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TERATOLOGICAL STUDY OF THE RECOMBINANT HUMAN INTERFERON-αA(rHuIFN-αA) IN RATS

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ABSTRACT: *A teratogenicity study was carried out on Sprague-Dawley rats which have been given the intravenously or intraperitonealy injections of rHuIFN-αA, an available therapeutic agent, at dose levels of 1×10^5, 4×10^5 and 1.2×10^6 I.U/Kg/day for a period of 11 days from day 7 to day 17 of gestation. Two-thirds of the pregnant females in each group were sacrificed on day 20 of gestation and their fetuses were examined. The remaining dams were allowed to litter naturally, and the postnatal development of the off springs was observed. No changes were observed in all aspects of parameters between the treated and the control dams. The incidence of external, internal, and skeletal anomalies were not significantly increased in the fetuses of any treated groups. The rHuIFN-αA caused no effects on parturition, lactation, and postnatal growth.*

INTRODUCTION

The present paper deals with the effects of rHuIFN-αA administered via tail vein or intraperitonium during the period of organogenesis in pregnant rats. This study was carried out in accordance with the "Guide-lines for Reproduction examinations to Evaluate the Safety of Drugs" issued by the Ministry of Health and Welfare, Korea National Institute of Health.

MATERIALS AND METHODS

Materials

Injectable rHuIFN-αA, which was produced by Genetic Engineering Division, Cheil Sugar co., Ltd. was used, and dose levels were divided into two groups, 1×10^6 and 3×10^6 I.U/vial.

Methods

Male rats, 6 weeks of age (163-177 g.), and female rats, 6 weeks of age (132-145 g.), of Sprague-Dawley strain were used under the approval of specific pathogen free-rats by serological tests.

The animals were housed in polycarbonate cages (26 × 42 × 18 cm) bedded with autoclaved wood-shavings at an ambient temperature of 23 ± 3° and a relative humidity of 55 ± 10%.

The ventilation of the breeding room was maintained in order to get optimal air condition, and the room was lighted by 12 hour photoperiod. They were allowed free access to Sam-Yang Laboratory Animal Diet (Sam-Yang Feedstuff Co., Korea) and water bottles. Water was

changed daily. Bottles and cages were autoclaved per three days. Attenuation period was 2 weeks.

Animals which did not show any abnormalities in estrus cycle identified by the presence of vaginal copulation plugs and sperms in vaginal smears were used. During the mating period, the female rats were paired at 1 : 1 basis with the male rats. The day on which mating was confirmed at 9 : 00th day after mating was designated day 0 of gestation.

Dosage range was determined, based on three kinds of treated groups-one vehicle as physiological saline and three treated groups were divided by high dose level (1.2×10^6 I.U/Kg/day), medium dose level (4×10^5 I.U/Kg/day) and low dose level (1×10^5 I.U/Kg/day).

All pregnant rats were kept individually in a cage. Each pregnant rat was dosed via tail vein or intraperitonium during the period of organogenesis.

On day 20 of gestation, two-thirds of pregnant females in each group (18 to 20 rats in each group) were autopsied by Cesarean section. The remaining dams in each group were allowed to deliver spontaneously.

After the weaning period, autopsies were carried out on day 22.

Maternal

1) All visible responses of treated animals were observed once a day. Body weights of female rats were recorded per 7 days during the attenuation period and were recorded at O, 7th, 9th, 11th, 13th, 15th, 17th, 19th and 20th day of gestation. During the weaning period, body weights were also recorded on O, 4th, 7th, 14th and 21th day post-partum.
2) On day 20 of gestation, pregnant females were anesthetized with sodium pentobarbital and autopsied. The number of corpora lutea, implantations, viable and dead fetuses, and absorbed embryos (deaths, macerations, absorptions, immaturities, placental traces) was counted. The weights of maternal liver, kidneys, spleen, and ovaries were also measured. We confined that the weights of underdeveloped fetuses which were 60% lighter than that of control fetuses.
3) The observation of the offsprings (F_1) in the spontaneous delivery, such as the number and weights of viable and dead fetuses, were carried out individually and the offsprings were examined for external abnormalities and sex.
4) In the case of spontaneous delivery, maternal conditions were observed pre-partum and post-partum. The number of implantations, the rate of birth, and period of pregnancy were also calculated on day 22 of parturition.

