

HELPFUL HINTS

before starting or doing any part of the procedure, here are a few things that can greatly improve the chances of getting good results

Speed is Important

Results will improve as you become faster at performing each step.

Water is Important

The choice of water depends upon local availability of highly-purified water. We make up stock solutions using Tissue Culture Water purchased from Sigma. For all other media, we use deionized water that is also distilled.

Oil is Important

Often when IVP fails, the oil is the culprit. Water-soluble contaminants in oil can kill embryos. Oil can be cleaned by incubating it with water or medium in some sort of shaker or mixer. We have had good luck using Sigma's Embryo-Tested Mineral Oil but there are many other oils available, with Ovoil probably the best (and most expensive).

Keep Incubators Set Properly

Check incubators regularly for accuracy of temperature and CO₂ readings and to ensure the air is humidified (i.e. reservoir of water inside the incubator is about ¾ full). Water-jacketed incubators respond to changes in setpoint slowly so make adjustments well before the incubator will be used. Nitrogen can run out very frequently if not continuously monitored.

Use Supplies for IVP Only

Set aside glassware, plasticware, and instruments that will only be used for IVP to prevent any residue from another application to contaminate media and affect the oocytes/embryos. This could be done easily by having a set of glassware that is different than that regularly used in the laboratory.

Keep the Work Area Warm to Prevent Cold Shock

1. Air-conditioning vents should be covered during IVP procedures. If the room is devoted for IVP only, air-conditioning vents can be permanently sealed.
2. A space heater may be used to warm up the air near the work site as well as microscope stage and bench surfaces. Placement of a dish on a cold lab bench or microscope stage could result in a rapid cold shock of embryos. Don't get too close to the space heater though or you can cook your embryos.
3. Before looking at embryos using a microscope, make sure stage has been pre-warmed (by turning on the space heater). Metal is an excellent conductor of cold from one material to another.
4. When washing oocytes, place beakers on plastic mesh or Styrofoam and not on a cold surface (i.e., countertop).
5. Always place dishes (i.e. petri dishes, X-plates) on a slide warmer set at 39°C.

Pre-Warm Media at 38.5°C

- a) Media designed for use in air or 5% CO₂ in air should be pre-warmed for at least 2 to 3 h in an oven, incubator or water bath set at 38.5°C. Make sure lid is on tight. Media to be pre-warmed in this manner includes transport saline, OCM, H-SOF, ISOLATE and PHE.
- b) Incubator - Media designed for use in a 5% CO₂ environment should be pre-warmed to 38.5°C in an incubator. In order for the pH of the medium to be equilibrated, it is important to loosen the lid of any bottle/tube of medium placed in the incubator. Leaving a bottle of medium buffered for a specific

