ratio (no. of male/no. of female)		0.76 (13/17)	1.07 (15/14)	0.79 (11/14)			
Sex of offsprings		male	female	male	female		
Body weight (g, Mean $\pm$ S.D.)	Day 1 <sup>a</sup>	$60.1 \pm 7.3$ (13)	$57.2 \pm 8.6$ (17)	$51.9 \pm 9.0$ (15)	$51.6 \pm 9.4$ (14)	$52.3 \pm 8.8$ (11)	$50.9 \pm 8.0$ (14)
	Week 1 <sup>b</sup>	$140.1 \pm 16.1$ (12)	$133.9 \pm 15.4$ (17)	$117.1 \pm 13.2$ (14)	$116.3 \pm 15.9$ (14)	$125.0 \pm 15.5$ (11)	$125.2 \pm 14.7$ (12)
	Week 2	$267.8 \pm 29.7$ (12)	$250.4 \pm 20.7$ (16)	$234.5 \pm 14.8$ (14)	$238.2 \pm 18.9$ (13)	$238.9 \pm 16.4$ (11)	$126.7 \pm 22.0$ (12)
	Week 3	$407.1 \pm 25.5$ (12)	$404.4 \pm 27.1$ (16)	$371.4 \pm 30.3$ (14)	$377.3 \pm 25.4$ (13)	$379.3 \pm 35.9$ (11)	$384.4 \pm 29.9$ (12)
	Week 4	$540.8 \pm 33.3$ (12)	$536.1 \pm 25.7$ (16)	$514.6 \pm 28.0$ (14)	$515.0 \pm 26.1$ (13)	$512.5 \pm 25.9$ (11)	$510.9 \pm 22.9$ (12)

<sup>a</sup>Day of lactation period; Birthday is regarded as day 1 of lactation period. <sup>b</sup>Week of lactation period. <sup>c</sup>Values in the parentheses represent the number of offsprings.

offsprings (F1) were shown in Table 4. In all the groups, no significant differences in the number of offsprings, and sex ratios were observed as compared with those of vehicle-treated group. Even though not shown in Table 4, the mean  $\pm$  S.D. of the gestation period was  $30.0 \pm 0.0$  in all the groups. Offsprings (dead or live) with external abnormalities were not observed. The body weights of the offsprings were not significantly different among the groups upto week 4 of lactation period. The body weight gain of the male or female offsprings from birthday to week 4 of lactation period was 464.2 or 467.9 g in nontreated group, 468.2 or 464.7 g in vehicle-treated group, 474.8 or 465.4 g in LBD-001 ( $0.35 \times 10^6$  I.U./kg)-treated group, 480.7 or 478.9 g in LBD-001 ( $0.69 \times 10^6$  I.U./kg)-treated group, 462.7 or 460.4 g in LBD-001 ( $1.38 \times 10^6$  I.U./kg)-treated group, and 460.2 or 460.0 g in hydrocortisone (0.3 mg/kg)-treated group, respectively. No special abnormalities were observed in the general behaviors, the developmental status of the offsprings in all the groups.

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