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Effects of Cigarette Smoke Exposure on Early Stage Embryos in the Rat

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It is well recognized that cigarette smoking in pregnant women exerts many deleterious effects on their progenies; intrauterine growth retardation, and increases in perinatal mortality and premature births (Lowe 1959; Frazier et al. 1961; Comstock et al. 1971; Butler et al. 1972; Meyer et al. 1974). The fetal growth retardation also has been reported in animals exposed to cigarette smoke (Essenberg et al. 1940; Younoszai et al. 1969; Reznik and Marquard 1980).

We previously demonstrated that cigarette smoke exposure in pregnant rats retarded the growth of fetuses from mid to late stages of pregnancy (Tachi and Aoyama 1983). In addition, the weight of uteri containing embryos in animals inhaling the smoke was smaller, although not significant, than that in the control on day 7 of pregnancy. Based on these findings, it was suggested that the growth of embryos in early stage seemed to be harmfully affected as well as during mid and late stages of pregnancy.

However, since the uterine weight in early pregnancy was measured in the previous study instead of the direct observation of early stage embryos, it remained unclear whether the early development of embryos was really influenced by cigarette smoke exposure or not. Moreover, there have been few data dealing with such phenomena.

The present study was designed to observe the effects of cigarette smoke inhalation by pregnant rats on early development of embryos from fertilization to implantation.

MATERIALS AND METHODS

Female Wistar rats were used, and maintained under the condition of lights (12L12D, lights on 0600h) and temperature at 22.5–26.5 ℃. Female

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rats were mated overnight with proven males. Vaginal smears were examined for the presence of sperm to determine day 0 of pregnancy.

Dams were exposed to cigarette smoke for 81 minutes, twice a day, on days 0 to 3 of pregnancy, according to the manner previously mentioned (Tachi and Aoyama 1983). Controls were exposed to room air.

Animals were sacrificed at noon on days 0, 1, 2, and 3 of pregnancy after being anesthetized with diethyl ether. Uteri and oviducts were removed, trimmed, and the uteri split at the cervical bifurcation. A 27 gauge hypodermic needle square-off at the needle end was inserted into the infundibulum of each oviduct to flush the oviduct with 0.5 ml of physiological saline. The needle (26 gauge) was also inserted into the tubal end of each uterine horn and the horn flushed with 1.0 ml of saline.

At noon on days 4 and 5, pregnant rats were injected with 0.5 ml of 1% Evans' blue dye from jugular vein under light ether anesthesia, and autopsied 20 minutes later with excess dose of ether. The number of dye accumulated points in the uterus were determined. Uterus and oviducts were removed for flushing as described aboved.

Specimens retreived by flushing were inspected with a light microscope, and the number and stage of the embryos were recorded.

Chi square and student's t test were employed to evaluate differences in the distribution of embryos at each stage and in the mean between groups, respectively.

RESULTS AND DISCUSSION

Figure 1 shows the distribution of embryos at each stage, expressed by percentage, from days 0 to 5 of pregnancy. On days 0 and 1 of pregnancy, there was no significant difference between groups. The development of embryos somewhat delayed in the group of cigarette smoke exposure on day 2 of pregnancy, although not significant. The percentage of 2-cell embryos still stood at 35% on day 2 of pregnancy in the group of cigarette exposure, while it was only 17% and 60% of embryos was at the stage of 4-cell in the control group. Chi square test revealed a significant difference ($P<0.01$) on day 3 of pregnancy. Almost equal percentage (48 and 46%, respectively) of embryos stood at the stages of 8-cell and morulla in the cigarette smoke group, in contrast to the group of control in which more than 80% reached the stage of morulla.

Yoshinaga et al. (1979) reported that daily nicotine administration (7.5 mg nicotine tartrate daily) to pregnant rats caused a delay of approximately 12h in ovum cleavage from the 2-cell to the 4-cell stage,