Fetuses

On day 20 of gestation, all viable fetuses were collected by Cesarean section. The half viable fetuses per litter were used in skeletal examination and the remained fetuses were used in organ examination.

1) Organ examination

Collected fetuses were fixed in Bouin's solution for 7 days, and were examined by the modified method of Wilson's and method of Nishmura (Wilson, 1965).

All fetuses were examined under the vertical microscope.

2) Skeletal examination

The half viable fetuses per litter were stained with alcian-blue & alizarin red S double staining method and examined for skeletal anomalies and development (Inouye, 1976). As an index of the state of ossification, ossifying rate of vertebra, ribs, sternebrae, metacarpus, metartarsus, hind paw, and forepaw were observed with the variation and anomalies of all skeletal system

(Hoshi *et al*, 1985).

Offsprings of spontaneous delivery

After parturition, the weights of viable and dead fetuses were measured. The weaning period was determined as 21 days. As for the survival rate of fetuses, the number of survived fetuses were recorded at O, 4th, 14th and 21th day post-partum.

1) During the test period, all visible responses of the fetuses were observed daily, newborn weights of fetuses were measured by O, 4th, 7th, 14th and 21th day post-partum.
2) Eye openings of fetuses were observed from the day 13 to the day 17 post-partum.
3) Data were analyzed statistically by one-way ANOVA and Student's t-test. the differences between treated groups and control groups were tested at the levels of 95% ($p < 0.05$) and 99% ($p < 0.01$).

RESULTS

1) Maternal body weights.

As indicated in Fig. 1, the body weights changes of treated groups and control groups were not significantly different ($p < 0.05$).

2) The number of corpora lutea, implantations, and the rate of implantation.

As seen in Table 1, the number and rate of implantation of low dose treated group were significantly different compared to non-treated group, but significant differences were not seen compared to vehicle treated group.

3) Rate of immatured fetuses.

All of the treated groups did not represent the significant differences, as indicated in Table 1,

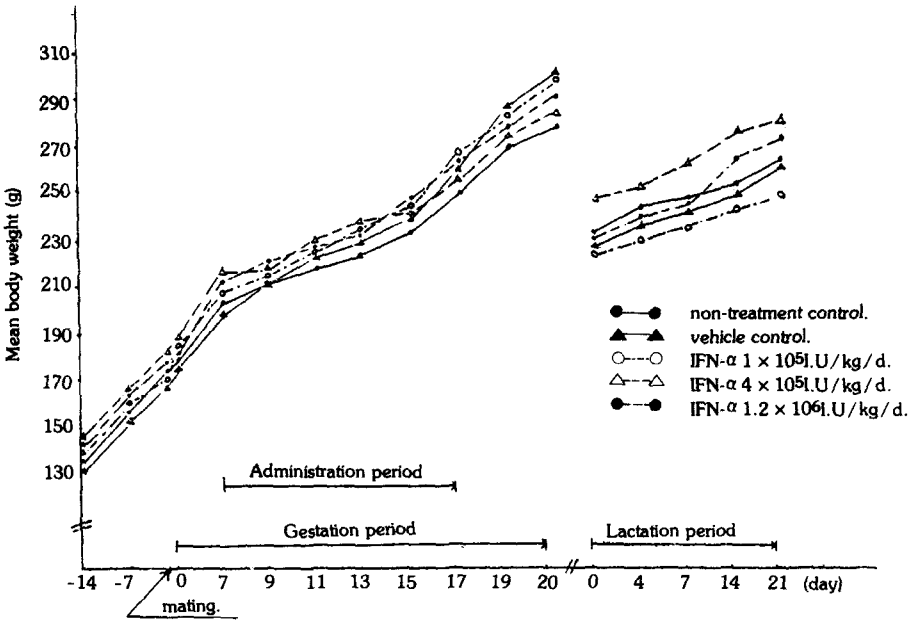

Fig. 1. Body weight changes of dams treated intraperitoneally with α-Interferon in teratogenicity study.

Table 1. Influence of IFN-α on embryonic development (F_1) in teratogenicity study.

