Washing mineral oil reduces contaminants and embryotoxicity

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Objective: To determine if washing improves the quality of mineral oil used for embryo culture.
Design: A 2 × 3 factorial experimental study.
Setting: University hospital-based infertility center.
Animal(s): Mice.
Intervention(s): The chemical nature of contaminants present in two lots of mineral oil was determined. Effect of washing on toxicity and amount of toxin present in media was determined.
Main Outcome Measure(s): The effect of washing was determined by a quality control bioassay or by directly determining the level of contaminant in oil-conditioned culture media.
Result(s): Water, culture media, and media plus albumin were equally effective in reducing toxicity and concentration of toxin. Temperature did not affect washing results. Peroxide, aldehydes, and alkenals were present in one lot of oil, and Triton X-100 was identified in the other lot. Washed oil containing peroxide passed the one-cell mouse embryo bioassay, and washing reduced the amount of Triton X-100 by 25%.
Conclusion(s): Mineral oil is the least defined component used for in vitro fertilization and embryo culture; therefore, it is important to determine if washing oil is beneficial. This study provides clear evidence that washing reduces toxicity of mineral oil. (Fertil Steril® 2010;94:2747–52. ©2010 by American Society for Reproductive Medicine.)

Key Words: mineral oil, embryo culture, quality control, toxicity, in vitro fertilization

Mineral oil is commonly used for embryo culture in human-assisted reproduction and embryo research applications. Although there are benefits to performing embryo culture in microdrops under oil (1, 2), mineral oil is a petroleum product that can vary widely in quality. Zinc (3), peroxidation (4, 5), and undefined contaminants (6–10) have been described as toxins in oil used for embryo culture. Although mineral oil used for IVF is commonly tested by manufacturers with a mouse embryo bioassay (MEA), quality of oil can vary by manufacturer, lot number, and storage conditions (4, 5, 9, 11). Thus, oil that passes the MEA may deteriorate during transit or storage.

Washing oil may reduce potential toxicity. However, washing is not practiced by all manufacturers of oil used for human IVF, nor is there a consensus among embryologists on the utility of washing oil. Fleming et al. (6) were the first to demonstrate that embryotoxic mineral oil could be detoxified by washing. Similarly, Lee et al. (10) observed improved mouse embryo development using washed oil.

In the present study, we used two distinct lots of embryotoxic mineral oil to determine the effect of washing on embryotoxicity. The aims of this study were to identify the contaminants responsible for toxicity and determine the effect of washing conditions using quantitative and qualitative measures of toxins and toxicity.

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MATERIALS AND METHODS

Mineral Oil

Two lots of embryotoxic mineral oil (M8410; Sigma-Aldrich, St. Louis, MO) were provided by embryologists from other laboratories. Lot 155K0071 (SMO71) was responsible for poor live birth rates in a transgenic mouse facility. Lot 114K0044 (SMO44) killed spermatozoa from nonhuman primates. Oil purchased from Sigma, Fisher Scientific (Pittsburgh, PA), and Vitrolife (Denver, CO) passed internal QC assays and were used as controls during toxin identification. Sigma oil was used as the control for QC assays and washing experiments.

Mouse Embryo Biossay

Cryopreserved one-cell and two-cell embryos from F1 hybrid mice were obtained from a commercial source (Embryotech Laboratories, Haverhill, MA) and thawed according to the manufacturer's instructions. Thawed embryos were equilibrated in synthetic human tubal fluid (HTF) with HEPES (HTF-HEPES; Irvine Scientific, Irvine, CA) with 5% human serum albumin (HSA, Irvine Scientific) for 10 minutes at room temperature. Embryos were cultured at 37°C in 6.5% CO_2 in 25 μL of HTF (In Vitro Care, Frederick, MD) without protein. Each experiment was performed in triplicate with 10 to 11 embryos per treatment. Embryos were graded at 24-hour intervals and the rate of blastocyst formation was determined at 72 and 96 hours postthaw for the two-cell and one-cell embryos, respectively. The blastocyst rate was defined as the percentage of embryos that developed to the expanded, hatching, or hatched blastocyst stage.

Human Sperm Motility Assay

The human sperm motility assay (HSMA) was performed as previously described (12) to test toxicity of different lots of mineral oil using donated sperm per institutional review board approval. Sperm

motility assays were performed using HTF conditioned by incubating five parts mineral oil with one part protein-free HTF for 24 hours at 37°C in 6.5% CO_2 in air. Fifty microliters of washed sperm suspension was added to 450 μL protein-free control or oil-conditioned HTF to yield a final concentration of 5×10^6 motile sperm/mL. All tubes were capped loosely and cultured at 37°C in 6.5% CO_2 in air. Motility and forward progression (grade) were determined at 24 and 48 hours. The Sperm Motility Index was calculated by dividing the percentage of motile sperm of the test item by the percentage progressive motile sperm of the negative control at the specified time intervals. Three tubes were used per test item and the experiments were repeated in triplicate.

Toxin Identification

Toxicity of the two lots of oil was confirmed using the HSMA and the two-cell MEA (see Results); therefore, steps were undertaken to identify toxins in these oils.

