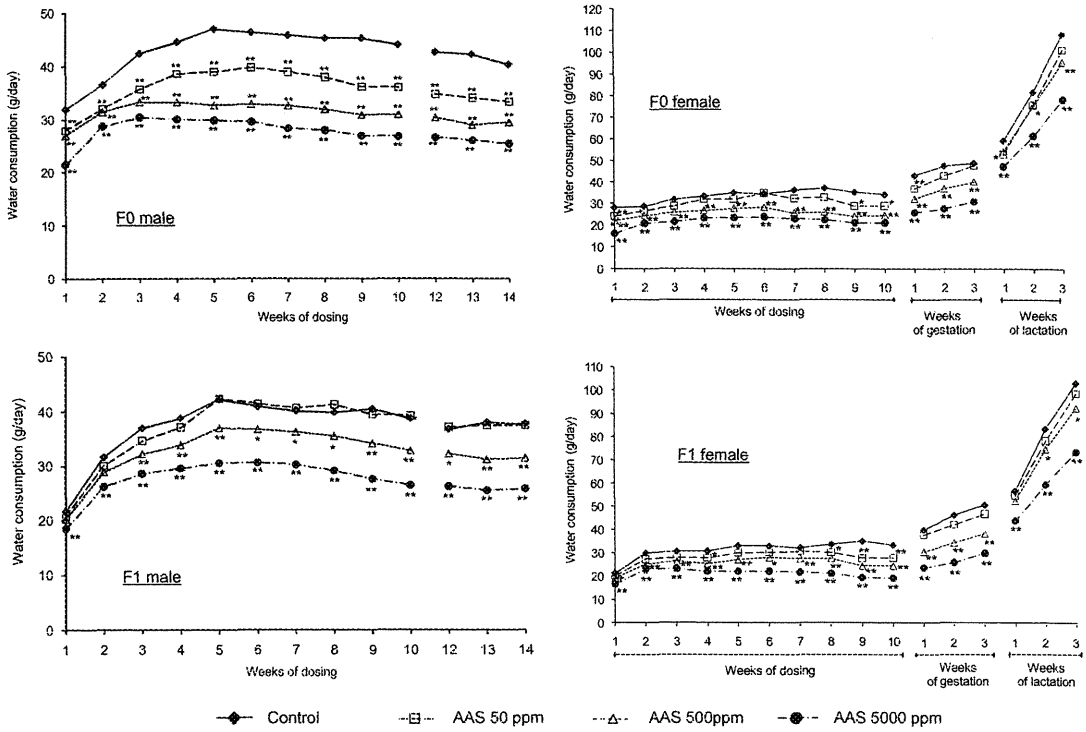

Fig. 2. Water consumption of F0 and F1 parental animals. *Significantly different from the control. $P < 0.05$, **Significantly different from the control, $P < 0.01$.

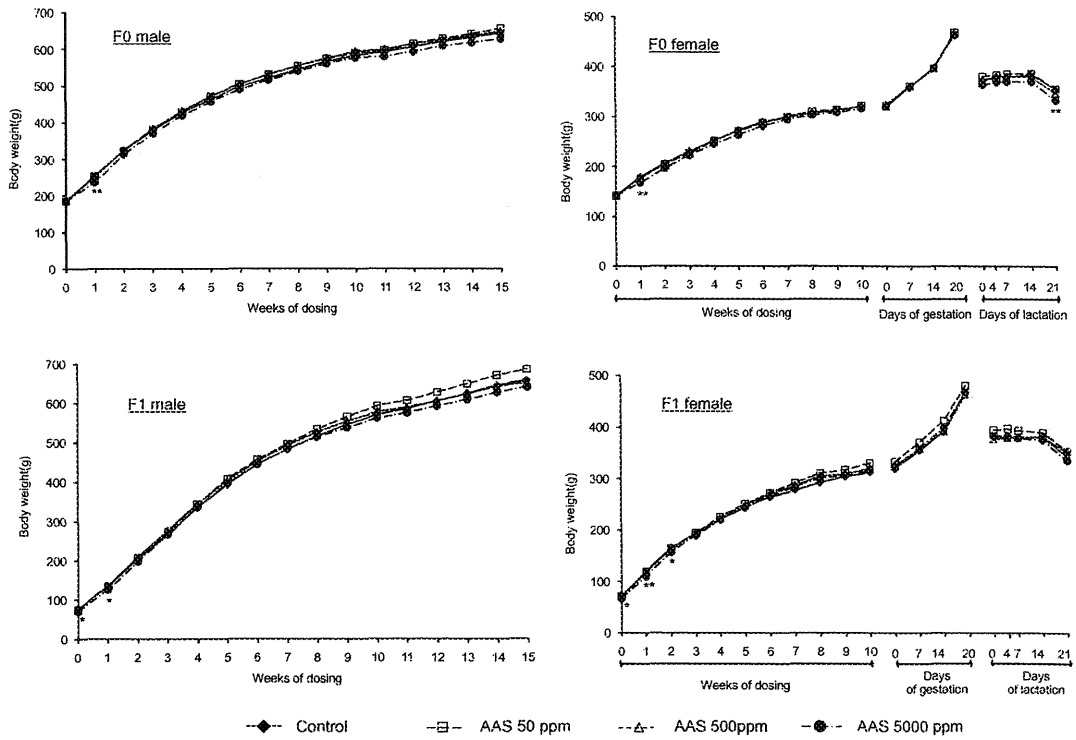

Fig. 3. Body weight change of F0 and F1 parental animals. *Significantly different from the control, $P < 0.05$. **Significantly different from the control, $P < 0.01$.

on right hindlimb and crushing of incisor/malocclusion in a few F1 pups in control and AAS-treated groups; however, there were no significant differences in incidence between the control and AAS-treated groups (data not shown). No gross abnormalities were found in any F2 pups. While there were no significant differences in birth weight between the control and AAS-treated groups, the

Table 1
Reproductive performance of F0 and F1 parental animals.

AAS (ppm)		0 (control)	50	500	5000
F0 generation					
No. of rats (male/female)		24/24	24/24	24/24	24/24
Copulation index (%)[a]	Males	100	95.8	91.7	100
	Females	100	100	100	100
Precoital interval (days)[b]		2.2 ± 1.0	2.5 ± 1.6	2.3 ± 1.2	2.8 ± 1.6
Fertility index (%)[c]	Males	100	91.3	100	100
	Females	100	87.5	100	100
Gestation index (%)[d]		100	100	100	100
Gestation length (days)[b]		22.3 ± 0.5	22.4 ± 0.6	22.3 ± 0.5	22.4 ± 0.5
F1 generation					
No. of rats (male/female)		24/24	24/24	23/24	24/24
Copulation index (%)[a]	Males	91.7	91.7	91.3	95.8
	Females	100	95.8	100	100
Precoital interval (days)[b]		2.7 ± 1.8	3.0 ± 2.1	3.3 ± 2.4	3.1 ± 1.3
Fertility index (%)[c]	Males	90.9	77.3	95.2	100
	Females	91.7	78.3	95.8	95.8
Gestation index (%)[d]		100	100	95.7	100
Gestation length (days)[b]		22.3 ± 0.5	22.3 ± 0.5	22.2 ± 0.4	22.2 ± 0.4

[a] Copulation index (%) = (No. of animals with successful copulation/No. of animals paired) × 100.
[b] Values are given as the mean ± S.D.
[c] Fertility index (%) = (No. of animals that impregnated a female or were pregnant/No. of animals with successful copulation) × 100.
[d] Gestation index (%) = (No. of females that delivered live pups/No. of pregnant females) × 100.

body weight of F1 males on PND 21 and of F1 females on PNDs 14 and 21 was significantly lower in the 5000 ppm group than in the control (Table 2). A similar decreasing trend was found in the body weight of male and female F2 pups around the time of weaning in the highest dose group, although no statistical significance was found.

In F1 and F2 pups, there were no significant differences in the completion rate of pinna unfolding, the age at completion of incisor eruption and eye opening, and AGD and AGD per cube root of the body weight ratio between the control and AAS-treated groups (data not shown). All male and female F1 and F2 pups in all groups achieved the surface righting reflex on PND 5, negative geotaxis reflex on PND 8 and mid-air righting reflex on PND 18, and no significant changes were found in the response time of surface righting and negative geotaxis reflex (data not shown). In F1 female animals, vaginal opening was significantly delayed at 5000 ppm (32.3 ± 1.8 days of age, compared with 30.2 ± 2.1 days of age in controls, $P \leqslant 0.01$). Body weight at the time of attainment was not significantly, but was slightly heavier in this 5000 ppm group (122.0 ± 15.7 g, compared with 115.8 ± 12.6 g in control). There were no significant differences in age at preputial separation or body weight at the time of completion in F1 males between control and AAS-treated groups (data not shown).

3.5. Necropsy, organ weight and histopathology of adults (F0 and F1)

No dose-related gross lesions were found in either F0 or F1 adults. In F0 females in the 500 and 5000 ppm groups and in F1 males and females in the 5000 ppm group, relative kidney weight was increased significantly. A significant decrease in the absolute weight of the pituitary gland was found in F0 females and in F1 males and females at 5000 ppm. In F1 females, there was also a significant decrease in the absolute thymus weight at 5000 ppm. Further, significant decreases were found in the relative weight of the seminal vesicle in 50 ppm-treated F1 males and in the absolute brain weight in 500 ppm-treated F1 females, but no dose-dependency was found in these changes (data not shown). There were no treatment-related alterations in the histopathology of male or female reproductive organs. No significant differences were found in the number of primordial follicles in the ovary of F1 females between control and 5000 ppm groups (data not shown).

3.6. Necropsy, organ weight and histopathology of weanlings (F1 and F2)

Body weight at scheduled sacrifice and absolute and relative organ weight of male and female F1 and F2 weanlings are shown in Table 3 and 4. In either generation, 5000 ppm-treated males and females had significantly lower body weights, and the absolute and relative weights of the spleen in both sexes and of the thymus in males were significantly decreased in this 5000 ppm group. A decrease in the absolute thymus weight was also observed in F1 females given 500 and 5000 ppm and in F2 females given 5000 ppm, but there were no significant changes in relative weight in F1 or F2 females. The absolute liver weight was significantly decreased in F1 and F2 males and females, accompanied with a decrease in the relative weight in F1 males and F2 females in the 5000 ppm group. The relative weights of the brain and kidney were increased significantly in F1 and F2 males and females given 5000 ppm. Further, a significant decrease in the absolute weight of the kidney, adrenal, testis, epididymis, ovary and uterus was found at 500 and/or 5000 ppm.