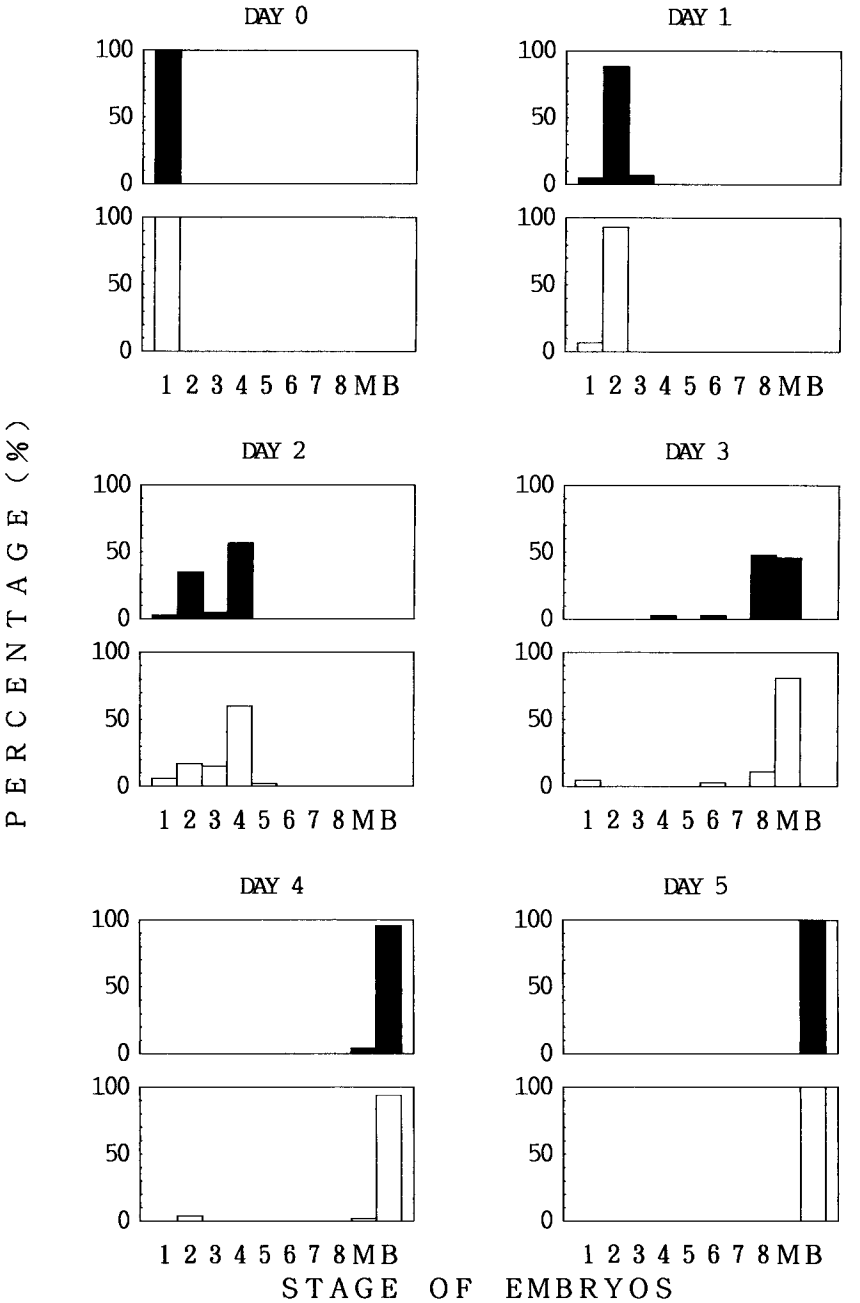

Figure 1. The distribution of embryos at each stage. Numerals and M and B represent the cell number of embryos and morulla and blastocyst, respectively. Solid or open column respectively indicates the group of cigarette smoke exposure or control. The number of embryos retrieved was more than 35 from 5-7 dams on any days except for day 5 of pregnancy on which it was less than 20.

and that each step of embryonic development after the 4-cell stage was thereby delayed. The delay of development in embryos with the injection of nicotine was also observed by Mitchell and Hammer (1985). The agreement in results between the earlier and present studies that the development of ova was delayed by nicotine administration or cigarette smoke exposure in pregnant rats seemed likely to indicate that nicotine in the cigarette smoke might be responsible for the event observed by cigarette smoke exposure in the present study.

Whereas the earlier results also showed the delay of the developed embryos after day 3 of pregnancy, the present study failed to demonstrate the delay of embryonic development on days 4 and 5 of pregnancy in the group exposed to the smoke (Figure 1). However, inner cell mass formation was slightly retarded in the smoke-exposed group, compared with the control (data not shown). This finding seemed to suggest that embryonic development on days 4 and 5 was delayed in this study, similar to the earlier ones. It remained unclarified whether cigarette smoke exposure affected the embryonic development on days 4 and 5 of pregnancy, at present. It should be undertaken to more precisely observe the embryonic development after day 3 of pregnancy to show the delay of development in these periods.

The appearance of embryos in the uterus was significantly delayed by cigarette smoke exposure in pregnant rats on day 3 of pregnancy ($P<0.01$, Table 1). More than 50% of embryos was retrieved from uterus in the control, while about 90% was recovered from oviducts in the cigarette exposed group. The previous expriment of nicotine administration to pregnant rats indicated a similar finding (Yoshinaga et al. 1979).

Nicotine has been reported to prolong gestation (Becker et al., 1968), probably due to the delay of implantation in rats (Yoshinaga et al. 1979;Card and Mitchell 1979). The present experiment could not find out a delay of implantation, since the number of implantation sites was not significantly different between groups on day 5 of pregnancy in addition to the fact that no dye accumulating point which reflected the implantation site was visible before day 5 of pregnancy (Table 2).

While many studies have been indicated that cigarette smoking in pregnant women resulted in harmful effects on their children such as intrauterine growth retardation, and increases in perinatal mortality and premature births (Lowe 1959; Frazier et al. 1961; Butler et al. 1972; Meyer et al. 1974), the effect of cigarette smoke exposure on early stage embryos remained unclear. The present results demonstrated delays in cell cleavage of embryos and in passage of embryos through the oviducts on day 3 of pregnancy. These findings suggested that cigarette smoke exposure deleteriously affected the development in

Table 1. The percentage of embryos retrieved from oviduct and uterus.

Day of Pregnancy	Treatment	Oviduct	Uterus
Day 0	CIG	100%	0%
	CONT	100%	0%
Day 1	CIG	100%	0%
	CONT	100%	0%
Day 2	CIG	98%	2%
	CONT	100%	0%
Day 3	CIG	87%	13%
	CONT	43%	57%
Day 4	CIG	0%	100%
	CONT	0%	100%
Day 5	CIG	0%	100%
	CONT	0%	100%

CIG and CONT represent the groups of cigarette smoke exposure and control, respectively.

Table 2. The number of implantation sites on days 4 and 5 of pregnancy.

	Day 4	Day 5
CIG	0	10.8±6.4
CONT	0	12.6±2.4

Mean ±S.E. Number of samples is five on any days in both groups.

embryos at early stage of pregnancy as well as at mid and late stages, and it was implied that nictoine in the smoke might be, at least in part, responsible for these events observed in this study.

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