Dose (IU1kg1d)	non-treatment control	Vehicle control	1 ± 10^5	4 ± 10^5	1.2 ± 10^6
No. of dams	20	18	18	19	20
No. of corpora lutea	218	224	214	228	222
mean ± S.D.	10.9 ± 2.2	12.4 ± 1.3	11.7 ± 1.9	12.0 ± 1.5	11.1 ± 1.2
No. of implantations	182	190	192	182	194
mean ± S.D.	9.1 ± 2.1	10.6 ± 1.2	10.7 ± 1.1*	9.6 ± 1.5	9.7 ± 1.0
Implantation rate(%) mean ± S.D. a)	83.1 ± 0.1	85.1 ± 0.1	89.9 ± 0.1*	80.0 ± 0.1	88.0 ± 0.1
No. of undeveloped embryos.	4	0	8	5	8
mean ± S.D.	0.20 ± 0.62	0.00 ± 0.00	0.44 ± 0.70	0.25 ± 0.45	0.38 ± 0.50
in early stage b)	0	0	2	2	3
in late stage c)	4	0	6	3	5
Undvelopment rate(%) d)	2.20	0.00	4.17	2.75	3.85
No. of live fetuses	192	190	190	174	186
mean ± S.D.	9.1 ± 2.1	10.6 ± 1.2	10.6 ± 1.2*	9.4 ± 1.9	9.3 ± 1.2
Live fetus rate(%) mean ± S.D. e)	100.0 ± 0.00	100.0 ± 0.00	98.99 ± 0.03	96.53 ± 0.06	96.31 ± 0.07
Sex ratio (male/female) f)	0.98	0.76	1.32	0.97	1.08
Body weight(g) g) male	3.997 ± 0.377	3.859 ± 0.462	4.011 ± 0.306	3.956 ± 0.550	3.904 ± 0.339
female	3.742 ± 0.277	3.808 ± 0.411	3.6777 ± 0.456	3.687 ± 0.384	3.645 ± 0.411
No. of external anomalies	0	2	2	0	0
External anomaly h) rate(%), mean ± S.D.	0.00 ± 0.00	0.11 ± 0.32	0.00 ± 0.00	0.00 ± 0.00	0.16 ± 0.34

a) : (No. of implantations / No. of corpora lutea) × 100.
b) : Includes implantation site. placental remnant & early resorption.
c) : Includes late resorption, macerated fetus & dead fetus.
d) : (No. of undeveloped embryos / No. of implantations) × 100.
e) : (No. of live fetuses / No. of implantations) × 100.
f) : (No. of male fetuses / No. of female fetuses).
g) : mean ± S.D. calculated from values of each fetus.
h) : (Number of fetuses showing external anomalies / No. of live fetuses) × 100.
* : Significantly different from non-treatment control ($P<0.05$), ** : ($P<0.01$).
\+ : Significantly different from vehicle control ($P<0.05$), + + : ($P<0.01$).

Table 2. Relative organ weights of dams on day 20 of gestation.

Dose (IU/kg/d.)	non-treatment control	Vehicle control	1×10^5	4×10^5	1.2×10^6
Liver (g/100g B.W)	4.508 ± 0.513	4.273 ± 0.316	4.432 ± 0.556	4.555 ± 0.230	4.484 ± 0.193
Kidneys (mg/100g B.W)	670.2 ± 65.6	624.5 ± 39.2	606.1 ± 43.1*	600.7 ± 42.6*	639.8 ± 37.7
Spleen (mg/100g B.W)	269.9 ± 46.5	253.3 ± 15.3	241.7 ± 37.2	257.9 ± 24.1	235.0 ± 50.4
Ovaries (mg/100g B.W)	101.6 ± 24.0	92.9 ± 23.8	85.3 ± 19.2	88.6 ± 16.0	94.1 ± 23.5

Table 3. Some aspects of fetuses born of rats after I.V or I.P administration of IFN-α on day 7-17 of gestation.

