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Short Communication

Background Data on Developmental Parameters During the Gestation Period in Rats

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Abstract: Background data during the gestation period were obtained from 128 Wistar Hannover GALAS rats and 26 Crl:CD(SD) pregnant rats in the control groups of our previous toxicity studies. The body weights of dams in the Wistar Hannover GALAS rats were significantly lower throughout the gestation period than those in the Crl:CD(SD) rats. In contrast, the time-dependent change in the body weight gain (%) of dams showed very similar trends in both strains. The mean number of live embryos/fetuses in the Wistar Hannover GALAS rats was 12.0, and was lower than that (14.5) in the Crl:CD(SD) rats. The placental weights gradually increased with pregnancy progression and reached a plateau on gestation day (GD) 19, although the embryo/fetal weights rapidly increased from GD 17 to GD 21. The embryo/fetal weights in the Wistar Hannover GALAS rats were significantly lower on only GD 21 than those in the Crl:CD(SD) rats. It is considered that this fetal weight difference between the strains develops during the fetal period, but not during the organogenesis period. In contrast, there were no differences in the placental weights between the two strains. Microscopically, the thickness of the labyrinth zone in the Wistar Hannover GALAS rats was thicker throughout the gestation period than that in the Crl:CD(SD) rats. (DOI: 10.1293/tox.26.83; J Toxicol Pathol 2013; 26: 83–88)

Key words: background data, Crl:CD(SD), development, rat, Wistar Hannover GALAS

Reproductive and developmental toxicity studies in rats are necessary for safety evaluation of pharmaceutical drugs, pesticides and food additives. There have been many reports on background data on gestation day (GD) 21 in pregnant rats[1–6]. However, there have been few reports on background data of cesarean section during the gestation period[7]. Therefore, we put the data concerning fetal and placental development from the pregnant rats in the control groups in our previous 3 fetal toxicity studies[8–10] and 7 placental toxicity studies[11–17] together in order to obtain background data during the gestation period in Wistar Hannover GALAS rats and Crl:CD(SD) rats. In addition, we compared the developmental parameters between the two strains.

These data were obtained from 128 Wistar Hannover GALAS and 26 Crl:CD(SD) pregnant rats that were the control animals in the above mentioned 10 toxicity studies and a few other toxicity studies. The Wistar Hannover GALAS rats (CLEA Japan, Inc., Japan) and Crl:CD(SD) rats (Charles River Laboratories Japan, Inc., Japan) were purchased at approximately 10-14 weeks of age. A female rat was housed together with a male rat of the same strain and source for mating. The occurrence of copulation was established by daily inspection for a vaginal plug. GD 0 was designated as the day when the presence of vaginal plug was identified. The maternal animals were housed individually in plastic cages on softwood chip bedding in an air-conditioned room (22 ± 2ºC; humidity, 55 ± 10%; light cycle, 12 hr/day). Feed (CRF-1, Oriental Yeast Co., Ltd., Japan) and water were available *ad libitum*. Maternal body weights were recorded on GDs 0, 6–17, 19, and/or 21. The dams were sampled on GDs 11, 13, 15, 16, 17, 19, and 21 in the Wistar GALAS Hannover rats and on GDs 13, 15, 17, and 21 in the Crl:CD(SD) rats. The dams were euthanized by exsanguination under anesthesia and necropsied. All embryos/fetuses were removed from the placentas. About 1/2 to 1/3 of the placentas were separated between the basal zone and the decidua basalis, and removed from the uterus wall. The embryos/fetuses and removed placentas were weighed, and the embryo/fetal-placental weight ratio was calculated individually. Some placentas in the Crl:CD(SD) rats were measured along the major axis and minor axis, and for thickness. The fetuses on GD 21 were macroscopically examined for external malformations. All placentas were fixed in 10% neutral buffered formalin. Four placentas per dam were obtained randomly from the live embryos/fetuses. The selected placentas were embedded in paraffin, sectioned at a thickness of 4-μm, and

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stained routinely with hematoxylin and eosin (H&E). The thicknesses of the labyrinth zone, basal zone, decidua basalis and metrial gland close to the central portion were measured in placentas from each dam with the aid of an image analyzer (WinROOF, Mitani Corporation, Japan, or IPAP, Processor for Analytical Pathology, Sumika Technoservice Corporation. Japan).

