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O-092 High-viscosity mineral oils provide enhanced protection to embryo culture systems against volatile organic compounds-induced embryotoxicity

Q. Matia Algué[1], M. Acacio[1], E. Mestres[1], A. Flores-Saiffe[2], A. Martínez Casado[1], A. Villamar[1], A. Franco-Roig[1], A. Casals[1], C. Castelló[1], L. Romera[1], J. Cohen[2], N. Costa-Borges[1]

[1]Embryotools, Research&Development, Barcelona, Spain
[2]Conceivable Life Sciences, Research, Guadalajara, Mexico

Study question: Can the viscosity of mineral oils used in embryo culture systems influence their protective effect against the embryotoxic impact of volatile organic compounds (VOCs)?

Summary answer: Embryonic developmental outcomes in sub-optimal environmental conditions with high VOC concentrations are protected more efficiently by high-viscosity mineral oils compared to low-viscosity oils.

What is known already: VOCs are gaseous emissions of organic compounds (including aldehydes [HCHO], alcohols, and hydrocarbons) commonly found in indoor and outdoor environments. Two of the most widely used VOCs are isopropyl-alcohol (solvent frequently used in cleaning products) and paraformaldehyde (a commonly used fixing agent). Whilst their embryotoxic effect is known, their presence in embryo culture systems is common and well documented. Mineral oil acts as a protective layer between the VOCs in the environment and the embryos in culture, but the extent to which the viscosity of the used mineral oils plays a role in its protective effect against VOCs remains unclear.

Study design, size, duration: Two isopropyl-alcohol wipes or 250µL of 4% paraformaldehyde were used as VOC sources. For each positive test, three dishes with 5mL of oil (high, medium or low viscosity) and culture medium droplets were placed under a glass bell jar with the VOC source nearby for 16h. For each oil, a negative control (without VOC exposure) was prepared in parallel. After incubation, dishes were equilibrated for 24h at 6%CO_2 and 7%O_2 before starting the mouse-embryo-assays.

Participants/materials, setting, methods: Oils with 102.1cP, 43.1cP and 22.3cP at 30°C were selected as high (HVO), medium (MVO) and low viscosity (LVO), respectively. Experiments were performed in triplicate with 21 freshly collected mouse zygotes each (n = 846). Cleavage rate was assessed after 24h, and expanded blastocyst formation rate (EBFR) was determined at 96 and 120hours. Resulting blastocysts were fixed and stained for cell counting, and lab VOC/HCHO levels were monitored throughout. Statistical analysis was performed using Fisher's or Kruskal-Wallis.

Main results and the role of chance: In the experiments with isopropyl-alcohol (>5 ppm), no differences in cleavage rate were observed across the experimental groups ($p > 0.05$). The EBFR was heavily reduced by VOCs in the MVO and LVO groups (46.0% and 36.5%, respectively) compared to their non-exposed controls (95.2% and 98.4%; $p < 0.0001$); by contrast, the HVO was able to avoid this reduction (98.4% EBFR in the VOC-exposed group and 100% in the non-exposed control; $p = 1$).

Mean cell counts indicated a decrease in blastocyst quality in the VOCs-exposed groups compared to their corresponding controls with all three oils: 167.90 (±37.47 SD) vs. 187.95 (±39.51 SD) with HVO ($p = 0.0361$); 126.59 (±33.99 SD) vs. 188.47 (±36.35 SD) with MVO ($p < 0.0001$); 101.57 (±38.73 SD) vs. 179.45 (±35.16 SD) with LVO ($p < 0.0001$).

Exposure to paraformaldehyde (>5 ppm) resulted much more embryo-toxic, already producing significant differences in cleavage rate on day 2 in all oil groups (23.8% HVO, 0% MVO, 0% LVO) compared to their corresponding non-exposed controls (100%, 100% and 96.83%, respectively; $p < 0.0001$). The EBFR was 0% in all paraformaldehyde-exposed groups, while in all negative controls more than 90% of the embryos reached this stage ($p < 0.0001$).

Limitations, reasons for caution: Further studies are needed to assess the effects of different VOC types and concentrations on embryo development, as their impact may vary. Additional replicates are necessary to enhance the robustness of the findings. Moreover, the results in mice may not fully translate to human embryos.

Wider implications of the findings: This study suggests that high-viscosity mineral oils confer higher protection to embryos against VOC exposure, optimizing embryo culture conditions. It also offers insights towards the development of sensitive mouse-embryo-assays to assess VOC effects in culture systems, highlighting the importance of mineral oil viscosity to improve success rates in fertility treatments.

Trial registration number: No