External and internal gross observations did not reveal any treatment-related alterations either in F1 and F2 weanlings or pups found dead during the lactation period. No dose-related changes were found in the histopathology of the liver and spleen in both sexes and of the thymus in males in either generation.

3.7. Behavioral effects (F1)

Spontaneous locomotor activity for 10 min intervals and for a total of 60 min was not significantly different between the control and AAS-treated groups in F1 males (Fig. 4). In F1 females, a significant decrease in spontaneous activity was found during the 40–50 min and 50–60 min after the start of recording in the 500 ppm group, but no significant changes were found in total activity for 60 min in this group. There were no significant differences in spontaneous locomotor activity for 10 min intervals or for a total of 60 min between the control and the other AAS-treated groups in females. In the water-filled T-maze test, pre-test swimming trials in the straight channel revealed that all male and female F1 rats in each group could swim satisfactorily, and no significant changes were observed in the elapsed time to traverse

Table 2
Developmental findings for F1 and F2 offsprings.

AAS (ppm)	0 (control)	50	500	5000
F0 parents/F1 offspring				
No. of F0 pregnant females	24	21	24	24
No. of implantations[a]	14.7 ± 3.1	14.3 ± 2.1	15.0 ± 3.3	15.1 ± 1.5
No. of litters	24	21	24	24
No. of pups delivered[a]	13.6 ± 3.1	13.5 ± 2.5	13.8 ± 3.1	14.4 ± 1.6
Delivery index (%)[a,b]	92.4 ± 8.0	94.2 ± 10.3	92.3 ± 7.8	95.4 ± 5.4
Sex ratio of pups[c]	0.509	0.493	0.476	0.487
Viability index of pups (%)[a]				
On PND 0[d]	99.5 ± 2.7	99.0 ± 2.4	99.5 ± 1.7	99.2 ± 2.3
On PND 4[e]	98.3 ± 5.0	98.0 ± 5.4	95.6 ± 20.4	99.2 ± 2.3
On PND 21[f]	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Male pup weight during lactation (g)[a]				
On PND 0	6.93 ± 0.66	6.96 ± 0.68	6.91 ± 0.48	6.90 ± 0.69
On PND 4	11.13 ± 1.88	10.84 ± 1.47	10.72 ± 0.94	10.68 ± 1.33
On PND 7	19.14 ± 2.30	18.86 ± 2.30	18.71 ± 1.51	18.49 ± 1.70
On PND 14	38.45 ± 3.57	38.32 ± 3.96	37.88 ± 2.31	36.51 ± 2.20
On PND 21	63.83 ± 5.93	62.59 ± 7.09	61.71 ± 4.94	58.67 ± 3.91**
Female pup weight during lactation (g)[a]				
On PND 0	6.66 ± 0.82	6.57 ± 0.61	6.58 ± 0.57	6.43 ± 0.63
On PND 4	10.70 ± 2.02	10.34 ± 1.25	10.22 ± 1.13	10.13 ± 1.28
On PND 7	18.40 ± 2.49	17.96 ± 2.02	17.97 ± 1.74	17.38 ± 1.79
On PND 14	37.23 ± 3.65	36.97 ± 3.30	36.59 ± 2.74	35.07 ± 2.35*
On PND 21	61.65 ± 6.05	60.03 ± 5.55	59.34 ± 5.22	56.13 ± 4.07**
F1 parents/F2 offspring				
No. of F1 parent females	22	18	23	23
No. of implantations[a]	15.0 ± 1.6	14.7 ± 1.7	14.7 ± 3.5	14.1 ± 2.2
No. of litters	22	18	22	23
No. of pups delivered[a]	13.9 ± 1.8	13.7 ± 2.4	14.0 ± 3.8	13.5 ± 2.1
Delivery index (%)[a,b]	92.7 ± 9.4	93.0 ± 11.2	90.9 ± 20.4	95.6 ± 5.7
Sex ratio of pups[c]	0.435	0.500	0.492	0.506
Viability index of pups (%)[a]				
On PND 0[d]	98.3 ± 4.5	97.6 ± 4.2	98.9 ± 3.3	99.7 ± 1.5
On PND 4[e]	97.9 ± 6.0	99.4 ± 2.4	99.4 ± 1.9	99.0 ± 2.9
On PND 21[f]	99.4 ± 2.7	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Male pup weight during lactation (g)[a]				
On PND 0	6.97 ± 0.62	7.03 ± 0.65	6.89 ± 0.53	6.97 ± 0.75
On PND 4	10.64 ± 1.62	11.31 ± 1.22	10.95 ± 1.32	11.16 ± 1.87
On PND 7	17.97 ± 2.18	19.19 ± 1.73	18.82 ± 1.90	18.42 ± 2.39
On PND 14	36.89 ± 3.26	38.99 ± 3.14	38.28 ± 3.26	36.40 ± 3.67
On PND 21	61.07 ± 6.06	64.40 ± 5.58	63.20 ± 5.51	58.65 ± 5.90
Female pup weight during lactation (g)[a]				
On PND 0	6.46 ± 0.47	6.68 ± 0.67	6.54 ± 0.50	6.51 ± 0.63
On PND 4	9.91 ± 1.26	10.62 ± 1.18	10.30 ± 1.20	10.59 ± 1.72
On PND 7	17.15 ± 2.06	18.28 ± 1.77	17.73 ± 1.68	17.58 ± 2.34
On PND 14	35.58 ± 3.00	37.47 ± 2.74	36.65 ± 2.69	35.20 ± 3.44
On PND 21	58.47 ± 5.33	61.83 ± 4.40	60.05 ± 3.82	56.72 ± 5.39

* Significantly different from the control, $P < 0.05$.
** Significantly different from the control, $P < 0.01$.
[a] Values are given as the mean ± S.D.
[b] Delivery index (%) = (No. of pups delivered/No. of implantations) × 100.
[c] Sex ratio = total No. of male pups/total No. of pups.
[d] Viability index on PND 0 (%) = (No. of live pups on PND 0/No. of pups delivered) × 100.
[e] Viability index on PND 4 (%) = (No. of live pups on PND 4/No. of live pups on PND 0) × 100.
[f] Viability index on PND 21 (%) = (No. of live pups on PND 21/No. of live pups on PND 4 after cull) × 100.

the straight channel (Fig. 5). On days 2–4 of the T-maze test, no significant changes were observed in the elapsed time and number of errors in both sexes.

4. Discussion

The present study was performed to provide general information concerning the effects of AAS on the integrity and performance of the male and female reproductive systems, and on the growth and development of the offspring. AAS administered via drinking water to male and female rats at 50, 500 or 5000 ppm resulted in decreased water consumption in all dose groups. This could be attributed to the astringent taste of AAS (Korea Food and Drug Administration, 2004), which would decrease the palatability of drinking water in AAS-treated groups. The change in water consumption was associated with transient decreases in food consumption in the 500 and 5000 ppm groups and in body weight in the 5000 ppm group. Nevertheless, the reproductive performance (i.e. copulation, fertility or gestation indices) was not affected up to the highest dose tested, at which average aluminium intake from food and drinking water was estimated to be 36.3–61.1 mg Al/kg bw/day. In addition, adverse effects were not found in estrous cyclicity or sperm parameters, or in the histopathology of reproductive tissues in male and female parental animals.

Previous studies demonstrated that water-soluble aluminium compounds given by oral gavage caused male reproductive toxicity, including changes in the number of spermatozoa and their motility,

Table 3
Absolute and relative organ weight of F1 male and female weanlings.

AAS (ppm)		0 (Control)	50	500	5000
Males					
No. of animals		24	20	23	24
Body weight	(g)	94.1 ± 9.1	90.8 ± 10.7	91.3 ± 9.8	80.9 ± 7.5**
Brain	(g)	1.72 ± 0.08	1.71 ± 0.07	1.70 ± 0.06	1.68 ± 0.07
	(g/100 g b.w.)	1.84 ± 0.16	1.90 ± 0.17	1.88 ± 0.16	2.09 ± 0.15**
Thymus	(mg)	392 ± 67	373 ± 72	360 ± 57	301 ± 48**
	(mg/100 g b.w.)	417 ± 61	411 ± 55	396 ± 61	372 ± 52*
Liver	(g)	4.32 ± 0.54	4.15 ± 0.55	4.12 ± 0.53	3.52 ± 0.43**
	(g/100 g b.w.)	4.58 ± 0.29	4.57 ± 0.17	4.51 ± 0.27	4.34 ± 0.25**
Kidney[a]	(g)	1.08 ± 0.13	1.04 ± 0.14	1.05 ± 0.10	0.98 ± 0.10*
	(g/100 g b.w.)	1.15 ± 0.10	1.15 ± 0.08	1.15 ± 0.06	1.21 ± 0.08*
Spleen	(mg)	421 ± 75	399 ± 66	403 ± 91	292 ± 49**
	(mg/100 g b.w.)	447 ± 64	441 ± 60	439 ± 78	361 ± 43**
Adrenal[a]	(mg)	26.4 ± 3.4	24.5 ± 2.7	25.5 ± 3.2	24.0 ± 3.4*
	(mg/100 g b.w.)	28.2 ± 3.6	27.2 ± 2.9	28.0 ± 3.0	29.8 ± 3.5
Testis[a]	(mg)	591 ± 69	571 ± 74	573 ± 72	532 ± 78*
	(mg/100 g b.w.)	628 ± 38	630 ± 41	628 ± 49	656 ± 61
Epididymis[a]	(mg)	80.7 ± 9.3	76.2 ± 10.7	78.9 ± 10.0	67.8 ± 9.9**
	(mg/100 g b.w.)	86.0 ± 8.1	84.3 ± 10.4	86.6 ± 8.3	84.2 ± 11.6
Females					
No. of animals		24	21	23	24
Body weight	(g)	87.0 ± 7.2	85.5 ± 7.6	83.3 ± 7.1	76.2 ± 7.0**
Brain	(g)	1.68 ± 0.12	1.64 ± 0.06	1.65 ± 0.06	1.62 ± 0.06
	(g/100 g b.w.)	1.93 ± 0.16	1.93 ± 0.16	1.99 ± 0.14	2.14 ± 0.16**
Thymus	(mg)	382 ± 58	365 ± 48	342 ± 51*	316 ± 41**
	(mg/100 g b.w.)	437 ± 46	429 ± 56	411 ± 54	416 ± 54
Liver	(g)	3.79 ± 0.38	3.80 ± 0.39	3.73 ± 0.42	3.28 ± 0.43**
	(g/100 g b.w.)	4.36 ± 0.38	4.45 ± 0.28	4.48 ± 0.31	4.30 ± 0.28
Kidney[a]	(g)	0.98 ± 0.10	0.97 ± 0.11	0.96 ± 0.09	0.93 ± 0.08
	(g/100 g b.w.)	1.13 ± 0.08	1.14 ± 0.06	1.15 ± 0.05	1.22 ± 0.07**
Spleen	(mg)	362 ± 63	351 ± 44	356 ± 59	272 ± 47**
	(mg/100 g b.w.)	416 ± 72	412 ± 49	428 ± 63	356 ± 46**
Adrenal[a]	(mg)	25.5 ± 3.9	23.6 ± 3.0	22.7 ± 3.0**	22.3 ± 2.6**
	(mg/100 g b.w.)	29.4 ± 4.0	27.8 ± 3.8	27.3 ± 3.5	29.4 ± 3.1
Ovary[a]	(mg)	24.6 ± 4.5	24.6 ± 4.4	23.8 ± 2.9	22.0 ± 4.0
	(mg/100 g b.w.)	28.2 ± 4.6	29.0 ± 4.6	28.9 ± 4.6	29.2 ± 6.4
Uterus	(mg)	67.3 ± 15.3	66.4 ± 21.4	64.6 ± 15.9	50.4 ± 10.9**
	(mg/100 g b.w.)	77.3 ± 16.6	77.1 ± 20.5	77.2 ± 15.8	66.2 ± 12.8