Means and standard deviations (SD) of the individual litter values were calculated. Comparison between the Wistar Hannover GALAS rats and the Crl:CD(SD) rats was analyzed with the Leven's test. When variances were homogeneous, the Student's *t*-test was performed. The Aspin-Welch *t*-test was performed when variances were not homogeneous. Comparison of parameters between GD 21 and other sampling points was analyzed with the Bartlett's test. When variances were homogeneous, the Dunnett's multiple comparison test was performed. The Steel's multiple comparison test was performed when variances were not homogeneous. The levels of significance were set at $P<0.05$ and $P<0.01$. All these experiments were conducted according to the Guidelines for Animal Experimentation, Japanese Association for Laboratory Animal Science, 1987.

There were no notable clinical signs in any dams during the gestation period. The body weights and body weight gains (%) of dams (based on the body weight on GD 6 as 100%) are shown in Table 1 and Fig. 1, respectively. The body weights of dams in the Wistar Hannover GALAS rats were significantly lower throughout the gestation period than those in the Crl:CD(SD) rats. In contrast, the time-dependent change in the body weight gain (%) of dams showed very similar trends in both strains, although there was a significant transient increase on GD 10 in the Wistar Hannover GALAS rats, as compared with the Crl:CD(SD) rats.

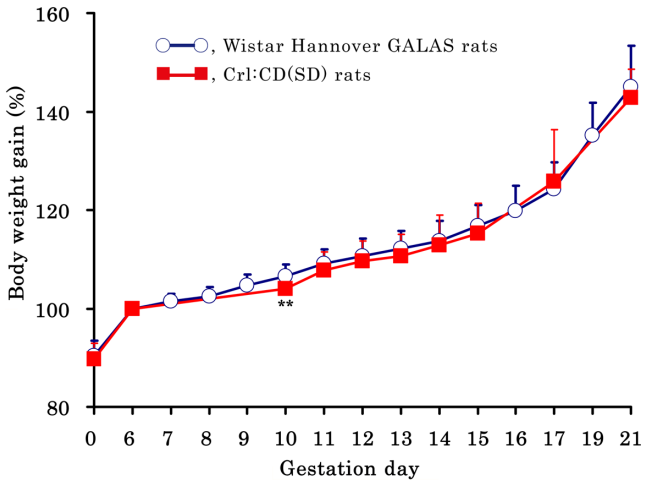

Fig. 1. Body weight gain (%) of dams during the gestation period. The body weight gain (%) of dams shows very similar trends in both strains, although there is a significant increase on GD 10 in the Wistar Hannover GALAS rats, as compared with the Crl:CD(SD) rats. Each value represents the mean ± SD. ** Significantly different from Wistar Hannover GALAS rats at $P<0.01$ (Student-*t* test).

The findings at cesarean section are shown in Table 2. There were no external abnormalities in the fetuses of both strains. The mean numbers of implantation sites and live embryos/fetuses during the gestation period were 12.5 and 12.0 in the Wistar Hannover GALAS rats, and 14.9 and 14.5 in the Crl:CD(SD) rats, respectively. Both parameters in the Wistar Hannover GALAS rats were significantly lower than those in the Crl:CD(SD) rats. The mean of the dead embryo/fetus ratio during the gestation period was 3.8% in the Wistar Hannover GALAS rats and 2.6% in the Crl:CD(SD) rats, with no significant difference between the two strains. In addition, there were no significant differences in these 3 parameters between GD 21 and other sampling points in both strains.