Dose(IU 1kg 1day)		non-treatment control	Vehicle control	1×10^5	4×10^5	1.2×10^6
No. of dams (F_o)		8	10	8	9	10
Gestation period(day), mean ± S.D.		22.0 ± 0.00	21.2 ± 0.42	22.0 ± 0.00	21.3 ± 0.52	21.8 ± 0.46
Total born (=live born)	Total	76	108	84	96	89
	male	50	52	44	42	45
	female	26	56	40	54	44
Implantation	Total	86	110	88	108	100
	mean ± S.D.	10.8 ± 1.58	11.0 ± 2.00	12.0 ± 1.55	10.0 ± 3.12	
Delivery rate(%), mean ± S.D.		89.1 ± 0.07	98.3 ± 0.04	95.0 ± 0.09	89.2 ± 0.09	89.2 ± 0.12
Sex ratio (male/female)		0.92	0.93	1.05	0.78	1.05
Viability index at birth(%)		100	100	100	100	100
0 day	male	50	52	44	42	45
	female	26	56	40	54	44
4 day	male	50	52	44	42	42(3) a)
	female	26	56	40	54	44
7 day	male	50	52	44	42	42
	female	26	56	40	54	43 (1)
14 day	male	50	52	44	42	42
	female	26	56	40	54	43
21 day	male	50	51(1)	44	40(2)	42
	female	25(1)	56	40	53(1)	43
Nursing rate[b] (%)	7th mean ± S.D.	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	95.5 ± 0.21
	21th mean ± S.D.	97.22 ± 0.05	98.18 ± 0.04	100.0 ± 0.00	96.3 ± 0.06	100.0 ± 0.00
External anomaly		0/76	0/108	0/84	0/96	0/89

a) : Each value in parenthesis represents the Number of dead newborn.
b) : Percentage of live offsprings vs 0 day.

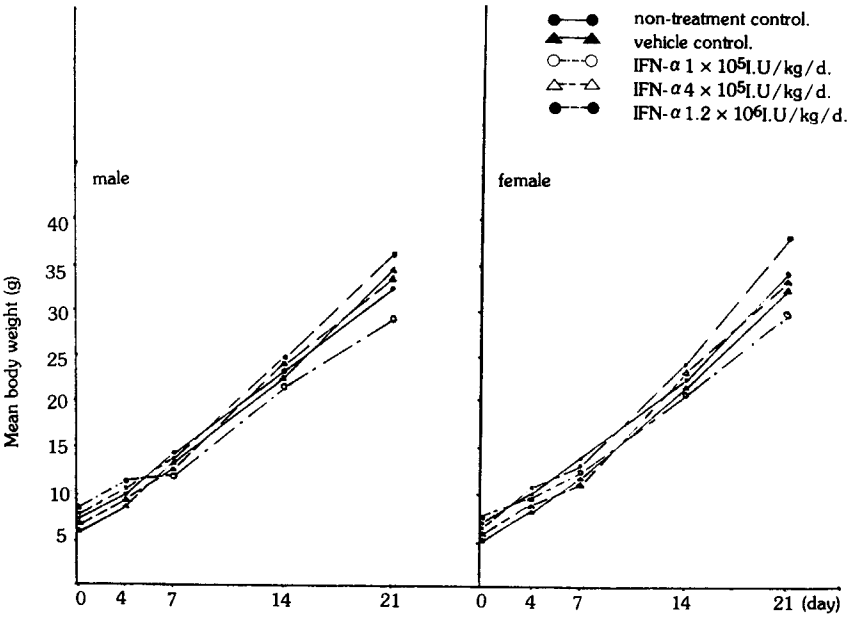

Fig. 2. Growth curves of male and female rats (F_1) in the teratogenicity study.

though the variations of each fetuses are recognizable.

4) Number and weights of viable fetuses.

The changes of low dose treated group had increasing trend ($p < 0.05$), but there were not any significant differences in comparison with the vehicle treated group (Table 1).

5) External anomalies in fetuses.

As seen in Table 1, no significant differences were observed.

6) Organ weight changes in dams.

The weights of liver, kidney, spleen, ovaries did show decreasing trend ($p < 0.05$), especially that of kidney in low dose treated group and medium dose treated group, but no significant differences were observed in comparison with the vehicle treated group (Table 2).

7) In the case of the spontaneously delivered dams, there were no significantly different in the gestation period, the number of offsprings, implantations, and the rate of birth etc.