Values are given as the mean ± S.D.
* Significantly different from the control, $P < 0.05$.
** Significantly different from the control, $P < 0.01$.
[a] Values represent the total weights of the organs on both sides.

at much lower doses [i.e. 2.5 mg Al/kg bw/day in a 6-month exposure study in rats (Krasovskii et al., 1979) and 3.4 mg Al/kg bw/day in a 13-week exposure study in rabbits (Yousef et al., 2005)]. However, the dose-relationship demonstrated in the oral gavage studies might be significantly-inaccurate because the dietary intake of aluminium was not considered. In addition, the relevance of these oral gavage studies for human risk assessment is unclear because the toxicokinetics after a bolus dose by gavage must differ significantly from those after actual continuous exposure via the diet in humans. As for the continuous exposure studies taking into account the aluminium content in the basal diet, germinal epithelial cell degeneration and atrophy in the seminiferous tubules were observed at 75 mg Al/kg bw/day in the 26-week feeding study of SALP basic in dogs (Pettersen et al., 1990), but no such effects on male reproductive organs were detected up to 88 mg Al/kg bw/day in a similar subchronic dietary study of SALP acidic in dogs (Katz et al., 1984). Difference in outcome of these subchronic studies using dogs is considered to come from the difference in the solubility of aluminium compounds [SALP acidic is insoluble in water, and SALP basic is a mixture of 70% of a complex of SALP (sparingly soluble) and 30% of disodium phosphate (very soluble)] because it is widely assumed that insoluble aluminium compounds are less bioavailable than soluble compounds (IPCS, 2007). Considering the relationship between the solubility and bioavailability of aluminium, the present continuous exposure study using water-soluble aluminum compound could provide more reliable data on the male reproductive toxicity of aluminium.

In the present study, the preweaning body weight gain of F1 and F2 pups was depressed in the 5000 ppm group. This change could be simply attributable to decreased water consumption of dams. Since rat pups commence drinking water during the last week of the lactation period, there is also a possibility that the decreased water intake of pups inhibited their body weight gain. However, similar effects on preweaning body weight were previously reported in offspring of mice given a diet containing aluminium lactate at 500 ppm and above during the gestation and lactation periods (daily aluminium intake during lactation: 94–273 mg Al/kg bw/day)(Golub and Germann, 2001; Golub et al., 1987, 1992). In this feeding study, food consumption was decreased, but preweaning growth inhibition at 1000 ppm was greater than that of the paired fed control (Golub et al., 1987), suggesting the possibility that the fall in body weight around weaning in the present study might not be explained only by a decreased intake of water. Aluminium ingested by pups themselves and/or via maternal milk might affect preweaning growth. Maternal nursing behavior abnormality or impairment of the lactation status could be considered another possible factor.

In F1 and F2 weanlings, various organ weight changes suggestive of treatment-related effects were found, among which, decreases in the absolute weight and/or increases in the relative

Table 4
Absolute and relative organ weight of F2 male and female weanlings.

AAS (ppm)		0 (Control)	50	500	5000
Males					
No. of animals		22	18	22	23
Body weight	(g)	89.9 ± 7.5	94.1 ± 8.3	91.7 ± 7.9	82.9 ± 10.2*
Brain	(g)	1.70 ± 0.06	1.73 ± 0.06	1.70 ± 0.06	1.67 ± 0.08
	(g/100 g b.w.)	1.90 ± 0.17	1.85 ± 0.14	1.86 ± 0.12	2.04 ± 0.23*
Thymus	(mg)	375 ± 69	379 ± 50	365 ± 50	296 ± 53**
	(mg/100 g b.w.)	417 ± 65	404 ± 53	399 ± 46	359 ± 58**
Liver	(g)	4.12 ± 0.55	4.49 ± 0.53	4.34 ± 0.44	3.69 ± 0.48*
	(g/100 g b.w.)	4.57 ± 0.32	4.77 ± 0.34	4.73 ± 0.19	4.46 ± 0.20
Kidney[a]	(g)	1.02 ± 0.10	1.08 ± 0.10	1.03 ± 0.10	1.01 ± 0.13
	(g/100 g b.w.)	1.13 ± 0.08	1.15 ± 0.08	1.13 ± 0.07	1.22 ± 0.07**
Spleen	(mg)	390 ± 86	387 ± 48	393 ± 40	292 ± 52**
	(mg/100 g b.w.)	435 ± 93	413 ± 54	430 ± 41	352 ± 46**
Adrenal[a]	(mg)	26.0 ± 3.8	25.2 ± 3.5	25.5 ± 3.3	24.7 ± 4.3
	(mg/100 g b.w.)	29.0 ± 4.1	26.7 ± 3.3	28.0 ± 3.9	29.8 ± 3.0
Testis[a]	(mg)	546 ± 83	571 ± 83	572 ± 70	515 ± 67
	(mg/100 g b.w.)	607 ± 72	605 ± 59	623 ± 51	624 ± 65
Epididymis[a]	(mg)	74.8 ± 7.5	76.1 ± 10.6	75.1 ± 9.9	68.7 ± 9.1
	(mg/100 g b.w.)	83.7 ± 10.2	80.7 ± 7.5	82.0 ± 9.0	83.6 ± 11.5
Females					
No. of animals		22	18	22	23
Body weight	(g)	85.3 ± 7.2	87.6 ± 6.5	84.0 ± 4.9	77.2 ± 5.7**
Brain	(g)	1.65 ± 0.05	1.65 ± 0.06	1.64 ± 0.06	1.62 ± 0.06
	(% of body weight)	1.95 ± 0.16	1.89 ± 0.13	1.95 ± 0.08	2.11 ± 0.15**
Thymus	(mg)	367 ± 68	354 ± 60	352 ± 47	300 ± 40**
	(mg/100 g b.w.)	432 ± 84	405 ± 66	419 ± 48	390 ± 55
Liver	(g)	3.93 ± 0.41	3.97 ± 0.37	3.80 ± 0.33	3.33 ± 0.34**
	(g/100 g b.w.)	4.61 ± 0.25	4.54 ± 0.19	4.52 ± 0.25	4.31 ± 0.31**
Kidney[a]	(g)	0.96 ± 0.08	0.97 ± 0.09	0.94 ± 0.06	0.93 ± 0.08
	(g/100 g b.w.)	1.13 ± 0.07	1.11 ± 0.07	1.12 ± 0.05	1.21 ± 0.07**
Spleen	(mg)	355 ± 53	330 ± 33	349 ± 52	276 ± 35**
	(mg/100 g b.w.)	416 ± 51	378 ± 35*	415 ± 59	358 ± 42**
Adrenal[a]	(mg)	23.3 ± 2.3	23.2 ± 2.3	23.2 ± 3.4	23.2 ± 2.4
	(mg/100 g b.w.)	27.4 ± 2.7	26.6 ± 2.9	27.5 ± 3.5	30.0 ± 3.1*
Ovary[a]	(mg)	24.6 ± 3.0	24.9 ± 4.0	24.2 ± 4.1	20.4 ± 3.2**
	(mg/100 g b.w.)	29.1 ± 4.3	28.5 ± 4.0	29.0 ± 5.5	26.7 ± 4.9
Uterus	(mg)	71.0 ± 55.7	66.8 ± 16.5	58.5 ± 11.8	53.5 ± 11.1*
	(mg/100 g b.w.)	82.3 ± 59.4	76.0 ± 15.4	69.6 ± 12.4	69.5 ± 14.8

Values are given as the mean ± S.D.
* Significantly different from the control, $P < 0.05$.
** Significantly different from the control, $P < 0.01$.
[a] Values represent the total weights of the organs of both sides.

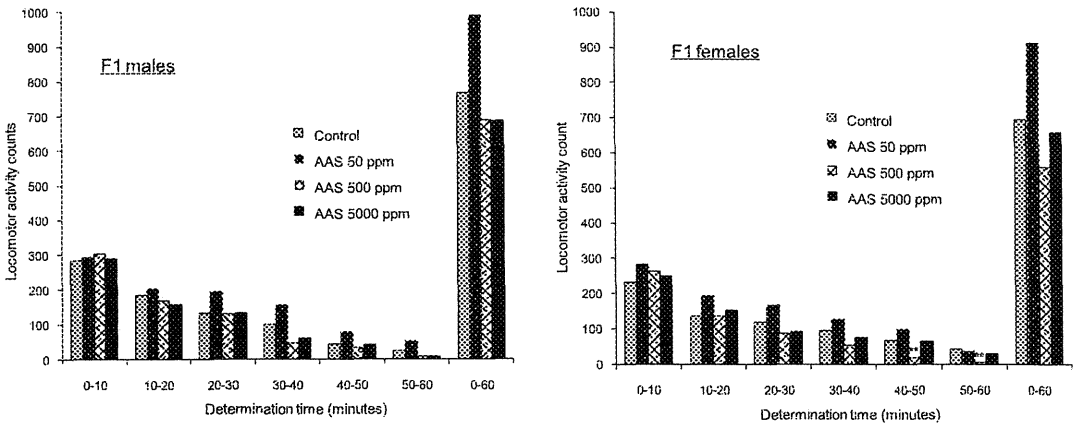

Fig. 4. Locomotor activity count in F1 parental rats. Data are presented as the mean of 10 animals/sex/group. **Significantly different from the control, $P < 0.01$.

weight of the brain, kidney, adrenal, testis, epididymis, ovary, and uterus are considered to be secondary changes that occur with the fall of body weight. On the other hand, decreases in the absolute and relative weights of the liver and spleen in both sexes and in the absolute and relative thymus weights in males in the 5000 ppm group could not be explained only by the fall of body weight. In these organs, no histopathological changes were detected, and further, there were no changes in the weights of these organs in F0 or F1 adults. Previously, Golub et al. (1987) demonstrated that gestational and lactational exposure of mice to excessive dietary aluminium (1000 ppm as aluminium lactate) markedly decreased the spleen weight of offspring at weaning age.

