Abstract title:
Evaluation of the effects produced by Styrene (volatile organic compound- VOC) over the human pre-embryo development.

Study question:
Can specific concentrations of Styrene, a very common IVF VOC, affect the human embryo development and ploidy?

Summary answer:
Embryos cultured along with IVF-ambient air-related concentrations of Styrene have worst survival rates and morphology score when evaluated at blastocyst stage (day 6).

What is known already:
Polystyrene, a synthetic aromatic hydrocarbon polymer made from the monomer styrene is present in the plastic-ware used in IVF laboratories. Styrene may persist even after the process of devolatilization, and its presence is known to affect the embryo development in animal models but no specific effects on humans have been described yet. Previous analysis have demonstrated that there are negative effects over human embryo development when exposing embryos to Benzene and Limonene.. Specific threshold levels at which most of the common pollutants in the IVF’s ambient air can cause harm to cultured human embryos, have not been determined either.

Study design, size, duration:
Prospective study with 115 day-3 (D3) human embryos. These have been allocated in four experimental groups; a. 40 embryos were cultured in the control (C) culture media. At the same time, 75 embryos were cultured and exposed to three Styrene concentrations based on previous IVF’s environmental characterizations: b. Exterior (SE: 165.8*10^-5ppm), c. Laboratory (SL: 981*10^-5ppm) and d. Double Laboratory (SDL: 1962*10^-5ppm). All embryos were cultured till day-6 (D6) so they could reach the blastocyst stage.

Participants/materials, setting, methods:
This developmental toxicity test includes D3 embryos, with 6 to 10 cells and less of 25% of fragmentation, which have been exposed by contaminating the blastocyst culture medium with three doses of Styrene (under 1% of the occupational limit value, OLV). After thawing, 25 embryos were exposed to each dose up to D6 of development, when embryo morphology was evaluated. Then biopsy of trophectoderm (TE) was performed for ploidy analysis of expanded blastocysts through NGS.

Main results and the role of chance:
A total of 115 D3 human embryos were donated for this VOCs research. In general, 66.7% of embryos developed to blastocyst stage after being exposed to Styrene while 90% of the embryos developed in C...
group \( (p = 0.006) \). Individually, all three doses of Styrene affected similarly the development: SE and SL 68\% \( (p = 0.0460) \) and SDL 64\% \( (p = 0.0228) \).

53.3\% of embryos exposed reached the expanded- hatched blastocyst stage compared to 75\% of the C group \( (p = 0.028) \). SDL doses affected significantly the expansion of the embryos: 48\% \( (p = 0.0350) \).

Inner cell mass (ICM) and trophoderm (TE) good quality scores (A-A, A-B, B-A, B-B) were only registered on 26.7\% of Styrene embryos group compared to 40\% of the control group, not being significant \( (p = 0.2048) \). A-A score embryos were obtained in both groups.

A total of 55 embryos that reached expanded- hatched state were analyzed for aneuploidies. Aneuploidy rates were not significant between Styrene \( (n=35) \) and Control \( (n=20) \) groups (28.6\% vs. 25\% respectively); however when analyzing the effects of specific doses, the embryos that were exposed to the to the highest dose of styrene (SDL) reached up to 40\% of aneuploidies compared to 25\% of the C group. \( (p = 0.4311) \).

**Limitations, reasons for caution:**
Developmental and ploidy rates were obtained by direct contamination of the culture medium with VOCs, specifically Styrene in this experiment. For this reason the results do not represent the exact conditions of an IVF laboratory.

**Wider implications of the findings:**
The association of the specific morphologic and chromosomal effects produced by common IVF VOCs, over the human pre-embryos will allow to establish a database with regulatory institutions, with accurate occupational limit values for human embryo culture.

**Trial registration number:**
Not applicable

**Keywords:**
IVF
VOCs
Embryo
development
Aneuploidies
Styrene