Tech Note No. 60

# Odor from Irradiated Polystyrene Has No Effect on Embryo and Sperm Survival

Over the years, many users have expressed concern that the unpleasant odor from radiation sterilized polystyrene products may influence cell growth and performance.

This has been of particular concern in the IVF field, where safety is at a premium.

In order to examine the effect of irradiated polystyrene on embryos and sperm, a Mouse Embryo Assay (MEA) and a Human Sperm Survival Assay (HSSA) were performed on irradiated polystyrene products.

#### **Methods**

## Description of the assays in 4 well dish

MEA: Embryos used in this test were derived from a cross between B6C3F1 female mice mated to B6D2F1 male mice (B6C3F1x B6D2F1). The resulting embryos were cultured directly in 'embryo tested' HTF medium overlaid with light culture oil in the 4 well dishes with 21 embryos per dish in triplicate. Three of the four wells received seven 1-cell stage embryos in 0.5 mL of medium. All incubations were done at 37°C with 5% CO<sub>2</sub>.

After 96 hours, the dishes were removed from the incubator and the embryos were examined microscopically. Those embryos that were determined to have reached the blastocyst stage were scored as viable (Fig. 1).

HSSA: Human semen samples were thawed for 30 minutes at room temperature before mixing with

pre-tested Ham's F10 medium supplemented with 2% BSA. After spinning and re-suspending in a fresh aliquot of the same medium, the swim up fraction was collected. The motile fraction was determined, and a suitable volume of the sperm suspension was added to the 4 well dish. Motility was recorded after 24 hours.

Since it is known that the odor is most intense immediately after irradiation and dissipates with time, dishes were tested at both 2 and 10 weeks\* after irradiation. It is also common practice to open packages and let them air overnight before use. Therefore, one pack of dishes from each set was opened 24 hours prior to MEA and HSSA testing, and one pack was opened immediately before testing. Three dishes from each of these sets were tested in each assay and monitored for embryo survival at the 2-cell and blastocyst stage in MEA, and for sperm cell motility in HSSA.

\* Due to the nature of the sterilization logistics, two weeks is the earliest possible time after irradiation that a user could receive products.

#### **Results**

MEA acceptance criterion ≥ 80% (Embryotech ≥ 70%) embryos have developed to blastocysts within 96 hours after fertilization.

HSSA acceptance criterion ≥ 70% (Embryotech ≥ 60%) sperm cells remain motile 24 hours after sample preparation.

These criteria were met for both assays under the described conditions (Figs. 2 and 3).

#### Conclusion

No significant differences in embryo or sperm survival were observed between groups, whether dishes were tested at 2 or 10 weeks, or the packs were opened to allow the odor to dissipate overnight. Furthermore, in both assays the product performance exceeded the acceptance criteria, confirming that odor has no detrimental effect on embryo survival and sperm motility.

All tests were carried out by an independent testing consultancy (Embryotech Laboratories, Mass, USA).

### **Early Embryonic Development**

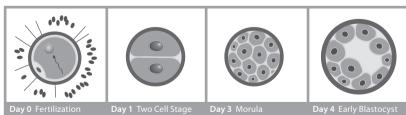
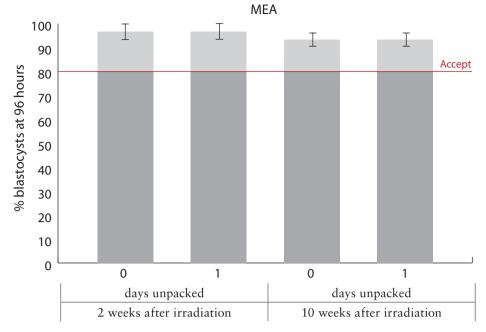
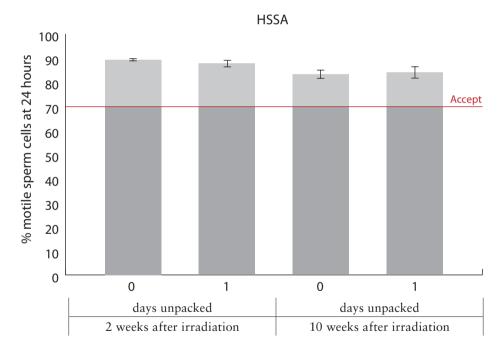


Fig. 1.
Diagrammatic representation of the developmental stages immediately after fertilization.



**Fig. 2.**Mouse Embryo Assay (MEA) in IVF dishes unpacked 0 or 1 day before use at 2 and 10 weeks after irradiation. No significant difference between these set-ups was seen in blastocyst formation 4 days after fertilization.



**Fig. 3.**Human Sperm Survival Assay (HSSA) in IVF dishes unpacked 0 or 1 day before use at 2 and 10 weeks after irradiation. No significant difference between these set-ups was seen in sperm motility 24 hours after sample preparation.

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