# Simple somatic cell assay to screen mammalian embryo toxicity caused by mineral oil

The success of *in vitro* fertilization (IVF), mammalian embryo culture and somatic cell nuclear transfer (SCNT) depend on stringent laboratory conditions and quality of chemicals, plastic ware, and glassware used to culture generated embryos. Therefore, all components and conditions need to be regularly monitored to avoid any kind of contamination and toxicity. Mineral oil (MO), a petroleum product, is extensively used to overlay the embryo cultures to maintain pH and osmolality of the culture medium. In human and farm animals, embryos are cultured with MO for 5 to 7 days to develop fertilized embryos to blastocyst stage[1]. Commercial suppliers claimed that MO is embryo safe by testing it with mouse embryo culture; however, MO quality can varied by a lot number and may deteriorate by improper storage and transport conditions. In addition, MO is an oil product that makes it susceptible to peroxidation and free radical formation[2,3]. Therefore, embryologist(s) should be more attentive to avoid MO-based embryo contamination and toxicity.

It has been reported that MO toxicity adversely affects the embryo development and IVF outcomes even though embryo safe MO was used[4,5]. The MO becomes crucial when embryologists have a very few and unique embryo type such as human IVF embryos, or SCNT and transgenic embryos of farm animals that are intended to produce babies. Therefore, procured MO needs to be screened for toxicity or contamination before its use in embryo culture. The commercial companies and some laboratories performed mouse embryo assay to screen MO for its toxicity. However, IVF clinics and many farm animal laboratories do not have facilities for mouse embryo culture which is laborious and expensive task. Therefore, a simple and cost-effective assay is needed so that embryologist(s) can easily screen MO in their laboratories.

Our laboratory has regularly been producing buffalo IVF and SCNT embryos[6–8], and for the last eight years, we used MO from the Sigma firm (Catalogue number M8410). Recently, we noticed embryo toxicity in IVF and SCNT experiments. We observed that arrested embryos have bleb membrane with a deformed morphology (Figure 1 *a*). To find the cause of this toxicity, we spent more than 20 trials in 5 months, in which all components such as culture medium, serum, antibiotics, vitamins, culture dishes, and MO were replaced one by one. When we replaced MO with a new lot, the normal embryonic development was recorded which indicates toxicity caused by MO.

To screen the MO toxicity, we developed a simple somatic cell assay. Briefly, somatic cells (approximately 5000 cells per well) were cultured in 4-well dishes in culture medium (DMEM supplemented with 20% FBS, 1% (v/v) non-essential amino acids, 1% (V/V) vitamin mix (Himedia, VA001, Mumbai, India), and 1 % (V/V) antibiotic solution (GIBCO, 15240-062, Grand Island, NY)) and seeded cells were overlaid with 200 µl

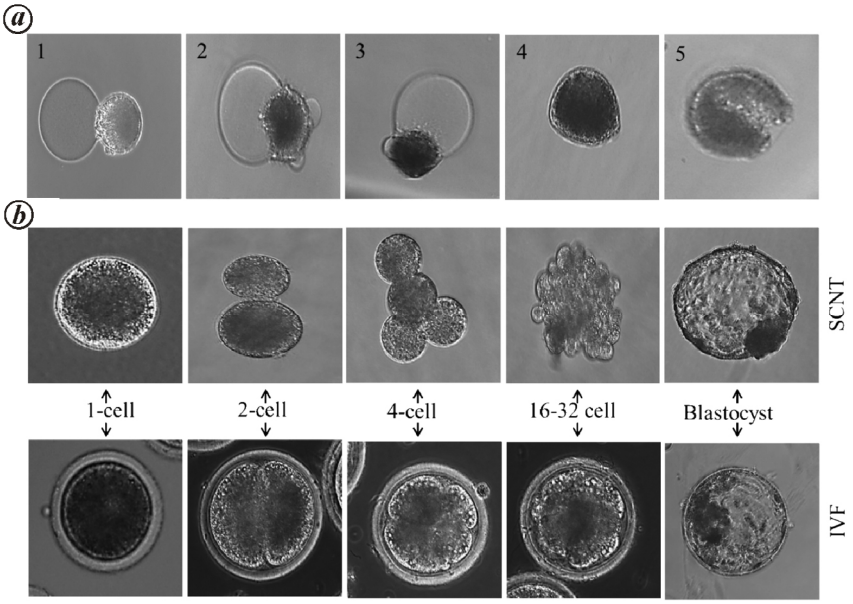


**Figure 1.** Embryo toxicity and developmental stages of IVF and SCNT embryo of buffalo. The bleb vesicle structures are visible on the membrane of one cell stage zona-free SCNT embryos (***a***) and were arrested at one cell stage. In IVF embryos, bleb vesicle structures were not distinguishable since IVF embryo has a tightly associated strong protective zona-membrane to embryo membrane. ***b***, Developmental stages of buffalo's zona-free SCNT and IVF embryo.

