Fertility, Reproduction, and Postnatal Survival in Mice Chronically Exposed to Isoflurane

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The effects on fertility and reproductive wastage of 110 female Swiss/Webster mice and postnatal survival of their offspring were examined after exposure to either air, 0.4% isoflurane, or 0.1% isoflurane. Treatments were for 4 h daily for 2 weeks before and during pregnancy. In a second experiment, the effects on fertility of 54 male Swiss/Webster mice and on reproductive wastage of their unexposed mates were examined after 4-h daily exposures to either air, 0.4% isoflurane, or 0.1% isoflurane throughout spermatogenesis and during mating. There were no adverse reproductive effects in either experiment. The lack of toxicity of isoflurane is consistent with the results of other reproductive studies in animals that have examined chronic intermittent exposure to subanesthetic concentrations of halothane, enflurane, methoxyflurane, and nitrous oxide. They suggest that these and lower (trace) levels of anesthetic gases may not be the cause of the harmful reproductive effects said to occur in operating room personnel. (Key words: Anesthetics, volatile: isoflurane; subanesthetic concentrations. Toxicity: fetal; reproductive; subanesthetic concentrations.)

The incidence of spontaneous abortion is reported to be, on the average, 30% higher among operating room personnel than it is among comparable control populations.1–7 It has also been reported that female physician anesthetists have a higher incidence of infertility than do nonanesthetist female physicians.8 The authors of these epidemiologic surveys have suggested that exposure to trace levels of waste anesthetic gases may be the cause of these adverse reproductive effects. However, this theory has not been supported by the negative results of animal studies from our laboratory8–11 and those of others,12–16 which have examined fertility, reproduction, and postnatal survival following intermittent exposure to subanesthetic concentrations of halothane, enflurane, methoxyflurane, and nitrous oxide. In the present study, the effects of exposure of Swiss/Webster mice to a low anesthetic (0.4%) and a subanesthetic (0.1%) concentration of isoflurane were examined using a protocol similar to those we have employed in our previous experiments.

Materials and Methods

Five-week-old virgin male and female Swiss/Webster mice† were individually marked with metal ear tags, observed for 7 days for signs of illness, then randomly divided into experimental groups. Mice were caged by sex and treatment group, four animals per cage, and bedded on ground corncob‡ in a room provided with artificial light from 0600 to 1900 h each day. No other animal species or mouse strain was housed in the same room during the experiment. No germicides or pesticides were used in the facility. Mice were allowed continuous access to animal chows§ and to water, except during inhalational exposures, and were weighed weekly.

Inhalational exposures were performed in stainless steel and Plexiglass® gas-tight chambers, each approximately 1,000 l in capacity. Isoflurane was vaporized in a Copper Kettle® with medical-grade compressed air and delivered to the chambers through latex rubber tubing at a total air flow of 10–12 l/min. A high-volume fan recirculated the atmosphere within each chamber in order to maintain uniform anesthetic vapor concentrations. Isoflurane levels were monitored every 5–15 min with a Miran IA-IF® infrared analyzer and were maintained within 10% of the desired concentrations. Oxygen concentration in each chamber was monitored continuously with IL 402 analyzers and remained between 20–21%. Carbon dioxide concentration was measured at random intervals, at least hourly, with a Beckman LB-2® infrared analyzer; it did not exceed 0.3% and usually was in the range of 0.1–0.2%. Temperature in the chambers ranged from 21° to 27° C.

Experiment 1—Female Fertility and Postnatal Survival of Offspring

One hundred ten female and 55 male mice were randomly distributed into three groups: two treatment groups and a control group. Treated mice were exposed to either 0.1% isoflurane (n = 32) or to 0.4% isoflurane (n = 32) for 4 h daily. The latter was the highest isoflurane con-

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centration Swiss/Webster mice would tolerate chronically without weight loss or increased morbidity or mortality (unpublished data). Control mice (n = 41) were exposed to compressed air in an identical exposure chamber. After the second week of treatment, female mice were recaged in pairs and one male mouse from the same treatment group was placed with each pair of females, nightly, for seven nights. Each morning following mating, male mice were returned to their original cages and female mice were examined for vaginal copulatory plugs. The day a copulatory plug was observed was considered day 0 of pregnancy. Female mice that did not show a copulatory plug after seven nights were remated with a new male mouse for seven additional nights. Daily isoflurane treatments were continued throughout mating and pregnancy. On day 18 of pregnancy, 1 day before expected parturition, approximately two-thirds of the female mice in each group were weighed, then killed by cervical dislocation. Their uteri immediately were exposed and examined to determine the number and position of live and dead fetuses, resorptions, and total implantation; the weight and sex of each fetus also were recorded. Treatment of dams was unknown to the examiner in this and in the other experiments.