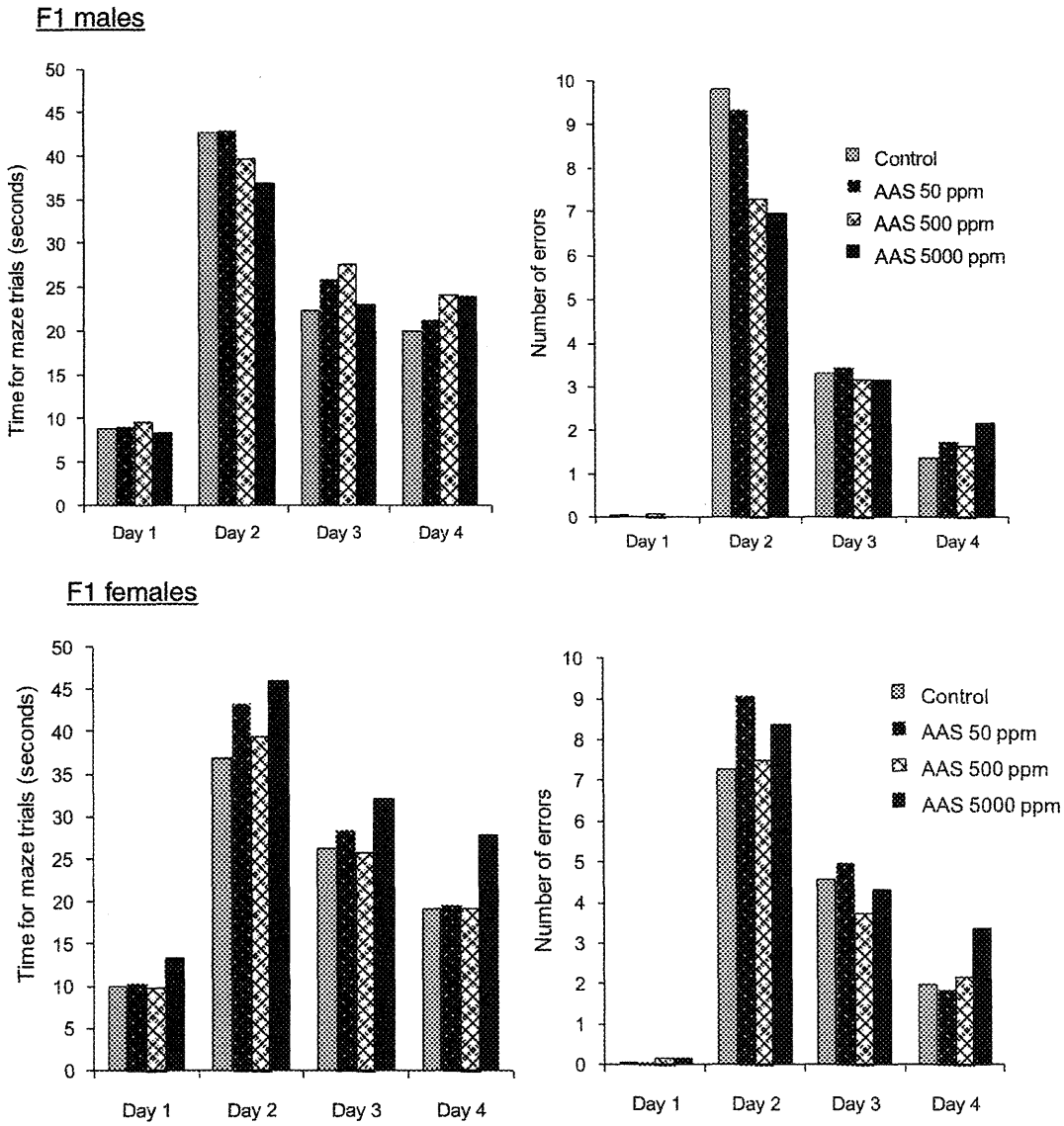

Fig. 5. Performance in a water-filled multiple T-maze in F1 parental rats. Data are presented as the mean of 10 animals/sex/group. There were no statistically significant differences between the control and AAS-treated groups.

Decreased concentrations of interleukin-2, interferon-γ and tumor necrosis factor-α in spleen cells were reported in mice exposed to a similar level of aluminum lactate via the diet from conception through 6 months of age (Golub et al., 1993). Although the association between such immunosuppressive effects and decreased spleen weight of weanlings is unclear, aluminium might have a certain effect on the developing spleen. Further study would be required to clarify the effects of developmental aluminium exposure on splenic function, including immune function. In the above-mentioned study conducted by Golub et al. (1987), dietary aluminium exposure did not affect the liver and thymus weight of weanlings significantly; therefore, the decreased weight of these organs observed in the present study might have resulted from reduced water consumption rather than ingested aluminium because water is essential for organ growth.

Vaginal opening is the initial sign of the estrogenic rise that accompanies the first ovulation followed by estrous cyclicity as the initial sign of the central drive of ovarian activity (Ramirez and Sawyer, 1965; Rasier et al., 2006); it is widely used as a marker of female puberty. In the present study, vaginal opening was slightly delayed (mean = 2.1 days) in F1 females at 5000 ppm and the age at completion was outside the normal range for this strain of rat in the laboratory in which the study was conducted (historical control data for the last seven years: 29.6–31.0 days). Although it is well known that decreased body weight can result in non-specific delays in puberty, the body weight at the time of vaginal opening was slightly heavier in the 5000 ppm group than in the control in the present study. Delayed age at vaginal opening is known to be caused by fetal and/or postnatal exposure to various chemicals disrupting steroid functions or hypothalamic–pituitary functions (Goldman et al., 2000; Rasier et al., 2006). The putative/suggested mechanism includes blockage of the response of estrogen-dependent tissues to the ovarian steroid hormone (lindane) (Cooper et al., 1989), inhibition of steroid synthesis (ketoconazole and fadrozole) (Marty et al., 1999) and decreased gonadotropin levels (luteinizing hormone-releasing hormone

antagonist, Org30276) (Meijs-Roelofs et al., 1990). In the present study, rats with delayed vaginal opening progressed to showing normal reproductive capacity and outcome. In addition, no effects were found on AGD, estrous cyclicity or on the weight and histopathology of reproductive organs in weanlings and adults. It seems unlikely that aluminium has a clear impact on hormonal events. In order to clarify the etiology of this slight delay in female sexual maturation, further studies are required.

Our previous two-generation study of aluminium sulfate administered via drinking water to rats gave the same results regarding parental toxicity and reproductive/developmental toxicity as the present study (Hirata-Koizumi et al., 2011); reduced water consumption in all 120, 600 and 3000 ppm groups (respective calculated aluminium intake: 2.96–4.72, 8.06–14.0, 31.2–55.6 mg Al/kg bw/day), and decreased body weight of parental animals, inhibition of preweaning body weight gain, decreased liver and spleen weight of weanlings, and a slight delay of vaginal opening in the highest dose group. In this two-generation study of aluminium sulfate, as well as in the present study of AAS, no treatment-related changes were found in reflex ontogeny, spontaneous locomotor activity or performance in a water-filled multiple T-maze, indicating that previous findings of developmental neurobehavioral effects were possibly related to the toxic effects of aluminium given at higher doses than those given in these two-generation studies. Some developmental effects observed in these two-generation studies could be considered to come from ionized aluminium in drinking water, but there is also a possibility that these are secondary effects due to decreased water consumption. In order to reach more definitive conclusions, further study including paired-comparison data is required to assess the effects of decreased water intake in the absence of AAS or aluminium sulfate exposure. Conservative evaluation of the present data led to the conclusion that the no observed adverse effect level of AAS for two-generation reproductive/developmental toxicity in rats is 500 ppm (5.35 mg Al/kg bw/day) primarily based on the effect on preweaning body weight gain.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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特集 妊娠と薬物療法

妊婦に対する薬物療法の考え方

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妊娠中は母体の生理的変化により薬物の体内動態が影響を受けて，投与した薬剤の血中濃度が変化する可能性がある。妊婦への薬剤投与ではこの点に留意した適切な投与設計が望まれる。また妊婦に投与された薬剤は胎児に対して，妊娠初期には催奇形性に作用し，中期以降は発育や機能発達を障害するような毒性を発揮するなど，胎児にとって大きな影響を及ぼす。これらの作用は薬剤により，また曝露される妊娠の時期によりさまざまであるため，薬剤師にはその回避のための正確な知識と情報提供が求められる。

Key word　母体の薬物動態，催奇形性，絶対過敏期，臨界期，胎児毒性，胎盤通過性

はじめに

妊婦に対する薬物療法を考える場合，臨床家は次の3つの事項に留意する必要がある。第一点は妊娠による影響で原疾患の病態が変化することにより，治療に必要な薬剤量が増減する可能性である。第二点は妊娠により母体の薬物動態が変化する結果，有効な血中濃度を維持するための薬物投与計画についてである。第三点は胎児への影響であり，催奇形性や胎児毒性の有無に基づいた薬剤の選択が必要となる。妊婦に対する薬物療法では以上の点を考慮して，疾病を有する妊婦自身への治療効果を保ちつつ，胎児への影響を最小限に回避する配慮が必要である。

妊婦に特徴的な薬物動態

妊娠中は胎盤からエストロゲン，プロゲステロンが大量に分泌され，循環血漿量の増量，血中アルブミン濃度の低下，遊離脂肪酸の増加などの変化が生じている。これらの生理的変化により薬物の体内動態が影響を受けることが報告されており，薬物によって非妊時よりも血中濃度が上昇するものがある一方，逆に低下するものもある。前者では副作用防止への配慮から，後者では治療効果が維持されるよう，適切な投与設計が望まれる。薬物動態は薬物の吸収，分布，代謝，排泄の4つの機序により規定される（表1）。