The placental weights gradually increased with pregnancy progression and reached a plateau on GD 19, although the embryo/fetal weights rapidly increased from GD 17 to GD 21. The embryo/fetal weights and embryo/fetal-placental weight ratio in the Wistar Hannover GALAS rats were significantly lower on only GD 21 than those in Crl:CD(SD) rats. In other background data[2–6], the fetal weights on GD 21 in the Crl:CD(SD) rats are more than 5 g (5.29-5.56 g in males and 5.06-5.30 g in females), and are also heavier than those in the Wistar Hannover GALAS rats in the present study. In addition, it has been reported that the fetal weights on GD 20 in the Crl:CD(SD)BR rats are more than those in Wistar Hannover (Tac:Glx:WIfBR) rats[18]. Therefore, we conclude that the embryo/fetal weights in the Wistar Hannover GALAS rats are lower at parturition than those in the Crl:CD(SD) rats. Furthermore, this fetal weight difference between the strains develops during the fetal period, but not during the organogenesis period. In contrast, the placental weights and embryo/fetal-placental weight ratio in the Wistar Hannover GALAS rats were significantly lower and higher on GD 17 than those in the Crl:CD(SD) rats, respectively. However, since these changes were transient, it appeared that there were no differences in the placental weights between the strains. The relationships between the embryo/fetal weights and the placental weights are shown in Table 3. In the present rat study, no clear correlation between them was detected at any sampling points under conditions with no distinction of gender, although it has been reported that a strong relationship is observed between the fetal and placental weights in humans[19]. This could be attributed to the fact that no dwarf fetus or small placenta was included in the present study and that the embryo/fetal and placental weights were within the range of normal variability.

Macroscopically, the diameter and thickness of the placenta in the Crl:CD(SD) rats are shown in Table 4 and Fig. 2. The thickness of the placenta reached a plateau on GD 17, although the diameter gradually increased until GD 21. Histologically, the thickness of each layer of the placenta is shown in Table 5 and Fig. 3. The placenta is composed of the fetal part and maternal part[20]. The fetal part of the placenta consists of the labyrinth zone and basal zone. The basal zone was fully developed on GD 15, leading to regression gradually before parturition. The labyrinth zone was developed

Table 1. Body Weight of Dams During the Gestation Period (g)

Strain		Gestation day															Gain (GD0-21)
		0	6	7	8	9	10	11	12	13	14	15	16	17	19	21	
Wistar Hannover GALAS	Mean	217.2	239.8	242.0	246.1	249.8	256.3	261.5	266.6	268.8	273.9	279.2	292.1	299.5	323.2	345.0	131.0
	SD	26.9	25.7	24.9	26.9	24.5	26.8	25.2	26.4	24.6	26.1	26.5	26.7	26.2	26.6	29.0	17.4
	No. of dams	120	128	91	67	91	83	104	100	120	87	98	54	44	23	32	31
Crl:CD (SD)	Mean	279.7**	311.7**				320.8**	335.3**	341.0**	344.2**	348.6**	355.1**		382.3**		431.8**	161.3**
	SD	29.9	30.3	ND	ND	ND	19.0	26.2	27.3	25.9	31.8	25.5	ND	35.7	ND	20.1	17.8
	No. of dams	26	26				11	26	26	26	14	22		6		8	8

ND: No data. ** Significantly different from Wistar Hannover GALAS rats at $P<0.01$ (Student's *t*-test).