In order to determine the effect of maternal exposure to isoflurane on postnatal survival, the remaining third of the dams were allowed to deliver and nurse their offspring; this group included pregnant female mice in which no copulatory plug had been observed. No animal was exposed to isoflurane after delivery. The total number of live pups at birth was determined, as the number and mean weight (±SD) of pups surviving on days 1 and 4. After 4 days, litters were randomly culled to a maximum of eight pups; survival and mean weight of pups then were determined on days 7, 14, and 21 after birth. Pups were weaned and weighed individually at 4 weeks of age.

All females that did not show signs of pregnancy were killed 14 to 18 days following the last day of mating and examined for implantations.

**Experiment 2—Male Fertility**

Five-week-old male Swiss/Webster mice were randomly allocated to three groups. Treated mice were exposed either to 0.1% isoflurane (n = 15) or to 0.4% isoflurane (n = 15) for 4 h daily; control mice (n = 24) were exposed to compressed air. After 6 weeks of treatment (the duration of spermatogenesis in mice is 35 days), male mice were placed nightly for seven nights with two untreated, 7-week-old, virgin female Swiss/Webster mice. Treatment of male mice continued throughout the mating period, however, female mice were never exposed to isoflurane or compressed air. Female mice that did not show a copulatory plug after 1 week were remated with a different male mouse for seven additional nights. Female mice were killed, and uterine examinations were performed on day 18 of pregnancy as in Experiment 1.

**Analysis of Data**

Copulatory rate (female mice with copulatory plugs/number mated), pregnancy rate among female mice with copulatory plugs, and overall pregnancy rate (number of pregnant dams/number mated) were determined for each group, as was the percentage of male mice siring litters. Mean weight gain (±SD) during pregnancy and mean fetal weight were determined for all pregnant dams with copulatory plugs killed on day 18 of pregnancy. Mean number of implantations (total of live and dead fetuses plus resorptions), fetuses per litter (live plus dead fetuses), and live fetuses per litter were determined for all pregnant dams killed before spontaneous delivery. Indices of reproductive wastage, i.e., resorptions plus fetuses dead in utero, equivalent to human abortions and stillbirths, respectively, were calculated for each female mouse as percentages of the total number of implantations, and the means for each group then were determined. Data from pregnant dams killed before delivery for which the precise day of copulation was not known (i.e., no copulatory plug was observed) were included in comparisons of implantation rates, litter sizes, and reproductive wastage, but not in comparisons of maternal weight gains or fetal weight.

For each female mouse allowed to deliver in Experiment 1, the number of live offspring on the day of delivery, the percentage of liveborn pups that survived until day 4 (viability index), the percentage of pups alive at 4 days that survived until weaning (lactation index), and the mean weight of surviving pups were determined and group means calculated. Isoflurane-treated groups were compared with each other and with the control group in that experiment.

Analysis of variance was used to determine if group means differed from each other. When a difference was found, Student’s t test, corrected for multiple tests, was used to identify the significantly different group. \( P < 0.05 \) was considered statistically significant.

**Results**

There were no apparent adverse effects due to isoflurane treatment. Weight gain was similar among the three groups, and all mice survived the experiment. Exposure
to 0.4% isoflurane resulted in light general anesthesia, but all mice were awake within approximately 10 min after treatment was discontinued.

The overall pregnancy rate in Experiment 1 was 92.4%; values for individual groups as well as the other indices of fertility and reproductive wastage are presented in Table 1. There were no differences among the three groups in any variable. Similarly, there were no differences in survival or in weight gain among the offspring of those dams allowed to deliver spontaneously (Table 2). Male fertility was unaffected by isoflurane treatment (Table 3). All male mice sired litters, and reproductive wastage in dams mated with treated mice was not increased above control values.