1．薬物の吸収の変化

血中プロゲステロン濃度上昇の結果，胃内容排出速度や腸蠕動が低下し薬物の吸収は遅延する。また胃内pHの上昇により，弱酸性や弱塩基性の薬物では溶解度の増減が吸収率の変化として現れる。

2．薬物の分布の変化

(1) 分布容積の変化

体水分量，循環血漿量は妊娠経過とともに増量し，妊娠第8カ月の末には非妊時に比べて約50％増加する。その結果，薬剤の分布容積は増大し，妊娠前と同じ投与量

虎の門病院産婦人科部長

表1　妊娠時における薬物動態に影響する生理的諸因子の変動

胃内pH↑	―胃酸分泌の減少と胃粘液分泌の減少
胃内容排出速度↓ 小腸運動↓	―第Ⅲ期に向かい30～50％の減少．プロゲステロンが原因とされる
血漿容積↑	―次第に増加し末期には約50％増加
心拍出量↑	―次第に増加し末期には約40％増加
肝血流量↑	―次第に増加し末期には約30％増加
糸球体濾過量↑	―次第に増加し末期には約50％増加
血漿蛋白量	―アルブミン量は増加するが，血漿容積の増加に及ばず含量としては減少．α_1-AG含量は軽度に低下する
血漿蛋白結合率	―ステロイドや胎児ホルモンなどによりアルブミン上の薬の結合部位が減少．含量の低下を伴い，多くの薬の蛋白結合率は低下
薬物代謝活性	―CYP1A2活性の低下．2D6活性の上昇．2A6および3A4活性の軽度の上昇．UGT活性の上昇

〔加藤隆一：臨床薬物動態学 改訂第4版．南江堂，p282，2009より引用〕

では薬剤の血中濃度は低下する。また，体脂肪量の増加により脂溶性薬剤の分布容積は増大する。

(2) 蛋白結合率の変化

血漿中の薬物は蛋白質と結合した蛋白結合型と，結合していない遊離型の双方の状態で存在するが，妊娠経過とともに循環血漿量が増加して血漿中のアルブミンやα_1酸性糖蛋白の濃度が低下する結果，薬物の蛋白結合率は低下する。妊娠末期には薬物とアルブミンとの結合に競合する遊離脂肪酸濃度が増加することも蛋白結合率の低下をもたらす。蛋白非結合型（遊離型）の薬剤濃度が増加すると薬効が高まる可能性がある。また遊離型の薬物は組織への移行が容易なため，結果として前述の分布容積が増大する。抗痙攣薬であるフェニトイン，カルバマゼピン，バルプロ酸ナトリウムなどは妊娠第3三半期に向かって蛋白結合率が低下することが知られており，留意する必要がある。

3. 薬物の代謝の変化

薬物の多くは肝の代謝酵素であるチトクロムP450（CYP）によって代謝される。妊娠による代謝酵素活性の変化は，低下するものと上昇するものとさまざまである。

活性が低下する酵素としてCYP1A2があげられる。CYP1A2は約半数の薬物の代謝酵素であるが，一例としてカフェインの妊娠中の半減期が延長することが知られている。一方，活性が上昇する酵素としてはメトプロロール酒石酸塩の代謝酵素であるCYP2D6やカルバマゼピンの代謝酵素であるCYP3A4，フェニトインの代謝酵素CYP2C9などが報告されている。

チトクロムP450による代謝以外ではグルクロン酸抱合があり，一般に妊娠中は亢進する。たとえばオキサゼパムやバルプロ酸ナトリウムはグルクロン酸抱合を受けるが，妊娠中の半減期は減少する。

4. 薬物の排泄の変化

(1) 腎からの排泄

循環血漿量の増加に伴い腎血流量が増加し，薬剤の腎でのクリアランスが高まることが知られている。アンピシリンやジゴキシンなどの腎排泄型の薬剤は非妊時よりも排泄速度が早くなり，血中濃度が低下する可能性がある。

(2) 肝からの排泄

妊娠中は肝血流量には大きな変化はなく，薬物の肝排泄速度は変化がないことが知られている。しかし肝で代謝されるクリンダマイシンの排泄速度が上昇しているという報告もあり，個々の薬物については確認が必要である。

5. 薬物の最高血中濃度や半減期の変化

薬物動態の指標である最高血中濃度（C_{max}）や半減期（$t_{1/2}$）は，前述の吸収，分布，代謝，排泄の4つの機序の総和として現れるため，妊娠による影響も個々の薬物により変化の程度がさまざまであるため複雑である。

妊娠時期に関する基礎知識

1. 妊娠週日についての規定

通常，妊娠の時期を表現するには直前の月経開始日（最終月経の開始日）から満で数えた週数・日数を用いている。たとえば妊娠8週5日のように表現する。月経周期が28日型の女性を基準としているため，排卵・受精日が妊娠2週0日であり，次の月経の開始予定日が妊娠4週0日，分娩予定日は40週0日となる。月経開始日から排卵までの期間が含まれているため，正味の妊娠期間

を求めるには2週間（14日間）を差し引く必要がある。ただし，排卵までの日数は女性によって異なり，同じ女性でも一定ではなく周期による変動があるため，同じ妊娠週日でも胎児の受精からの日数（胎齢）とは数日から10日間くらいの幅の誤差があると考える必要がある。

2. 胎　齢

胎齢は受精からの日齢を“数え”で表現する。受精当日は胎齢第1日であり，妊娠2週0日である。体外受精・胚移植などの生殖補助医療によって妊娠した場合は，受精日が確定しているため胎齢を正確に表すことができる。これに対して自然の妊娠では，排卵日（受精日）が不明であるか不確かな記憶をたどることが多いため，正確な胎齢を求めるのは難しい。妊娠初期の胎児計測値により，ある程度の精度で胎齢を知ることができる。たとえば，頭殿長が20mmならば妊娠9週0日であり胎齢は第50日となる。

3. 妊娠月数

妊娠月数は，妊娠週日と同様に最終月経の開始日を起点として数える。4週間（28日間）が1カ月に相当する点は理解しやすいが，数えで表されるため週数との対応上注意が必要である。妊娠第1カ月は最終月経初日から妊娠3週6日までであり，妊娠4週0日，すなわち次の月経の開始予定日が妊娠第2カ月の初日にあたる。女性が自らの月経が遅れて妊娠に気づくのは第2カ月に入ってからということになる。

4. 妊娠の三半期

妊娠期間を月数よりもさらに大まかに区分する考え方が妊娠の三半期である。三半期も数えで表し，日本産科婦人科学会では妊娠の開始から妊娠14週未満（妊娠13週6日まで）を第1三半期，妊娠14週から28週未満（妊娠27週6日まで）を第2三半期，妊娠28週から分娩までを第3三半期としているが，便宜的な分け方であり国内で統一された定義はない。海外でも区分は国によりまちまちであるので，海外の文献や資料を読む際には注意が必要である。

薬物の胎児への影響

1. 薬物の影響からみた妊娠時期の区分

配偶子（卵子と精子）から受精卵，受精卵（胚）から胎児へという成長過程から考えると，薬物の胎児への影響の類型には3つの種類がある。第一の影響は致死的な作用を受けるかまったく影響を受けないかで，“all or none”の法則とよばれる。受精前の配偶子の時期から受精後約2週間（妊娠3週6日まで）の時期が該当する。第二の影響は胎児形態異常（奇形）の発生で，器官形成期が該当する。妊娠4週0日から15週6日の時期である。第三の影響は胎児毒性であり，器官形成期以後出生までの胎内発育の期間が該当する（図1）。

2. all or noneの法則

(1) 受精から妊娠3週6日まで

最終月経の初日から33日間（受精から19日間），すなわち器官形成期までは受精卵（胚）は薬剤の影響が胎児に現れない。これをall or noneの法則という。薬剤の影響を受けて死滅するか着床しない，あるいは着床しても月経と区別できない初期の流産として終わるか，または受けた影響が完全に修復されて後遺症がまったくない状態で生まれてくるという意味である。

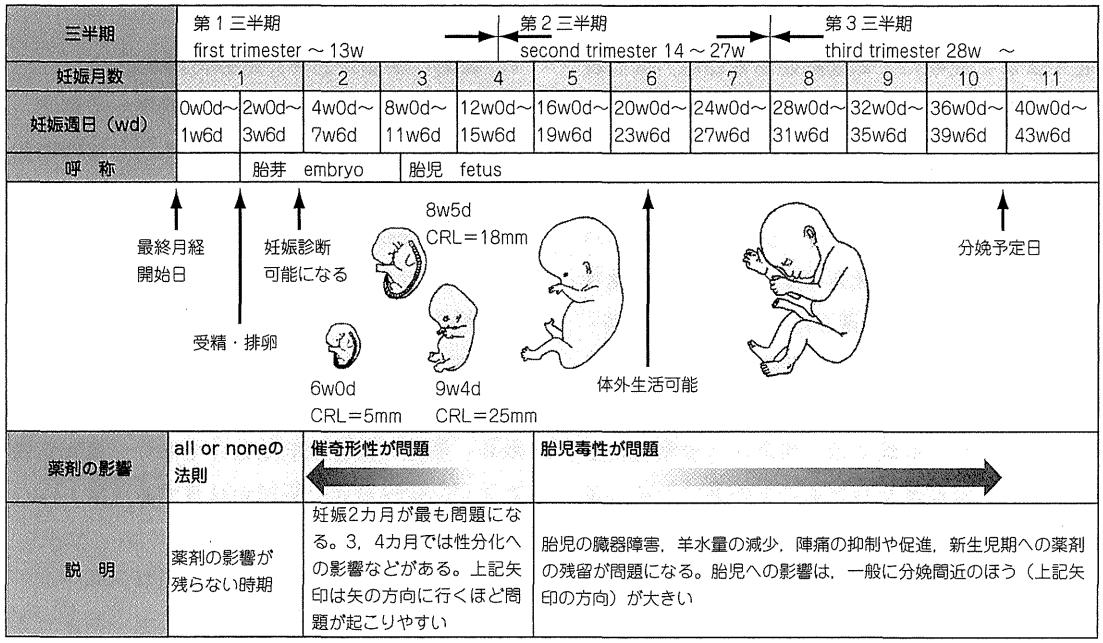
受精から19日間は妊娠週日では妊娠4週4日までとなるが，排卵日のずれを考慮して妊娠3週6日（予定月経開始日の前日）までと考えておいたほうが安全である。したがって，後で妊娠がわかった場合でも，この時期の薬剤の影響を心配する必要はない。また妊娠前から服用している薬剤については，妊婦での禁忌薬でも予定月経までは続けても差しつかえないことになる。