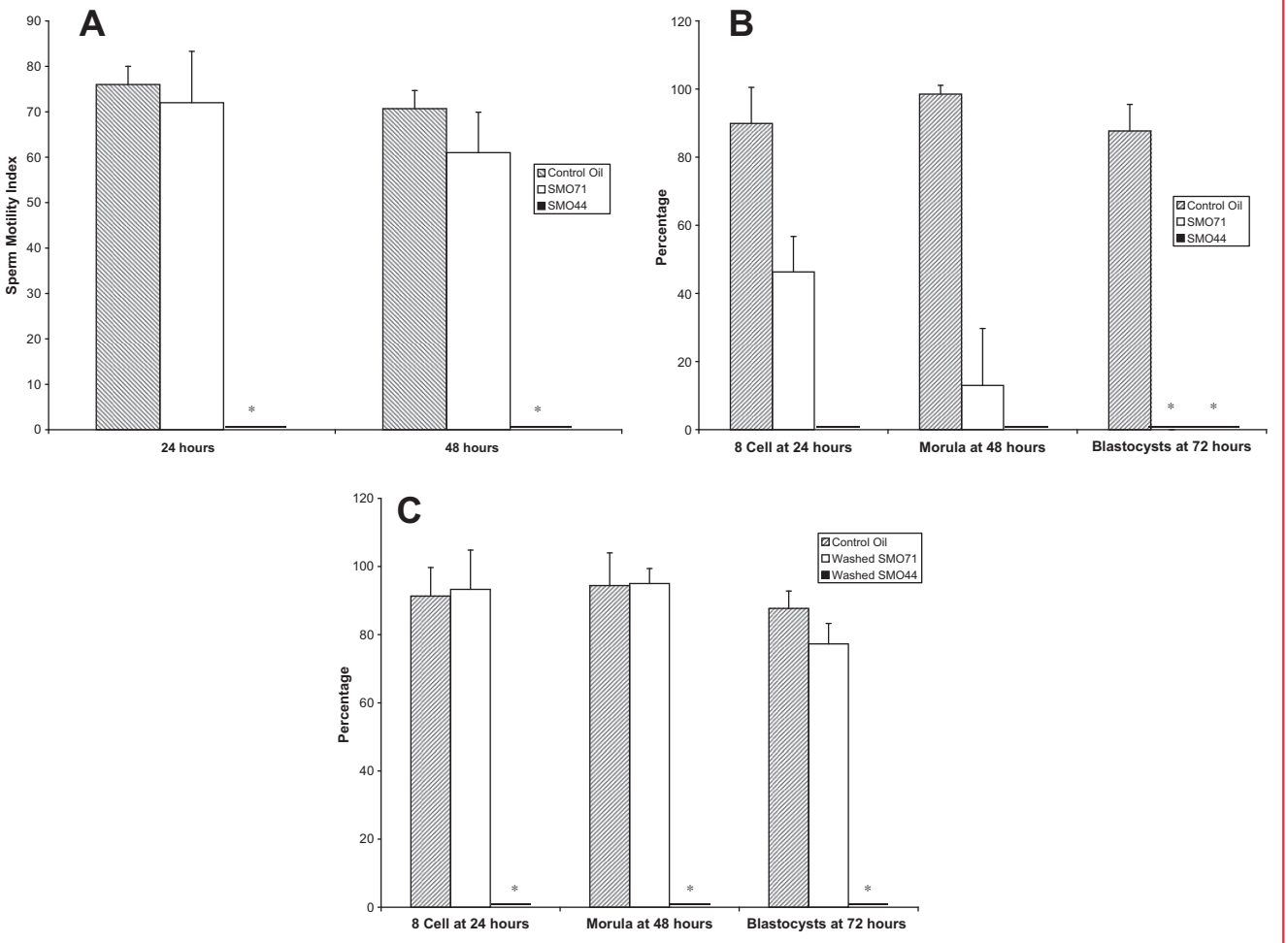
Peroxide assay The SafTest PeroxySafe kit (MP Biomedicals, Irvine, CA) was used to determine peroxide levels in both lots of mineral oil. The assay is a rapid, colorimetric assay that detects peroxides and byproducts of oil oxidation such as aldehydes and alkenals. The assay was performed by MP Biomedical per the manufacturer's instructions. The sensitivity of the PeroxySafe kit is 0.02 mEq/kg oil.

Tandem mass spectrometry Tandem mass spectrometry (LC-MS/MS) was used to further characterize the unknown contaminant in SMO71. Differences between control oil and Lot SMO71 were examined by performing LC-MS and LC-MS/MS on extracts of oil mixed with cell culture media. Lot SMO71 was extracted with aqueous cell culture media (HTF) by liquid–liquid extraction at a ratio of 5 parts oil to 1 part of media. The oil and aqueous media were mixed by hand and equilibrated for 24 hours at 37°C.

The LC-MS experiments were performed on a Waters Acquity UPLC liquid chromatography system coupled to a Waters Q-TOF

FIGURE 1

Response of two-cell mouse embryo assay (MEA) and Human Sperm Motility Assay (HSMA) to oils SMO71 and SMO44. (**A**) Sperm motility was not affected by SMO71-conditioned HTF when compared to control oil. Sperm did not survive to 24 hours when exposed to media conditioned with SMO44 ($P < .001$). (**B**) Development of two-cell mouse embryos to blastocyst was reduced for test oils at all times observed compared to control oil ($P < .01$). (**C**) Two-cell MEA results after contaminated oil lots (SMO71 and SMO44) were washed with human tubular fluid (HTF). Embryos passed at >70% blastocyst rate when they were cultured in media that was exposed to washed oil lot SMO71. No embryo growth was seen in the media that was exposed to washed oil lot SMO44 ($P < .01$).



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