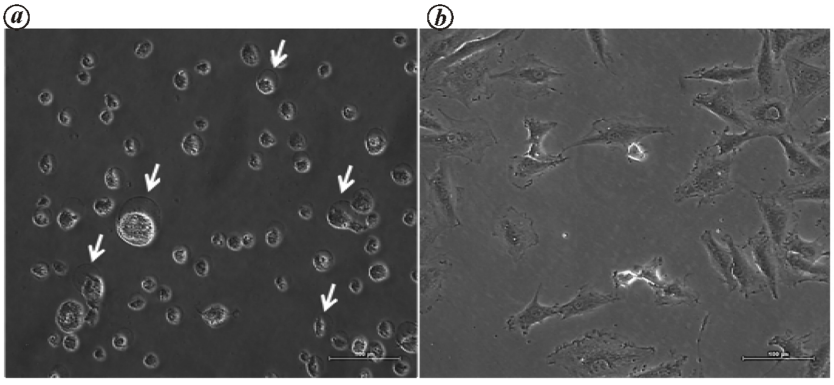


**Figure 2.** Somatic cell assay to screen the quality of MO. The somatic cells were cultured in four well dishes and overlaid with two different batches of the same catalogue number MO. In toxic MO, bleb vesicle structures were observed on the membrane of the seeded cells (***a***), that are indicated by arrow marks and seeded cells were not attached to the culture surface. The somatic cells cultured with non-toxic MO were attached and proliferated (***b***).

of MO. The seeded cells were cultured at 38.5°C under 5% $CO_2$ in the air for 24 h and monitored cell attachment rate and proliferation. The morphological changes after 24 h of culture were captured using an inverted microscope (Nikon, Tokyo, Japan) at 200× magnifications. We observed that seeded somatic cells were not attached to culture surface and cells have bleb membrane similar to embryos (Figure 2 *a*). Somatic cells were attached and proliferated when MO was not overlaid in the culture dishes. To reconfirm MO toxicity, above-mentioned cell assay was performed with a new lot of MO, and it was observed that seeded cells were attached and proliferated. This indicates that an old lot of MO was toxic to embryos. Hereafter, we have regularly been testing each lot of procured MO using the described somatic cell assay to avoid MO toxicity to embryos.

The present study describes a cheap and simple somatic cell assay to test the quality of MO. This assay can easily be performed by embryologists who are working in human IVF and farm animal laboratories. In addition to somatic cell assay, further analysis can be done to determine the actual level of peroxidation, alkenes and aldehydes, and residual Triton X-100 in each lot of MO. Since the human IVF embryos are not available in a large number and they are mainly used to produce babies, we suggest using the embryos of farm animals to screen the quality of MO and other compounds such as serum and antibiotics.


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# Infestation of bopyrid isopod parasite (Bopyridae) on 'coral banded boxing' shrimp *Stenopus hispidus* Olivier, 1811 (Stenopodidae) in the Lakshadweep archipelago

Bopyrid parasitic isopods (family Bopyridae Rafinesque) are unique and well known to utilize marine crustaceans as both intermediate and definitive host during their life cycle[1,2]. The family Bopyridae is the largest and highly diverse group with 605 species under 8 subfamilies[2,3]. The males are smaller than females (exhibiting reverse sexual dimorphism) and are attached to the ventral posterior region of the female abdomen[4]. Bopyrids used to infest either branchially or abdominally on a variety of crustaceans including brachyurans, anomurans, penaeids and carideans[1,2,5–7]. Infestation of parasites has deleterious effects on host such as reduction in growth, energy and mainly resulting in reproductive failures[8–11]. It can also affect the escape response of host to predators[12]. Extensive studies have revealed bopyrid parasite infestation on caridean shrimps[3]. However, the infestation of parasite on Stenopodidean shrimps has been rarely documented[10,13–15]. The present contribution is one such rare observation on the infestation of bopyrid isopod parasite on the marine ornamental shrimp *Stenopus hispidus* from Lakshadweep.

The 'barber pole' or 'coral banded boxing' shrimp *Stenopus hispidus* Olivier (Infraorder Stenopodidea) is recognized for its remarkable coloration and circumtropical distribution[16,17]. It is the only species among the family Stenopodidae to successfully pass through the major

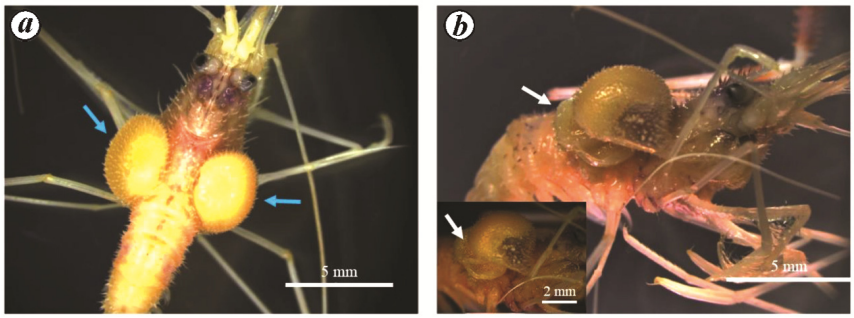


**Figure 1.** Boyprid parasite infested 'coral banded boxing' shrimp *Stenopus hispidus* Olivier. ***a***, Dorsal view of carapace region showing the bulbous structure (blue arrows) on the branchial region of cephalothorax; ***b***, Lateral view of carapace (right side) showing the presence of bopyrid parasite (female) (white arrow), inset: the adult female carrying eggs (white arrow).