(2) 妊娠前に投与された薬剤の影響

妊娠前の女性に投与された薬剤の場合にもall or noneの法則があてはまる。

卵巣内の卵子（未受精卵）はその女性が胎児期にあるときに卵母細胞から減数分裂によって形成され，出生したのち思春期になって順に排卵される。未受精卵は卵巣内の原始卵胞内で第1減数分裂の前期で停止した状態で排卵を待つわけであるため，薬剤の影響を受けにくい。

未受精卵が排卵するときは，停止していた減数分裂が



注：三半期の定義は一定したものがない。示したのは一例。
CRL＝Crown-Rump Length（頭殿長）

図1 妊娠の経過と薬剤の影響

〔林 昌洋，他・編：実践 妊娠と薬 第2版．じほう，p3，2010より引用〕

排卵前36時間に黄体化ホルモン（hCG）の刺激を受けて再開し，受精準備状態に入る。この減数分裂再開から受精までの期間は未受精卵が薬剤の影響を受けやすい時期であり，薬剤の体内での半減期を考慮すると排卵日の前2日間となる。しかしながら，この時期の薬剤の影響もall or noneの法則に従うとされており，受精能力を失って妊娠が成立しない可能性がある一方，妊娠が成立して発育してきた胎児に関しては薬剤の影響を考慮する必要はない。

(3) 男性に投与された薬剤の影響

妊娠相手の男性に投与された薬剤の影響については次のように考える。卵子とは異なり精子は思春期以後，絶えず精巣内で精母細胞から形成されている。その形成に要する日数は70～80日間であり，精子形成後は受精能獲得のため数日間精管から精嚢に貯留されたのちに射精される。したがって，薬剤の影響を考慮すべき期間は受精前3カ月間である。しかし，薬剤の影響を受けて受精能が低下すれば受精に関与できないこと，1回の射精で数千万～数億個の精子が射精されることを考慮すると，精子への薬剤の影響にもall or noneの法則が適用されると考えられている。実際に疫学的に精子への影響を示した報告はほとんどなく，過去に影響を指摘された薬剤であるエトレチナート（チガソン：乾癬，魚鱗癬などの治療薬）やコルヒチンについても催奇形性については否定的である。

3. 器官形成期

(1) 妊娠4週0日から7週6日まで

最終月経の初日から34日目（妊娠4週5日）から妊娠15週6日は，胎児の諸器官が形作られ器官形成期とよばれる。そのうち最終月経初日から数えて34日から50日，受精後20日から36日（妊娠4週5日から7週0日）は中枢神経系，心臓・循環器系，消化器系，四肢などの主要臓器・器官が形成される時期であり，薬剤の催奇形性の影響を最も受けやすく，催奇形性の絶対過敏期または臨界期（critical period）とよばれる。前述した妊娠週日と胎齢との誤差を考慮して，本稿では妊娠4週0日から7週6日までの4週間，ちょうど妊娠第2カ月にあたる

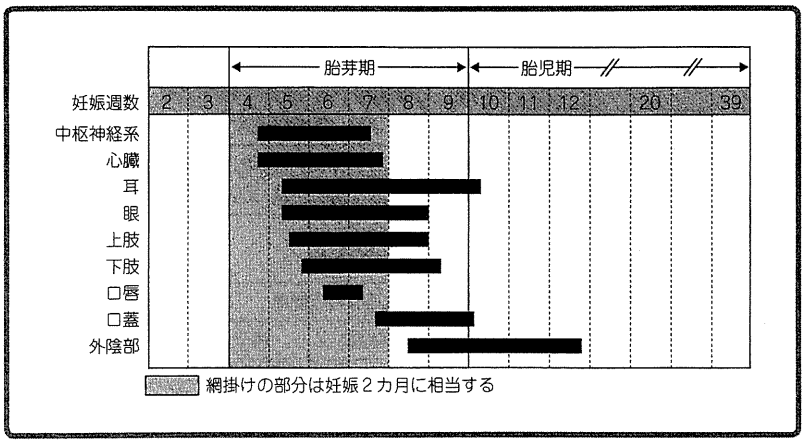


図2　胎児の器官形成期と薬剤が主に影響する時期

〔林　昌洋，他・編：実践 妊娠と薬 第2版．じほう，p5，2010より引用〕

時期を絶対過敏期と扱うこととする（図2）。

この時期では，催奇形性の知られている薬物の投与は原則として避けるべきであり，安全といわれている薬剤についても安易な使用は慎まなければならない。

また，薬剤の影響以外でも奇形発生の危険性があることに留意する時期でもある。糖尿病合併の妊婦がこの時期に高血糖を呈すると児に心奇形が発症することや，葉酸欠乏により二分脊椎や無脳症の発生がみられることなどが代表例であり，対策としては血糖調節や葉酸補充など妊娠前からの計画的な指導が必要である。

(2) 妊娠8週0日から16週0日まで

絶対過敏期を過ぎて妊娠16週までは，主要な器官の形成は終わっているものの体表の形態の完成期であり，外性器の分化や口蓋・口唇の癒合が行われる。奇形発生という意味での胎児諸器官の薬剤感受性は低下しつつあるが，催奇形性のある薬物の投与はなお慎重でありたい。

4. 胎児の機能獲得と薬剤の胎児毒性

器官形成期を過ぎた16週以降では胎児は各器官の機能の獲得期に入り，出生後の胎外生活が可能となるよう成熟が進行する。この時期には薬物の直接的な影響による奇形発生はみられないが，胎児の機能的発育に及ぼす影響や発育の抑制，子宮内胎児死亡のほか，出生後の新生児の適応障害や薬剤の離脱症状などが起こりうる。これらは胎児毒性とよばれる（表2）。

胎児毒性の例としてはアンジオテンシン変換酵素阻害薬（ACE阻害薬）やアンジオテンシンⅡ受容体拮抗薬（ARB）が代表的である。これらは妊娠第2三半期以降に投与されると胎児の低血圧と腎血流量の低下により腎機能障害を引き起こす。その結果，胎児の尿量減少から羊水量が減少して羊水過少症を発症し，胎児の肺低形成，四肢の拘縮，頭蓋の変形を生じる。

胎児毒性のもう一つの代表例が非ステロイド性抗炎症薬（NSAIDs）である。NSAIDsは妊娠第3三半期の後半に投与されると胎児の動脈管の収縮を起こし，肺動脈の血流が増大して肺高血圧症から右心不全を引き起こすことが知られている。

5. 薬物の胎盤通過性

妊娠の第2三半期以降，母体に投与された薬物はほとんどが胎盤を経由して胎児に到達する。一般に薬物は母体血中から単純拡散により胎盤を通過して胎児に移行する。したがって，母体血中濃度が高いほど胎児への移行量も多くなる。

それぞれの薬物の胎盤通過性は，胎児毒性を回避するために妊婦へ投与する薬物を選択するうえでの重要な要素である。胎盤通過性を左右する因子としては以下のものがある。

(1) 薬物の分子量

分子量が300～600程度の薬物は比較的容易に胎盤を通過し，1,000以上になると通過しにくい。抗凝固療法を行う必要のある妊婦では，胎盤通過性が高く催奇形性の

表2　胎児毒性があると考えられる主な薬剤

非ステロイド性抗炎症薬（NSAIDs）	第3三半期曝露で胎児動脈管早期閉鎖 後期曝露により，動脈管収縮，胎児循環遺残，羊水過少
アンジオテンシン変換酵素阻害薬 アンジオテンシンⅡ受容体拮抗薬	妊娠中期・後期曝露による胎児腎障害・無尿・羊水過少， 羊水過少による肺低形成・四肢拘縮・頭蓋変形
アルキル化薬 （ブスルファン，シクロホスファミド）	子宮内胎児発育遅延
アミノグリコシド系抗菌薬	非可逆的第Ⅷ脳神経障害
テトラサイクリン系抗菌薬	中期・後期曝露により，歯牙着色・エナメル質形成不全
ヨード	過剰摂取により，可逆的な甲状腺機能低下
抗凝固薬（ワルファリン）	頭蓋内出血
アルコール	胎児性アルコールスペクトラム障害
喫煙	子宮内胎児発育遅延

〔伊藤真也，他・編：妊娠と授乳．南山堂，p10，2010より引用〕

あるワルファリンを中止し，胎盤を通過しにくいヘパリンに変更する必要がある。

(2) 薬物の脂溶性

脂溶性の薬物は水溶性の薬物よりも胎盤通過性がよい。脂溶性の高い薬物の例として，ビタミンAやフェノバルビタールは容易に胎児に移行する。

(3) 薬物の蛋白結合率

薬物は遊離型のものが胎盤を通過するため，ジゴキシンやアンピシリンなどの蛋白結合率が低い薬物は胎盤通過性が高い。

(4) 胎児血pHによる通過性の差異

胎児血のpHは母体血よりもわずかに低く，このpHの差がイオントラッピングとよばれる効果を及ぼすことが知られている。pKa値が血液pHに近い弱塩基性の薬物は，母体血中では主に非イオン型で存在するため胎盤を通過しやすい。胎盤を通過した薬物は，より酸性の胎児血中でイオン化するため，胎児側では非イオン型の濃度が低下して濃度勾配が生じ，母体側から胎児側に向かってさらに薬物が移動することにつながる。逆に弱酸性の薬物では胎児から母体への移動が起こる。

おわりに

妊婦に投与された薬剤は，妊娠という母体のなかで生じる大きな生理的変化のなかで吸収され，代謝，排泄される。疾病をもった妊婦への投薬に際しては，この薬物動態の変化を理解して投与計画が適切かを判断する必要がある。一方，母体に投与された薬剤の胎児への影響は，生まれてくる子どもの一生を左右する問題となりうる。妊婦への薬剤投与に関わる者は，この点についての正確な知識を有し，常に最新の情報を取り入れることが求められる。

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P-15 Survey of pregnancy outcomes in women who used etizolam

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[Objective] There have been no epidemiological studies on children born to women who used etizolam (EZ) during pregnancy. Therefore, we analyzed pregnancy outcomes in women who visited the Counseling Clinic for Pregnancy and Medicine of Toranomon Hospital.
[Methods] In women who used EZ in the early stage of pregnancy, the gestational week of delivery, child's birth weight, and presence/absence of congenital anomalies were investigated. A cohort study was performed involving pregnant women who used acetaminophen without teratogenic effects as a control group.
[Results] During the organogenesis period, 224 pregnant women used EZ, and normal child delivery was observed in 196, spontaneous abortion in 12, artificial abortion in 9, and neonatal death in 1. The incidence of congenital anomalies was 2.97% (6/202) and the OR was 0.77 (95% CI: 0.24–2.42), showing no difference from the control group.
[Discussion] In this study, since exposure to EZ did not increase the risk of congenital anomalies, its use may not be an indication for artificial abortion.
This study was performed in conformity with the ethical principles of epidemiological studies with the approval of the Clinical Trial Review Committee.