Table 2. Observation at Cesarean Section of Dams During the Gestation Period

Strain	Gestation day	No. of dams	No. of implant site	No. of live embryo/ fetus	Dead embryo ratio (%)	Embryo/fetal weight (mg)[a]	Placental weight (mg)[a]	Embryo/fetal-placental weight ratio (mg/mg)[a]
Wistar Hannover GALAS	11	8	13.3 ± 1.7	13.3 ± 1.7	0.0 ± 0.0	ND	ND	ND
	13	17	12.8 ± 1.9	12.2 ± 2.3	3.8 ± 5.6	68.4 ± 14.5	109.3 ± 16.1	0.65 ± 0.11
	15	33	12.7 ± 2.2	12.3 ± 2.2	3.6 ± 5.8	260.7 ± 20.7	215.1 ± 32.8	1.26 ± 0.18
	16	14	12.9 ± 2.5	12.0 ± 2.9	7.9 ± 0.3	521.7 ± 125.6	276.7 ± 40.7	1.91 ± 0.32
	17	20	12.2 ± 1.9	11.8 ± 2.3	4.0 ± 7.1	750.6 ± 59.1	309.2 ± 38.6	2.47 ± 0.29
	19	4	11.3 ± 0.4	11.0 ± 0.6	2.5 ± 2.2	2062.2 ± 80.0	448.7 ± 13.0	4.84 ± 0.07
	21	32	11.9 ± 2.4	11.6 ± 2.7	2.6 ± 8.0	4884.7 ± 341.5	450.0 ± 34.4	11.03 ± 1.02
	Mean	128[b]	12.5 ± 2.1	12.0 ± 2.4	3.8 ± 6.9	NE	NE	NE
Crl:CD(SD)	13	4	14.3 ± 0.4	13.8 ± 0.4	3.6 ± 3.1	72.0 ± 1.9	125.9 ± 16.6	0.66 ± 0.10
	15	8	14.9 ± 1.8*	14.6 ± 1.9**	1.7 ± 3.2	276.6 ± 22.0	242.5 ± 42.8	1.18 ± 0.20
	17	6	15.3 ± 1.6**	14.8 ± 2.1**	3.5 ± 5.7	787.1 ± 40.7	363.2 ± 22.3**	2.22 ± 0.22*
	21	8	14.9 ± 1.1**	14.5 ± 1.2**	2.3 ± 0.4	5200.4 ± 345.9*	440.4 ± 31.2	12.01 ± 0.71*
	Mean	26[b]	14.9 ± 1.4**	14.5 ± 1.6**	2.6 ± 5.3	NE	NE	NE

Mean ± SD. ND: No data. NE: Not examined. [a] Mean of individual litter values. [b] Total number of dams. *, ** Significantly different from Wistar Hannover GALAS rats at $P<0.05$ and $P<0.01$, respectively (Student's *t*-test).

Table 3. Squared Correlation Coefficients (R^2) Between Embryo/Fetal Weights and Placental Weights

Strain		Gestation day					
		13	15	16	17	19	21
Wistar Hannover GALAS	R^2	0.281	0.146	0.105	0.191	0.065	0.092
	No. of embryos/fetuses	74	236	125	100	19	235
	No. of dams	17	41	10	20	4	32
Crl:CD(SD)	R^2	0.094	0.054	ND	6E-6	ND	0.469
	No. of embryos/fetuses	26	56		42		57
	No. of dams	4	8		6		8

ND: No data.

Table 4. Macroscopic Size of the Placenta During the Gestation Period

Strain	Gestation day	No. of dams	Length (mm)[a)]		
			Diameter		Thickness
			Major axis	Minor axis	
Crl:CD (SD)	15	4	11.38 ± 0.25	10.62 ± 0.26	2.58 ± 0.23
	17	6	13.47 ± 0.45	12.34 ± 0.38	3.47 ± 0.18
	21	4	14.22 ± 0.29	12.61 ± 0.30	3.41 ± 0.30

Mean ± SD. [a)] Mean of individual litter values.