8) Survival rate and external abnormalities of fetuses

As seen in Table 3, no significant differences were observed.

9) Body weight changes and Eye openings in fetuses.

No significant differences were recognized as indicated in Fig. 2 and Table 4.

10) Ossifications of fetuses.

No delayed ossifications in all treated groups compared to the nontreated control as seen.

Table 4. Effects of IFN-α on postnatal development of youngs(F_1) from dams I.P or I.V. ad. IFN-α on day 7-17 of gestation.

Dose (IU/kg/day)		non-treatment control		Vehicle control		1×10^5		4×10^5		1.2×10^6	
Sex		male	female	male	male	female	female	male	female	male	female
Body weights of youngs(g)	at birth.	6.97 ± 0.61	6.82 ± 0.38	6.48 ± 0.65	7.24 ± 0.78	7.00 ± 0.58	6.81 ± 0.65	6.65 ± 0.39	6.27 ± 0.64	7.13 ± 0.72	6.81 ± 0.653
	4 day	9.93 ± 0.91	9.96 ± 0.57	9.42 ± 0.84	10.16 ± 1.05	9.66 ± 0.79	10.06 ± 1.48	9.86 ± 0.73	9.35 ± 1.18	10.25 ± 1.57	10.06 ± 1.481
	7 day	14.17 ± 1.17	13.85 ± 0.93	13.12 ± 1.30	12.80 ± 2.78	12.83 ± 0.84	12.95 ± 2.24	12.92 ± 1.45	12.22 ± 1.84	13.56 ± 2.38	12.95 ± 2.24
	14 day	23.45 ± 1.34	22.92 ± 1.04	23.27 ± 2.40	21.69 ± 1.83	21.37 ± 1.44	23.99 ± 4.80	23.69 ± 3.37	22.98 ± 3.30	23.71 ± 5.25	23.99 ± 4.80
	21 day	33.01 ± 6.50	33.83 ± 5.30	34.25 ± 2.72	28.62 ± 3.95	29.90 ± 3.49	37.96 ± 7.82	34.01 ± 4.02	33.03 ± 4.88	36.29 ± 9.25	37.96 ± 7.82
Separation of eyelids (days)		14.53 ± 0.76		15.12 ± 0.63		14.62 ± 0.79		14.59 ± 0.94		13.94 ± 0.36	

Table 5. Influence of IFN-α on skeletal development of fetuses (F_1) in teratogenicity study.