P-16 Pregnancy outcome of women using betamethasone: A comparative study

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[Objective] There have been no epidemiological studies on children born to women who used betamethasone (BM) during the early stage of pregnancy. Therefore, we analyzed pregnancy outcomes in women who visited the Counseling Clinic for Pregnancy and Medicine of Toranomon Hospital. [Methods] In women who used BM in the early stage of pregnancy, the gestational week of delivery, child's birth weight, and presence/absence of congenital anomalies were investigated. A cohort study was performed involving pregnant women who used acetaminophen without teratogenic effects as a control group. [Results] During the organogenesis period, 258 pregnant women used BM, and normal child delivery was observed in 249, spontaneous abortion in 6, artificial abortion in 2, and stillbirth in 1. The incidence of congenital anomalies was 3.61% (9/249) and the OR was 0.94 (95% CI: 0.33–2.69), showing no difference from the control group. [Discussion] In this study, since exposure to BM did not increase the risk of congenital anomalies, its use may not be an indication for artificial abortion. This study was performed in conformity with the ethical principles of epidemiological studies with the approval of the Clinical Trial Review Committee.

Efficacy of Double Vaccination With the 2009 Pandemic Influenza A (H1N1) Vaccine During Pregnancy

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OBJECTIVE: To evaluate the efficacy of double vaccination with the 2009 pandemic influenza A (H1N1) vaccine during pregnancy.

METHODS: A study of the 2009 H1N1 vaccine was conducted in 128 pregnant women, who were between 8 and 32 weeks of gestation in October 2009, to monitor the immune response to vaccination and the change in antibody positivity rate and to assess the immune response. Furthermore, the study aimed to assess the changes in these parameters after the first and second vaccination, monitor the maintenance of antibody titers in maternal blood, assess antibody transfer to umbilical cord blood, and evaluate the vaccine.

RESULTS: The antibody positivity rate increased from 7.2% before vaccination to 89.5% after the second vaccination. The vaccine was efficacious, producing a sufficient immune response in 90% of patients, regardless of the stage of gestation. The antibody titers were maintained until delivery, and were higher in umbilical cord blood at delivery than in maternal blood. Although the second vaccination increased the antibody titers in 27% of patients, and the antibody titers in maternal and umbilical cord blood at delivery tended to be higher in the double vaccination group than in the single, the differences were not statistically significant.

CONCLUSION: Single vaccination induces sufficient immune response and transfer of immunity to the fetus in pregnant women with no pre-existing antibodies.

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DOI: 10.1097/AOG.0b013e31822e5c02

LEVEL OF EVIDENCE: III

Pregnant women are more vulnerable to infection than those who are not because the immune system is altered to tolerate the fetus, which is inherently antigenic. Moreover, early gestation is associated with an impaired maternal physical condition owing to nausea and vomiting, whereas middle and late gestation may be associated with impaired cardiopulmonary function owing to cardiac stress resulting from a decrease in lung capacity caused by the enlarging gravid uterus and increases of circulating plasma volume.[1] According to epidemiologic surveys of several previous influenza virus epidemics,[2–6] pregnant women are classified as at high risk[7–14]; influenza vaccination in all stages of gestation has been recommended by the American College of Obstetricians and Gynecologists and the Centers for Disease Control and Prevention since 2004. For the 2009 pandemic influenza A (H1N1) influenza strain, the percentage of patients hospitalized because of infection was more than four times higher in pregnant women than in those who were not, and pregnant women accounted for 13% of all the pandemic-related mortalities in the United States.[15] Despite the high risk of influenza-related complications reported in infants, vaccination is not indicated for those younger than 6 months, emphasizing the importance of vaccination of pregnant women. Accordingly, there are several reports on efficacy of vaccination from epidemi-

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ologic surveys of risk of infection or hospitalization or both in vaccinated pregnant women and their newborns.[16-23]

No immunologic studies have yet been conducted to support the epidemiologic surveys of the 2009 H1N1 vaccine and we expected that double vaccination might be better than single in women with no pre-existing antibodies during pregnancy. Because both maintenance of maternal antibody titers until delivery and antibody transfer to the fetus during pregnancy are important, the present study was designed to evaluate the efficacy of 2009 H1N1 vaccination during pregnancy by assessing the immunogenicity of the vaccine based on vaccination frequency-related change of antibody titers, maintenance of antibody titers during pregnancy, and antibody transfer to the fetus.

MATERIALS AND METHODS

This study was approved by the local ethics committee at the National Center for Child Health and Development and was conducted after informed consent was obtained from all study participants. A total of 128 pregnant women who were between 8 and 32 weeks of gestation in October 2009 were enrolled in this study. An additional 82 pregnant women who received a single vaccination during the same period were included in the study for comparison (Table 1). Women with complications involving immunologic abnormalities were excluded. Some of our procedures have been described previously.[24]

Before vaccination, the hemagglutination inhibition (HI) antibody titer against the HI antigen in the 2009 H1N1 virus was measured to determine the levels of pre-existing antibodies in maternal blood. In addition, white blood cell count, lymphocyte count, CD4, CD4/CD8, Th1/Th2 ratio, and natural killer (NK) cell activity were measured to investigate the maternal immune status. The 2009 H1N1 vaccine was subcutaneously injected into the upper arm twice at an interval of 3 weeks; antibody titers in maternal blood were measured 3 weeks after each vaccination and at the time of delivery. Antibody titers in umbilical cord blood were also measured at the time of delivery (Fig. 1).

Table 1. Characteristics of Patients

	Single (n=81)	Double (n=124)	P
Age (y)	35.7±3.6	34.8±4.1	.090
Gravidity	1.0±1.2	2.1±1.5	<.01
Parity	0.4±0.6	0.8±0.9	<.01
Interval between vaccination and birth (wk)	13.0±6.1	13.3±6.6	.844
Weeks of gestation at birth	38.6±1.9	38.5±1.7	.798

Data are mean±standard deviation unless otherwise specified.

The antibody titers in maternal blood and umbilical cord blood at time of delivery were measured according to the time from the first vaccination to delivery to monitor the immune response to vaccination and the resulting change in the antibody positivity rate, and to assess the immune response by Th1/Th2 ratio and stage of gestation, as well as assess changes of these parameters after the first and second vaccinations, to monitor the maintenance of antibody titers in maternal blood from the first and second vaccination to delivery, and to assess the differences in maternal–fetal antibody transfer.

The 2009 H1N1 vaccination during pregnancy was evaluated in mothers and fetuses by investigating the incidence of abortion or preterm delivery and the gestational age at delivery in mothers other than those with vaccination-related adverse reactions or multiple births, as well as the incidence of malformation, Apgar score, and birth weight in the newborns.

Specific staining of lymphocytes was performed by incubating whole blood with anti-CD4–PC5 or anti-CD8–PC5-conjugated monoclonal antibodies (Beckman Coulter). Red blood cells (RBCs) were removed by lysis (FACS Lysing solution; Becton Dickinson, BD Biosciences) and lymphocytes analyzed by flow cytometry (FACSCalibur; Becton Dickinson). After surface staining the activated whole blood samples with anti-CD4–PC5-conjugated monoclonal antibodies, RBCs were lysed and subsequently specific intracellular staining using FastImmune interferon γ fluorescein isothiocyanate/interleukin-4 phycoerythrin (Becton Dickinson) was performed according to the manufacturer's instructions. The stained cells were analyzed by flow cytometry; $CD4^+$ T lymphocytes that stained positive for interferon γ or interleukin-4 were used to assess numbers of Th1 and Th2 cells, respectively.

Serum samples from maternal or umbilical cord blood were treated with a receptor-destroying enzyme from *Vibrio cholerae* at 37°C for 18 hours to remove nonspecific inhibitors. After heat inactivation at 56°C for 30 minutes, the samples were diluted to ten times their volume with physiologic saline. To adsorb nonspecific agglutinins, receptor-destroying enzyme–treated serum samples were then incubated with 2.5% *volume/volume* chicken RBCs at 4°C for 1 hour. The serum samples used in subsequent hemagglutination inhibition tests were separated by centrifugation at 300×*g* for 5 minutes.

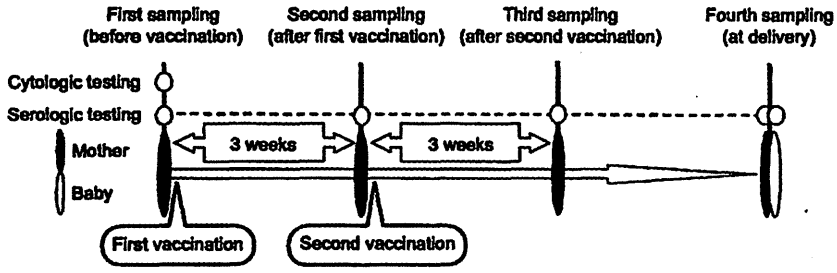

Fig. 1. Vaccination and protocol for blood sampling.
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The pretreated serum samples were serially double diluted in phosphate buffered saline using U-shaped 96-well microtiter plates and incubated with an equal volume of 4 U of various virus hemagglutinin antigens at room temperature for 10 minutes. An aliquot of 0.5% *volume/volume* chicken RBC suspension in phosphate buffered saline was added to each well and incubated at room temperature for 1 hour. The titers of the specific antibodies for the various strains were read by inverting the plates to produce a streak.[25,26]

The 2009 H1N1 vaccine (influenza hemagglutinin vaccine; Kitasato Institute Research Center for Biologicals) is a split vaccine made of the hemagglutinin fraction of viral proteins from concentrated and inactivated A/California/7/2009 virus, and contains neither an adjuvant nor preservative (thimerosal or 2-phenoxyethanol).