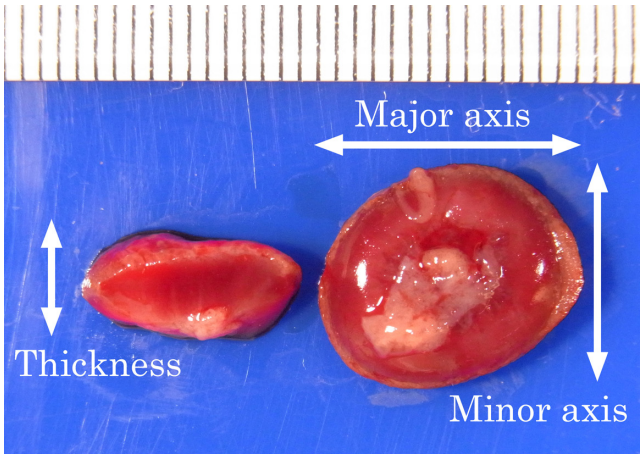

Fig. 2. Gross appearance of the placenta on GD 21.

with advancing pregnancy, and formed the majority of the fetal part of the placenta. The maternal part of the placenta consists of the decidua and metrial gland. The decidua basalis underwent regression after GD 11. The metrial gland was fully developed on GD 13 and was maintained until parturition. The metrial gland formed the majority of the maternal part of the placenta. The labyrinth zone in the Wistar Hannover GALAS rats was thicker throughout the gestation period than that in the Crl:CD(SD) rats, which appeared to result from strain differences. However, it appeared that there was no difference in the total volume of the labyrinth zone between the Crl:CD(SD) rats and the Wistar Hannover GALAS rats, because the placental weights, which mainly consisted of the labyrinth zone and basal zone, were almost the same. Therefore, it is considered that the difference in the thickness of the labyrinth zone between them is not a

Table 5. Thickness of Each Layer of the Placenta During the Gestation Period

Strain	Gestation day	No. of dams	Thickness (μm)[a)]			
			Fetal part of placenta		Maternal part of placenta	
			Labyrinth zone	Basal zone	Decidua basalis	Metrial gland
Wistar Hannover GALAS	11	8	329 ± 166	179 ± 36[1)]	1653 ± 321	1618 ± 286
	13	12	837 ± 199	596 ± 135	876 ± 173	2939 ± 559
	15	21	1525 ± 214	986 ± 217	468 ± 96[2)]	2639 ± 249[2)]
	17	20	2499 ± 256	809 ± 107	323 ± 77	2862 ± 144[3)]
	21	24	2854 ± 189	452 ± 71	244 ± 78[4)]	2551 ± 220[3)]
Crl:CD (SD)	13	4	583 ± 293	576 ± 74	1160 ± 56[††]	NE
	15	8	942 ± 151[**]	1077 ± 232	580 ± 103	NE
	17	4	1892 ± 90[**]	799 ± 154	377 ± 86	NE
	21	8	2438 ± 346[**]	527 ± 65[*]	243 ± 29	NE

Mean ± SD. [*, **] Significantly different from Wistar Hannover GALAS rats at $P<0.05$ and $P<0.01$, respectively (Student's *t*-test). [††] Significantly different from Wistar Hannover GALAS rats at $P<0.01$ (Aspin-Welch *t*-test). [a)] Mean of individual litter values. [1)] Data from 4 dams; [2)] Data from 17 dams; [3)] Data from 16 dams; [4)] Data from 20 dams data.