Dose (IU/kg/d.)		non-treatment control	Vehicle control	1×10^5	4×10^5	1.2×10^6
No. of dams		10	10	8	9	10
No. of fetuses observed		102	98	88	77	95
Degree of ossification: cervical vertebra	arch	7.0 ± 0.0[a]	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0
	body	0.12 ± 0.33	0.10 ± 0.30	0.11 ± 0.32	0.12 ± 0.32	0.13 ± 0.34
thoracic vertebra	arch	13.0 ± 0.0	13.0 ± 0.0	13.0 ± 0.0	13.0 ± 0.0	13.0 ± 0.0
	body	13.0 ± 0.0	13.0 ± 0.0	13.0 ± 0.0	13.0 ± 0.0	13.0 ± 0.0
lumbar vertebra	arch	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0
	body	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0
sacral vertebra		4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
caudal vertebra		2.8 ± 1.0	2.7 ± 1.3	3.3 ± 1.1$^+$	3.0 ± 1.2	3.7 ± 1.1$^+$
rib		13.0 ± 0.0	13.0 ± 0.0	13.0 ± 0.0	13.0 ± 0.0	13.0 ± 0.0
metacarpus	Lt.	3.7 ± 0.5	4.0 ± 0.4	3.8 ± 0.4*	4.0 ± 0.2*	3.8 ± 0.4
	Rt.	3.7 ± 0.5	3.9 ± 0.3	3.8 ± 0.3	3.8 ± 0.4	3.8 ± 0.4
Prox.phal of forepaw	Lt.	0.4 ± 0.6	0.6 ± 0.9	0.3 ± 0.7	0.4 ± 0.8	0.4 ± 0.7
	Rt.	0.5 ± 0.9	0.4 ± 0.7	0.4 ± 0.8	0.3 ± 0.6	0.4 ± 0.8
distal phal. of forepaw	Lt.	1.0 ± 1.3	1.5 ± 1.7	1.3 ± 1.6	1.3 ± 1.5	0.9 ± 1.2
	Rt.	0.9 ± 1.3	1.4 ± 1.6	1.2 ± 1.4	1.1 ± 1.2	0.9 ± 1.2
metatarsus	Lt.	3.9 ± 0.3	3.9 ± 0.5	3.9 ± 0.3	4.0 ± 0.0	3.9 ± 0.3
	Rt.	3.8 ± 0.4	3.8 ± 0.5	3.9 ± 0.2	4.0 ± 0.2	3.8 ± 0.5
prox. phal. of hindpaw	Lt.	0.2 ± 0.5	0.4 ± 0.7	0.3 ± 0.6	0.4 ± 0.9	0.6 ± 0.9
	Rt.	0.1 ± 0.4	0.4 ± 0.7	0.2 ± 0.4	0.4 ± 0.8*	0.5 ± 0.9*
dist. phal. of hindpaw	Lt.	0.7 ± 1.1	1.1 ± 1.2	1.3 ± 1.2*	1.4 ± 1.4*	1.4 ± 1.4
	Rt.	0.6 ± 1.1	1.1 ± 1.2	1.2 ± 1.3	1.4 ± 1.4*	1.3 ± 1.4*
Sternebra		4.0 ± 1.0	4.6 ± 1.0	42. ± 1.1$^+$	4.1 ± 1.2	4.0 ± 0.8$^+$
No.of fetuses showing variations		23	20	18	16	18
variation rate(%) mean ± S.D. b)		22.5 ± 0.5	20.4 ± 0.4	20.5 ± 0.4	20.6 ± 0.4	18.4 ± 0.5
fission of thoracic vertebral centra		19	18	16	11	10
Lumbar rib		3	2	0	3	4
cleft sternum		1	0	2	2	4

a) : Values are expressed as mean ± S.D. calculated from values per litter in No. of ossified bones.
b) : (No. of fetuses showing skeletal variations/No. fetuses observed) × 100.

But there were increasing ossification trends in coccygeal vertebra and right proximal phalanges of hindpaw of high dose and low dose treated groups, metacarpus of medium dose and low dose treated groups, and distal phalanges of hindpaw of all treated groups ($p < 0.05$) (Table 5).

Otherwise, compared to the vehicle control, coccygeal vertebra of high and low dose treated groups showed increasing ossification ($p < 0.05$). Ossification of sternebrae of all treated groups was significantly delayed compared to the vehicle control, but not to the non-treated control (Table 5).

11) Skeletal anomalies in fetuses.

Any significant differences were not recognized in all treated groups, as shown in Table 5.

Table 6. Influence of IFN-α on fetal viscera (F_1) in teratogenicity study.

Dose (IU/kg/d.)	non-treatment control	Vehicle control	1×10^5	4×10^5	1.2×10^6
No. of dams	10	8	10	10	10
No. of fetuses observed	78	90	106	83	93
No. of fetuses showing visceral anomalies	11	10	10	13	9
Visceral anomaly rate(%) mean ± S.D	14.1 ± 0.4	11.1 ± 0.3	9.4 ± 0.3	15.6 ± 0.4	9.6 ± 0.3
Dilatation of Lateral Ventricle	0	2	2	4	0
Dilatation of renal pelvis	8	6	8	7	7
Ventricular spetal defect	1	0	0	1	2
Renal displacement	2	2	0	1	0

12) Organ anomalies in fetuses.

Any significant differences were not seen in all treated groups (Table 6).

DISCUSSION

The present study was carried out to examine the effects of rHuIFN-αA which was produced by gene manipulated *E. coli*, as an available therapeutic agent, on Sprague-Dawley rats. The substance was administered via tail vein or peritonium at levels of 1×10^5, 4×10^5, and $1.2^{*}10^6$ IU/kg/day during the period of organogenesis.

No death of dams was observed in the control and treated groups.

No remarkable changes in gestation period were seen. There was no noticeable variation in body weights of newborns.

From the results mentioned above, it might be considered that rHuIFN-A has none of fetal toxicity and teratogenicity.

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