Values are expressed as mean±standard deviation. Differences between groups were assessed by χ^2 test. Other data were analyzed by analysis of variance, with a post hoc test (Dunnett's test) when the F value was significant. Statistical significance was concluded with a 2-tailed $P<.05$. Analyses were performed using StatMate software.

RESULTS

The maternal mean age, white blood cell count, lymphocyte count, CD4, and CD4/CD8 ratio were not significantly different among the stages of gestation. On the other hand, Th1/Th2 ratio and NK cell activity tended to decrease as gestation progressed (Table 2). The change in Th1/Th2 ratio during pregnancy was consistent with the tendency that we reported previously.[24] Natural killer cell activity was measured because, on the assumption that NK cells are normally inhibited during pregnancy because the fetus is immunologically foreign to the mother, elevated NK cell activity might result in abortion or preterm delivery.[27]

HI antibody titers of 1:40 or higher usually are regarded as protective and are an objective for successful vaccination.[28] We applied the following criteria for classification of the immune responses. Participants with a prevaccination HI antibody titer of less than 1:10 and a postvaccination HI antibody titer of 1:40 or higher or a prevaccination HI antibody titer of 1:10 or higher and a fourfold or more rise in postvaccination HI antibody titer were classified as responsive. Those with a twofold or less increase or with no increase in postvaccination HI antibody titer were classified as poorly responsive and nonresponsive, respectively.

For the 2009 H1N1 virus, the antibody positivity rate increased from as low as 7.2% before vaccination to 89.5% after the second vaccination Whereas the first vaccination produced a good immune response in the presence of low titers of pre-existing antibodies

Table 2. Maternal Condition at the Time of Vaccination

	Trimester			
	First (n=17)	Second (n=79)	Third (n=29)	*P*
Age (y)	34.2±4.0	34.9±4.0	35.2±4.4	.699
WBC (/mL)	8,312±2,413	8,655±1,791	8,724±2,200	.174
Lymphocytes (%)	20.5±7.7	19.1±6.1	18.1±5.7	.458
CD4 (/mL)	45.6±6.5	44.7±7.5	44.6±7.0	.880
CD4/CD8 (ratio)	1.5±0.5	1.4±0.5	1.4±0.4	.802
Th1/Th2 (ratio)	15.5±12.5	14.4±8.2	10.0±4.0	<.01
NK cell activity (%)	37.6±13.1	32.4±12.0	30.6±9.5	.141

WBC, white blood cells; NK, natural killer.
Data are mean±standard deviation unless otherwise specified.

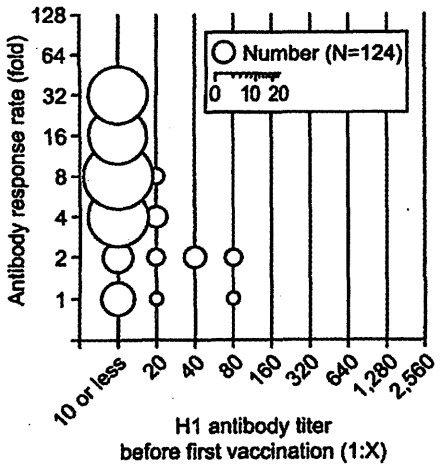

Fig. 2. Immune response after first vaccination. The fold-change in antibody titer is shown relative to the antibody titer before the first vaccination. Immune response to first vaccination was good because many participants had low titers of pre-existing antibodies.

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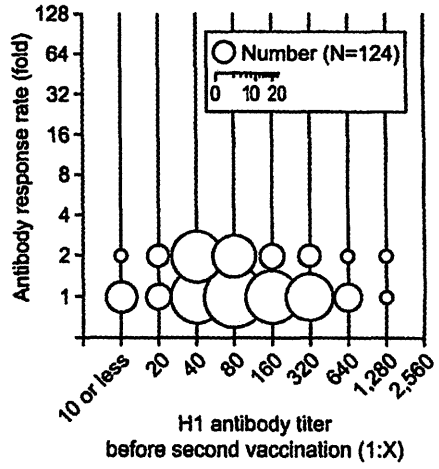

Fig. 3. Immune response after second vaccination. The fold-change in antibody titer is shown relative to the antibody titer before the second vaccination. The second vaccination was associated with a reduced postvaccination increase in antibody titers because many participants had high antibody titers before vaccination.

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(Fig. 2), the second vaccination was associated with a reduced postvaccination increase in antibody titers in participants with higher prevaccination antibody titers because of the presence of pre-existing antibodies (Fig. 3). Participants with an antibody titer of less than 1:4 after the first vaccination tended to demonstrate no increase after the second vaccination.

The vaccine produced a sufficient immune response in approximately 90% of participants regardless of Th1/Th2 ratio and stage of gestation (Table 3). No significant difference of responsive rate was observed among participants in each stratum of Th1/Th2 ratio (from 9.9 or less to 20 or more) both in the single-vaccination group (P=.352) and in those receiving double vaccination (P=.360) and among the participants in the first, second, and third trimester both in the single-vaccination group (P=.602) and in those receiving double vaccination (P=.685).

Participants with a poor response to the first vaccination had a poor response to the second vaccination as well, resulting in no significant increase in percentage of responsive participants after the second vaccination. However, 27% of participants had higher antibody titers after the second vaccination than after the first vaccination (Fig. 3).

The antibody titers tended to decrease over time after vaccination but were appropriately maintained until delivery, with a geometric mean antibody titer of 20 or more even 21 or more weeks after vaccination (Fig. 4). Antibody titers in umbilical cord blood at time of delivery showed a similar pattern to maternal antibody titers with regard to time from vaccination to delivery, and were higher than those in maternal serum. At the time of delivery, maternal antibody titers tended to be higher in the double vaccination group than in the single vaccination group regardless of gestational stage; those in umbilical cord blood tended to be higher in participants who received double vaccination within 10 weeks of delivery than in those who received a single vaccination during the same period (Fig. 5). However, statistical analysis revealed no significant difference in the antibody titers in maternal blood or umbilical cord blood between the two vaccination groups.

Redness at the vaccination site was the most common maternal adverse reaction after vaccination, followed by local symptoms such as pain and induration and systemic symptoms such as headache, malaise, fever, and nausea. However, no serious symptom requiring medical intervention was reported. These adverse reactions were not augmented or attenuated by the second vaccination, with no significant difference between the first and second vaccination detected (Fig. 6).

In the assessment of fetal adverse effects, there were no abortions, indicating that vaccination even at an early stage of gestation was unlikely to induce abortion. The incidence of preterm delivery was 7% in participants vaccinated in the first or third trimester

Table 3. Status of Immunity After Vaccination

	No. of Participants	Vaccination						
		Single			Double			
		R (n)	PR (n)	NR (n)	R (n)	PR (n)	NR (n)	P
All	106	87.7 (93)	5.7 (6)	6.6 (7)	88.7 (94)	4.7 (5)	6.6 (7)	.955
Th1/Th2 ratio								
9.9 or less	47	89.4 (42)	6.4 (3)	4.3 (2)	91.5 (43)	4.3 (2)	4.3 (2)	
10.0–14.9	22	91.0 (20)	0 (0)	9.1 (2)	91.0 (20)	0 (0)	9.1 (2)	
15.0–19.9	22	81.8 (18)	4.6 (1)	13.6 (3)	81.8 (18)	4.6 (1)	13.6 (3)	
20.0 or more	15	86.7 (13)	13.3 (2)	0 (0)	86.7 (13)	13.3 (2)	0 (0)	
Trimester								
First	15	86.7 (13)	6.7 (1)	6.7 (1)	86.7 (13)	6.7 (1)	6.7 (1)	
Second	69	85.5 (59)	7.2 (5)	7.2 (5)	87.0 (60)	5.8 (4)	7.2 (5)	
Third	22	95.5 (21)	0 (0)	4.5 (1)	95.5 (21)	0 (0)	4.5 (1)	

R, responsive; PR, poorly responsive; NR, nonresponsive.

and 2% in those vaccinated in the second trimester, with no significant difference detected. In addition, the incidence of preterm delivery in participants vaccinated at any time throughout gestation was similar to that from general statistics,[29] indicating that preterm delivery was unlikely to have been related to vaccination. In participants other than those with preterm delivery, the number of weeks of gestation at the time of delivery, Apgar score, and birth weight were within the standard ranges, showing no difference according to the gestational stage of vaccination (Table 4). The reasons for preterm delivery included maternal indication due to collagen disorder and spontaneous delivery, indicating no association with vaccination. Birth weight was similar to the standard body weight calculated based on the gestational age and sex and was not affected by vaccination. Five cases of malformation (three vaccinated in the second trimester and two in the third trimester) were observed, including one case each of ventricular septal defect, ear appendage, ankyloglossia, encephalocele and aplasia cutis congenita, and inguinal hernia. The incidence was not significantly different among

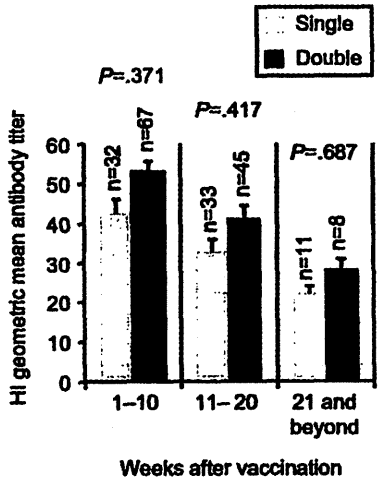

Fig. 4. Geometric mean antibody titer in maternal blood at time of delivery. The geometric mean antibody titer decreased as the time from vaccination to delivery increased, but high antibody titers of 20 or more were maintained until delivery. High antibody titers tended to be maintained more efficaciously after double vaccination than after single vaccination.

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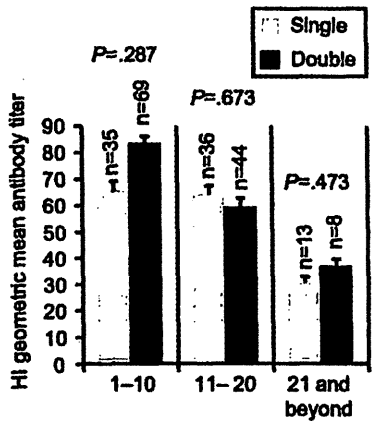

Fig. 5. Geometric mean antibody titer in umbilical cord blood. The geometric mean antibody titer in umbilical cord blood was higher than in maternal blood. Antibody titers tended to be higher in participants who received double vaccination within 10 weeks of delivery than in those who received a single vaccination during the same period.

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