Title : MOUSE SPERM MORPHOLOGY FOLLOWING EXPOSURE TO ANESTHETICS DURING EARLY SPERMATOGENESIS

Authors : P. C. Land, M.D., E. L. Owen, and H. W. Linde, Ph.D.

Affiliation : Department of Anesthesia, Northwestern University Medical School, Chicago, IL 60611

Introduction. Epidemiological surveys conducted in the U.S. (1) and the UK (2) have shown that the children of male anesthetists are at increased risk for the development of congenital abnormalities. This suggests the possibility that genetic defects could be transmitted via the sperm. Indirect investigations of male reproductive toxicity from inhalation anesthetics have been performed for halothane with the dominant lethal assay in mice (3,4). These studies have not indicated which, if any, general anesthetics could produce sperm abnormalities. However, recent evidence suggests that this type of assay may have a high index of false-negative results (5). We have chosen a more direct approach to this question by examining the sperm of (C57Bl/C3H) F_1 mice for morphological abnormalities following exposure to general anesthetics.

Methods. The mouse sperm morphology assay was adapted from that devised by Wyrobek and Bruce (6). Thirteen-week-old male mice of genotype (C57Bl/C3H) F_1 (Cumberland View Farms, Jackson, TN.) were used for each assay. Mice were randomized and caged, 5 per cage, throughout the assay. Each cage constituted one exposure group. Each group was exposed to one anesthetic at one concentration for 4 hours daily for 5 consecutive days. The anesthetic agents tested are as follows: nitrous oxide, chloroform, trichloroethylene, methoxyflurane, enflurane, halothane, diethyl ether, and isoflurane. Each agent was administered at 1 MAC and 0.1 MAC concentrations in glass exposure chambers. Those agents which produced greater than 20% mortality with 1 MAC exposure for 20 hours were retested at 0.5 MAC. Three control groups of 5 mice each were exposed to compressed air alone under similar conditions. Twenty-eight (28) days following the first day of exposure, the mice were sacrificed by cervical dislocation, and both cauda epididymides were removed. These were minced into 2 ml physiologic buffered saline solution, strained through stainless steel gauze, and stained with 1% Eosin Y(H_2O). Slides were prepared from these preparations, mounted, coded and evaluated by counting 1000 sperm per slide at 400 x magnification. Data were reported as percent abnormal sperm. The means for percent abnormalities were calculated for each group and compared with the controls by the 2 sample t-test.

Results. The results are listed in Table I. Of the general anesthetics tested, trichloroethylene, chloroform, and enflurane resulted in statistically significant elevations of the percentage of morphologically abnormal sperm. Exposure to halothane, isoflurane, methoxyflurane, diethly ether, and nitrous oxide had no effect of sperm morphology in this assay system.

Discussion. Direct investigation of the effects of general anesthetics on reproductive cells may be necessary to determine the reproductive toxicity of these agents. This initial survey was designed to determine if any of the agents which have enjoyed general use have a recognizable effect on sperm morphology in the mouse model. After prolonged exposure to high concentrations of trichloroethylene, chloroform, and enflurane, damage to the early spermatocyte during the period of meiotic cell division occurred to a small but significant percentage of cells. The mechanism of this phenomenon will require further investigation.

TABLE I. LEVELS OF SPERM ABNORMALITIES

AGENT	CONC. (%)	N	% ABN SPERM (+SEM)
AIR (CONTROL)	-	15	1.42 (0.08)
NITROUS OXIDE	80	5	1.64 (0.15
NITROUS OXIDE	8	5	1.44 (0.19)
CHLOROFORM	0.08	4	1.88 (0.39)*
CHLOROFORM	0.04	4	1.95 (0.31)*
TRICHLOROETH.	0.20	5	2.58 (0.29)**
TRICHLOROETH.	0.02	5	1.68 (0.17)
MOF	0.10	5	1.24 (0.17)
MOF	0.01	5	1.24 (0.05)
ENFLURANE	1. 2	5	2.02 (0.04)**
ENFLURANE	0.12	5	1.50 (0.18)
HALOTHANE	0.80	5	1.40 (0.18)
HALOTHANE	0.08	4	1.13 (0.17)
DIETHYL ETHER	1. 6	5	1.24 (0.11)
DIETHYL ETHER	0.32	4	1.70 (0.23)
ISOFLURANE	1. 0	5	1.70 (0.18)
ISOFLURANE	0.10	5	1.20 (0.24)

* P less than 0.05
** P less than 0.001

References.
1. Cohen EN, et. al.: Occupational disease among operating room personnel. A national study. Anesthesiology 41:321-340, 1974.
2. Knill-Jones RP, et. al.: Anaesthetic practice and pregnancy. Controlled survey of male anaesthetists in the United Kingdom. Lancet 2:807-809, 1975.
3. Bruce DL: Murine fertility unaffected by traces of halothane. Anesthesiology 38:473-477, 1973.
4. Wharton RS, et. al.: Fertility, reproduction and postnatal survival in mice chronically exposed to halothane. Anesthesiology 48:167-174, 1978.
5. Generoso WM, et. al.: Genetic lesions induced by chemicals in spermatozoa and spermatids of mice are repaired in the egg. Proc. Natl. Acad. Sci. USA 76:435-437, 1979.
6. Wyrobek AJ, Bruce WR: Chemical induction of sperm abnormalities in mice. Proc. Natl. Acad. Sci. USA 72:4425-4429, 1975.