Anesthesiology
67:42-44, 1987

The Effect of Nitrous Oxide on In Vitro *Fertilization Success Rate*

Mark A. Rosen, M.D., Michael F. Roizen, M.D.,† Edmond I. Eger II, M.D.,‡ Robert H. Glass, M.D.,§ Mary Martin, M.D.,¶ Pramila V. Dandekar, M.Sc.,** Patricia A. Dailey, M.D.,†† Lawrence Litt, M.D.,‡‡*

The authors studied the effect of nitrous oxide on success rates for *in vitro* fertilization and pregnancy in women undergoing laparoscopy for oocyte retrieval. Ninety-eight patients in an *in vitro* fertilization program were randomly assigned to an anesthetic regimen including either 0.7% (end-tidal) isoflurane with 60% nitrous oxide in oxygen, or 1.4% (end-tidal) isoflurane in oxygen. Success rates for fertilization and pregnancy in 44 additional patients who declined randomization were also studied. Among the 51 randomized patients who did not receive nitrous oxide, 192 oocytes were obtained and 122 fertilized (63.5%), resulting in eight pregnancies (16.3%). From the 47 randomized patients given nitrous oxide, 168 oocytes were retrieved and 114 fertilized (67.9%), resulting in nine pregnancies (19.1%). No significant differences between rates of fertilization or pregnancy emerged between groups. Such differences would have been found with an 80% probability had nitrous oxide had a 20% effect on oocyte fertilization. (Key words: Anesthesia, obstetrics: *in vitro* fertilization. Anesthetics, gases: nitrous oxide. Anesthetics, volatile: isoflurane.)

IN 1978, THE FIRST CHILD was born of an externally fertilized human egg.[1] Now, the techniques for external (*in vitro*) fertilization are widely applied. The first step is to stimulate development of multiple oocytes. In the *In Vitro* Fertilization Program at the University of California, San Francisco (UCSF), follicular growth is monitored by sonography and by measurement of serum hormone levels.§§ Oocytes are retrieved before ovulation, transferred to culture media, incubated, then fertilized. The resulting embryos are transferred into the patient's uterus through the cervix.

In most *in vitro* fertilization programs, oocytes are retrieved *via* laparoscopy during a general anesthetic that often includes nitrous oxide. Success in securing fertilization varies from 0–80%, and success in achieving pregnancy from 0–30%.[2,3] Because clinical,[4] experimental,[5,6] and epidemiological[7] data suggest that nitrous oxide has toxic effects, we speculated that the use of this drug during laparoscopy may adversely affect fertilization and pregnancy. Accordingly, we undertook the following study to determine whether nitrous oxide administration during oocyte retrieval influences the incidence of *in vitro* fertilization or of pregnancy initiated by transfer of developing embryos.

Methods

With approval from the UCSF Committee on Human Research, we studied patients undergoing oocyte retrieval *via* laparoscopy. Ninety-eight patients gave written informed consent and were randomly assigned to receive general anesthesia either including or excluding nitrous oxide. Forty-four patients declined randomization; 35 of these received nitrous oxide and nine did not. In all patients, anesthesia was induced by administration of thiopental and succinylcholine, preceded by a small dose of d-tubocurarine. The trachea of each patient was intubated and the lungs were mechanically ventilated to maintain an end-tidal carbon dioxide partial pressure of 30–35 mmHg measured by mass spectrometry. An infusion of succinylcholine provided muscle relaxation when necessary. Randomized patients were assigned to receive either approximately 0.7% end-tidal isoflurane with 60% nitrous oxide in oxygen (n = 47), or approximately 1.4% end-tidal isoflurane in oxygen (n = 51). The inspired concentrations of nitrous oxide and end-tidal concentrations of isoflurane were measured by mass spectrometry. Patients in both groups were given 0–4 μg/kg fentanyl before oocyte retrieval.

The peritoneal cavity of each patient was inflated with carbon dioxide. Oocytes were retrieved by aspiration through a laparoscope, transferred to culture media, and incubated with sperm using techniques previously described.[8] The duration of anesthetic exposure before oocyte retrieval ranged from 20–150 min. When laparoscopy was concluded, the trachea of each patient was extubated and the patient allowed to awaken fully in the recovery room before discharge.

Fertilization was assessed by light microscopy, revealing cell division. Forty-eight hours after oocyte retrieval, the developing embryos were transferred at the

* Assistant Professor of Anesthesia and Obstetrics, Gynecology, and Reproductive Sciences.