**Discussion**

The results of the present study indicate that intermittent exposure of Swiss/Webster mice to a low anesthetic (0.4%) and to a subanesthetic (0.1%) concentration of isoflurane does not result in increased female or male infertility, reproductive loss, or early postnatal fetal wastage. There are no other reproduction studies employing isoflurane with which to compare our results. However, our results generally are in agreement with the reports in the literature that have examined the effects of the other inhaled anesthetics on reproduction. Studies in our laboratory employing intermittent exposure to halothane, enflurane, nitrous oxide, and methoxyflurane have been uniformly negative at subanesthetic concentrations while showing only a few adverse reproductive effects at low anesthetic (0.3–0.5 MAC) levels. Thus, 4-h daily exposures of female Swiss/Webster mice to 0.3% halothane for 9 weeks before and during pregnancy resulted in statistically significant decreases in pregnancy rate, number of implantations per dam, and number of live fetuses per litter compared with air-treated control mice. In experiments employing similar exposures of male mice, no changes were seen in fertility or in postnatal survival and weight gain of offspring of their unexposed mates. Exposure of female mice to 0.025% and 0.1% halothane was without adverse effects.

Exposure of female Swiss/Webster mice to 1% enflurane for 4 h per day for 3 weeks before and during pregnancy resulted in decreased fetal weight gain without an increase in fetal wastage. Exposure of male mice to 1% enflurane for 11 weeks before mating was not associated with adverse effects. Exposure of male and female mice to two subanesthetic enflurane concentrations, 0.01% and 0.1, also was without adverse effects.

In studies employing nitrous oxide, we found no adverse reproductive effects following exposure of female Swiss/Webster mice to 0.5%, 5%, or 50% concentrations for 4 h on days 6–15 of pregnancy (MAC for nitrous oxide in mice is approximately 150%). Exposure of male mice was not carried out. However, 24-h exposure of female Sprague-Dawley rats to 75% nitrous oxide throughout day 9 of pregnancy resulted in increased early and late resorptions and fewer live fetuses per dam. There were no adverse effects from similar 24 h exposures to 0.75%, 7.5%, and 25% nitrous oxide. Finally, we detected

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**Table 1. Experiment 1, Female Fertility, Reproductive Indices (mean ± SD)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Females Mated</th>
<th>Copulatory Rate (%)</th>
<th>Pregnancy Rate in Copulated Females (%)</th>
<th>Overall Pregnancy Rate (%)</th>
<th>Litters</th>
<th>Implantations/Dam</th>
<th>Live Fetuses/Litter</th>
<th>Resorbed (%)</th>
<th>Dead In Utero (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment control</td>
<td>41</td>
<td>85.3</td>
<td>97.1</td>
<td>97.6</td>
<td>24</td>
<td>9.1 ± 2.0</td>
<td>8.2 ± 2.0</td>
<td>9.2 ± 8.8</td>
<td>0.8 ± 3.2</td>
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<tr>
<td>0.1% Isoflurane</td>
<td>32</td>
<td>90.6</td>
<td>96.6</td>
<td>93.8</td>
<td>20</td>
<td>8.7 ± 2.3</td>
<td>8.0 ± 2.4</td>
<td>9.2 ± 9.5</td>
<td>0.0</td>
</tr>
<tr>
<td>0.4% Isoflurane</td>
<td>32</td>
<td>87.5</td>
<td>85.7</td>
<td>84.4</td>
<td>17</td>
<td>8.1 ± 2.0</td>
<td>7.4 ± 2.3</td>
<td>9.2 ± 13.6</td>
<td>0.0</td>
</tr>
</tbody>
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**Table 2. Experiment 1, Postnatal Survival and Pup Weight (Mean ± SD)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Litters</th>
<th>Live Fetuses/Litter</th>
<th>Viability Index (%)</th>
<th>Lactation Index (%)</th>
<th>24 h</th>
<th>4 Days</th>
<th>7 Days</th>
<th>14 Days</th>
<th>21 Days</th>
<th>28 Days</th>
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<tbody>
<tr>
<td>Treatment control</td>
<td>16</td>
<td>8.3 ± 2.0</td>
<td>94.6 ± 21.5</td>
<td>91.6 ± 25.3</td>
<td>4.5 ± 0.5</td>
<td>7.3 ± 0.6</td>
<td>10.3 ± 1.1</td>
<td>17.2 ± 1.4</td>
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<td></td>
</tr>
<tr>
<td>0.1% Isoflurane</td>
<td>10</td>
<td>8.9 ± 1.8</td>
<td>99.0 ± 3.2</td>
<td>88.8 ± 31.4</td>
<td>4.3 ± 0.6</td>
<td>7.4 ± 0.7</td>
<td>10.0 ± 0.9</td>
<td>17.7 ± 1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.4% Isoflurane</td>
<td>10</td>
<td>9.4 ± 2.4</td>
<td>96.2 ± 9.5</td>
<td>96.3 ± 8.4</td>
<td>4.2 ± 0.3</td>
<td>7.1 ± 0.9</td>
<td>9.0 ± 1.5</td>
<td>16.8 ± 1.7</td>
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</table>
no adverse reproductive effects following 4-h exposures of female Swiss/Webster mice to 0.0002% and 0.006% methoxyflurane on days 6–15 of pregnancy.