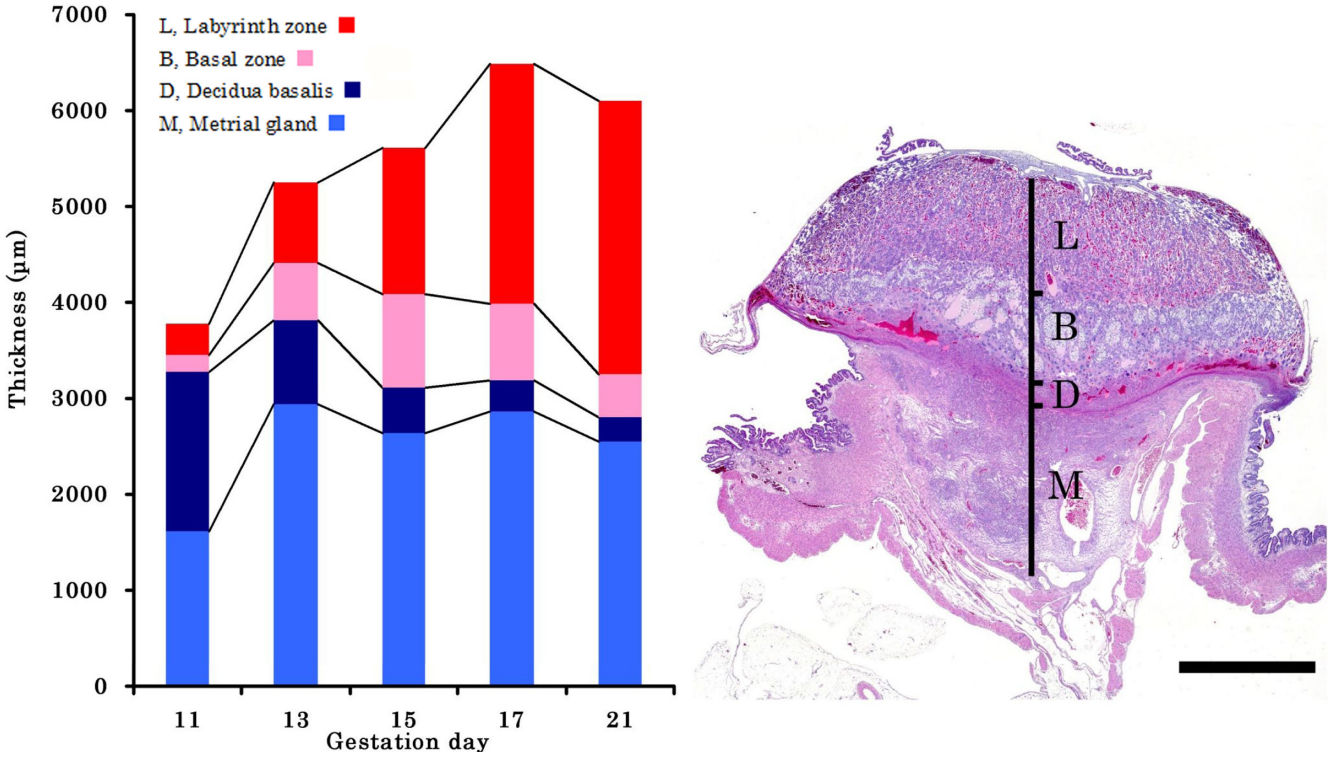

Fig. 3. Time-dependent change in thickness of each placental layer in Wistar Hannover GALAS rats and microscopic appearance of the placenta on GD 15. H&E stain, Bar=2000 μm. The labyrinth zone developes with advancing pregnancy. The basal zone is fully developed on GD 15, leading to regression gradually before parturition. The decidua basalis undergoes regression after GD 11. The metrial gland is fully developed on GD 13, and is maintained until parturition.

major cause of the strain difference in reproductive and developmental toxicity. In contrast, the basal zone and decidua basalis in the Wistar Hannover GALAS rats were significantly thicker on GD 21 and thinner on GD13, as compared with those in the Crl:CD(SD) rats, respectively. Since these changes in the basal zone and decidua basalis seemed to be transient, it is necessary to examine many more placentas in the Crl:CD(SD) rats.

In conclusion, this study indicated that there were some differences in parameters between the Wistar Hannover GALAS rats and the Crl:CD(SD) rats. The data in the present study would contribute to evaluate the results in reproductive and developmental toxicity studies in both rats. Further accumulation of background data during the gestation period should be performed for detailed consideration and better understanding of the mode of action in chemical-induced developmental toxicity.

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