† Professor of Anesthesia and Chairman, Department of Anesthesia and Critical Care, Professor of Medicine, University of Chicago.

‡ Professor and Vice Chairman of Research, Department of Anesthesia.

§ Professor of Obstetrics and Gynecology.

¶ Assistant Professor of Obstetrics and Gynecology.

** Laboratory Scientist, *In Vitro* Fertilization Program.

†† Assistant Professor of Anesthesia.

‡‡ Assistant Professor of Anesthesia and Radiology.

Received from the Departments of Anesthesia and Obstetrics, Gynecology, and Reproductive Sciences, University of California, San Francisco. Accepted for publication February 17, 1987. Presented in part at the 59th Congress of the International Anesthesia Research Society, Houston, Texas, March 1985.

Address reprint requests to Dr. Rosen: Department of Anesthesia, University of California, San Francisco, California 94143-0648.

§§ Martin MC, Glass RG, Dandekar P, Shriock E, Lewis V: Comparison of various stimulation regimes in an *in vitro* fertilization program (unpublished data).

four-cell stage into the uterine cavity through the cervix of the nonanesthetized mother. Pregnancy was assessed initially by measurement of serum hormone levels and confirmed later by sonography. When pregnancy spontaneously aborted, or did not result from embryo transfer, the products of conception were not available for evaluation.

Fertilization data were analyzed using chi-square analysis with a Yates correction, and an unpaired *t* test, in order to compare the nitrous-oxide and no nitrous-oxide groups for the percent of oocytes fertilized and the percent fertilized per patient. The percent of pregnancies following transcervical placement of the embryos was analyzed using Fisher's exact test. Power analysis was used to determine the probability that the null hypothesis would be found were it true. A *P* value of less than 0.05 was considered significant for all findings.

Results

Results for the randomized and nonrandomized groups are presented separately. The overall fertilization rate for both randomized and nonrandomized patients given isoflurane alone was 63.4%; the overall rate for patients given nitrous oxide and isoflurane was 63.1%.

Randomized Patients

The nitrous-oxide and no nitrous-oxide groups did not differ in age, weight, or amounts of thiopental or fentanyl administered. One hundred ninety-two oocytes were obtained from 49 of the 51 patients anesthetized without nitrous oxide. One hundred twenty-two of these (63.5%) were fertilized. No oocytes were retrieved from two patients. In the nitrous-oxide group (n = 47), 168 oocytes were retrieved, 114 of which were fertilized (67.9%) (table 1). Chi-square analysis of these data using the Yates correction revealed no significant differences between the groups. An unpaired *t* test was used to compare the percent of eggs fertilized in each woman in each group. Again, no significant difference between groups was identified.

Eight pregnancies (16.3%) occurred among the 49 women not given nitrous oxide; two of these pregnancies subsequently aborted. Among the 47 women given nitrous oxide, nine pregnancies (19.1%) resulted, one of which was ectopic. No significant differences between groups resulted from comparison of these data using Fisher's exact test.

Nonrandomized Patients

Of the 44 patients who declined randomization, 35 were anesthetized with nitrous oxide and nine without.

TABLE 1. Oocyte Retrieval, Fertilization, and Pregnancy

	N	Number of Oocytes Retrieved	Number (%) of Oocytes Fertilized	Number (%) of Pregnancies
Randomized patients				
Nitrous oxide given	47	168	114 (67.9)	9 (19.1)
No nitrous oxide	51*	192	122 (63.5)	8 (16.3)
Nonrandomized patients				
Nitrous oxide given	35	114	64 (56.1)	—
No nitrous oxide	9	35	22 (62.9)	—

* No oocytes were retrieved from 2 of 51 patients.

From those given nitrous oxide, 114 eggs were retrieved, 64 of which were fertilized (56.1%). From the nine patients given isoflurane and oxygen alone, 35 eggs were retrieved, 22 of which were fertilized (62.9%) (table 1). Chi-square analysis revealed no significant difference in the rate of fertilization for the two groups. No pregnancies resulted.

Discussion

In vitro fertilization is now performed at many medical centers. Success rates for fertilization range from 0–80%, and, for pregnancy, from 0–30%. The success rates for *in vivo* conception and pregnancy may not be higher. It is possible that oocyte exposure to differences in Pa_{O_2} due to differences in FI_{O_2} during anesthesia contribute to differences in success rates with *in vitro* techniques; however, this seems unlikely. Although we did not measure Pa_{O_2} during these procedures, it is unlikely that clinically significant differences in oxygen content resulted from the differences between groups in FI_{O_2}, or that oocyte function which normally occurs at lower Pa_{O_2} was impaired by exposure to elevations in Pa_{O_2}.

