

## WASHED PARAFFIN OIL BECOMES TOXIC TO MOUSE EMBRYOS UPON EXPOSURE TO SUNLIGHT

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Mammalian embryos are typically cultured under paraffin oil in microdrops of medium. Paraffin oil permits the use of small volumes (40  $\mu$ l) by preventing microdrop dehydration. Micromanipulation of embryos is also often done in microdrops covered with paraffin oil. It is a common problem to find that a batch of paraffin oil is toxic to embryos resulting in failure of growth. Therefore, prudent laboratories routinely check each batch of oil for embryotoxicity before use. It is possible sometimes to remove the toxic component(s) from the paraffin oil by washing the oil repeatedly with a saline solution. Some laboratories wash and maintain their paraffin oil in equilibration with saline for added assurance. It is unknown what might cause paraffin oil previously found to be toxin-free to change and become toxic. We were interested in the effect of sunlight on paraffin oil. In an initial experiment, washed paraffin oil in glass media bottles was either exposed or not to sunlight for approximately 4 h. CF1 females were superovulated and mated with B6D2F1 males. Two-cell embryos were flushed 48 h later from the oviducts and used to determine the toxicity of the paraffin oil. Criterion for survival was growth to blastocyst stage after 72 h of culture. Data were binomial with embryos either succeeding or failing to develop to expanded blastocysts. When washed, sun-exposed, paraffin oil was used, embryo survival was 0 of 114 embryos. Survival of embryos cultured with washed, unexposed, paraffin oil was 117 of 126. The two treatments differed significantly ( $P < 0.001$ ;  $z$  score). A second experiment was conducted to determine if unwashed paraffin oil would also be affected by sunlight exposure. Embryos were collected from 4 superovulated females, selected for good quality and arbitrarily assigned to each of the 4 treatments shown in the below table.

Embryos surviving in oil exposed to sunlight or not exposed, in either washed or unwashed oil.

Sunlight Exposed	Unwashed Paraffin Oil	Unexposed
<sup>a</sup> 13/15		12/12
0/13	Washed Paraffin Oil	13/13

Only the washed, sunlight exposed treatment differed significantly from all other treatments ( $P < 0.001$ ; binomial statistical analysis using  $z$  score). <sup>a</sup> Numerators are number of embryos surviving; the denominators are total per treatment.

It is interesting that the very procedure used to eliminate toxicity from toxic paraffin oil, renders the oil more vulnerable to becoming toxic upon exposure to sunlight. Although the sunlight exposure that caused the oil to become toxic was high, the toxicity of the oil prevented any embryo from developing for even a single cell division. It would seem that lower exposure levels would have a similar although reduced effect. Paraffin oil is often transported outdoors to embryo transfer sites for embryonal biopsies and splitting procedures and might receive exposure to sunlight if attention is not taken. Our results suggest that during transport, the oil should be shielded from light. Washed oil should be stored in the dark and treated as a photoreactive compound.