Exposure of female Swiss/Webster mice to an anesthetic level of methoxyflurane, 0.2% (1 MAC), resulted in an increased incidence of fetuses that were dead in utero compared with control (1.7% vs. 0.0%) but no other harmful effects. Exposure of female mice to 0.0002% and 0.006% methoxyflurane was without ill effect. Male mice were not exposed.

In other laboratories, Bruce12 reported no adverse effects on fertility or reproduction in two purebred and one hybrid strain of mice exposed to 0.016% halothane 7 hours daily, for 6 weeks before mating. Basford and Fink13 exposed Sprague-Dawley rats to 0.8% halothane for 12-h periods at various times during pregnancy; no increase in fetal wastage was observed. Pope et al.14 reported no increase in fetal loss following exposure of Sprague-Dawley rats to either 0.16% or 0.32% halothane for 12 h daily throughout pregnancy. Similar negative results were reported by these investigators following exposure of Sprague-Dawley rats to the following: subanesthetic (0.01%) and low anesthetic (0.04 and 0.08%) concentrations of methoxyflurane; subanesthetic (1%, 10%, and 50%) concentrations of nitrous oxide; and the combination of 10% nitrous oxide and 0.16% halothane. Stout et al.15 and Pope and Persaud16 performed limited studies on the reproductive effects of enflurane in rats. The former group evaluated the effects of administration of 0.0011% and 0.0064% enflurane for 8 h per day, whereas the latter investigators administered 0.32% enflurane for the same period of time. Both studies had negative results.

Only the study of Vieira et al.,19 reported serious adverse reproductive effects at a low concentration (in fact, at a trace concentration) of an inhaled anesthetic. They found a higher incidence of fetal resorptions and fewer and smaller fetuses per litter in Wistar rats continuously exposed to 0.1% nitrous oxide almost throughout pregnancy (days 1–19). However, these results could not be duplicated by Rao et al.,20 who exposed Sprague-Dawley rats continuously to 5% nitrous oxide throughout gestation (days 1–21). Only after continuous exposure to 10% nitrous oxide did Rao et al.,20 observe increased fetal resorptions and decreased fetal weight. It is possible that both groups of investigators19,20 are correct and that the discrepancy in their results is due to a strain difference in toxicity; such differences are well known.21

Alternatively, unappreciated errors in design in one or both of the experiments may explain the lack of reproducibility of the results reported by Vieira et al.19 In either case, continuous exposure almost throughout pregnancy is an unusual method of treatment for a reproduction experiment and does not resemble the exposures that occur in occupational or clinical situations.

In conclusion, exposure of Swiss/Webster mice to a low anesthetic (0.4%) and to a subanesthetic (0.1%) concentration of isoflurane did not result in altered female or male fertility, reproduction, or postnatal survival of offspring. These results are in agreement with the vast majority of animal experiments in the literature. Given the limitations of extrapolation of animal data to humans, they suggest that intermittent exposure of operating room personnel to subanesthetic or to low anesthetic concentrations of isoflurane is not associated with adverse reproductive effects.

References