Alternatively, oocyte exposure to specific drugs given during retrieval may contribute to the low success rates for these techniques. We postulated that nitrous oxide might be such a drug because it inactivates methionine synthetase, thereby diminishing the amount of thymidine available for DNA synthesis in dividing cells.[9] Because nitrous-oxide inactivation of normal DNA synthesis reportedly lasts 24–72 h, this drug might be expected to affect oocyte function and embryologic development.

Our results do not support this hypothesis. Perhaps the amount of nitrous oxide that reaches the human oocyte in the limited time before retrieval is insufficient to cause harm. It is known that nitrous-oxide inactivation of methionine synthetase proceeds slowly in human liver.[10] In addition, the low solubility of nitrous oxide exposes the oocyte to this gas for only a short period, as rapid transfer to an oxygen-carbon dioxide media occurs after oocyte retrieval. A second possibility is that

the use of isoflurane may affect fertilization and pregnancy. If this is so, the use of nitrous oxide may actually increase the rate of *in vitro* fertilization by reducing the concentrations of other potentially toxic and less diffusable anesthetics. However, in a recent study, no teratogenic effect was observed in rats exposed to 1% isoflurane during gestation.[11] A third possibility is that nutrients provided in the culture media to which the oocyte is transferred provide protection against the consequences of nitrous-oxide inactivation of methionine synthetase. It has been shown that administration of folinic acid prevents nitrous oxide-induced megaloblastic changes in bone marrow.[12] The media used at UCSF, a modification of Ham's F-10,[9] contains vitamin B_{12} and folinic acid. Finally, our sample size may have been too small to reveal the effects of nitrous oxide. However, power analysis indicated that we would have an 80% chance of finding an effect of nitrous oxide had fertilization rate changed by 20%.[13] To demonstrate smaller differences will require investigation of more patients and pregnancies.

References

1. Steptoe PC, Edwards RG: Birth after the reimplantation of a human embryo (letter). Lancet 2:366, 1978
2. Jones HW Jr, Jones GS, Andrews MC, Acosta AA, Bundren C, Garcia J, Sandow B, Veeck L, Wilkes C, Witmyer J, Wortham JE, Wright G: The program for in vitro fertilization at Norfolk. Fertil Steril 38:14–21, 1982
3. Edwards RG: Test-tube babies, 1981. Nature 293:253–256, 1981
4. Amess JAL, Burman JF, Rees GM, Nancekievill DG, Mollin DL: Megaloblastic haemopoiesis in patients receiving nitrous oxide. Lancet 2:339–342, 1978
5. Lane GA, Nahrwold ML, Tait AR, Taylor-Busch M, Cohen PJ: Anesthetics as teratogens: Nitrous oxide is fetotoxic, xenon is not. Science 210:899–901, 1980
6. Koblin DD, Watson JE, Deady JE, Stokstad ELR, Eger EI II: Inactivation of methionine synthetase by nitrous oxide in mice. ANESTHESIOLOGY 54:318–324, 1981
7. Cohen EN, Brown BW, Wu ML, Whitcher CE, Brodsky JB, Gift HC, Greenfield W, Jones TW, Driscoll EJ: Occupational disease in dentistry and chronic exposure to trace anesthetic gases. J Am Dent Assoc 101:21–31, 1980
8. Dandekar PV, Quigley MM: Laboratory set up for human in vitro fertilization. Fertil Steril 42:1–12, 1984
9. Nunn JF, Chanarin I: Nitrous oxide inactivates methionine synthetase, Nitrous Oxide/N_2O. Edited by Eger EI II. New York, Elsevier, 1985, pp 211–234
10. Koblin DD, Waskell L, Watson JE, Stokstad ELR, Eger EI II: Nitrous oxide inactivates methionine synthetase in human liver. Anesth Analg 61:75–78, 1982
11. Mazze RI, Fujinaga M, Rice SA, Harris SB, Baden JM: Reproductive and teratogenic effects of nitrous oxide, halothane, isoflurane, and enflurane in Sprague-Dawley rats. ANESTHESIOLOGY 64:339–344, 1986
12. Amos RJ, Amess JAL, Nancekievill DG, Rees GM: Prevention of nitrous oxide-induced megaloblastic changes in bone marrow using folinic acid. Br J Anaesth 56:103–107, 1984
13. Fleiss JL: Statistical Methods for Rates and Proportions, 2nd Edition. New York, John Wiley and Sons, 1981, pp 258–280