

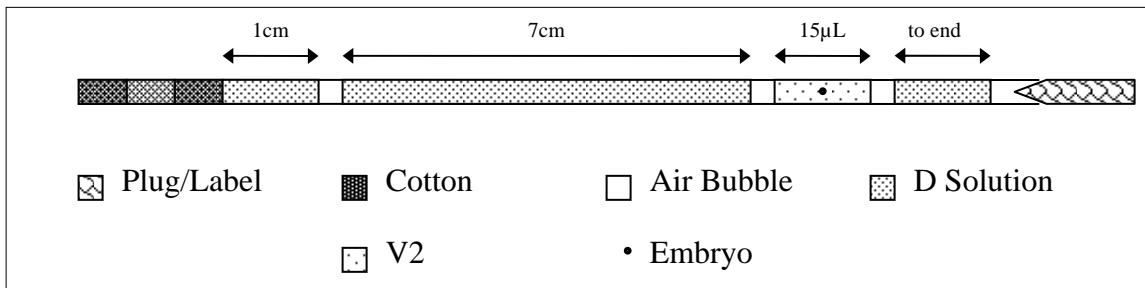
Vitrification of Bovine Embryos

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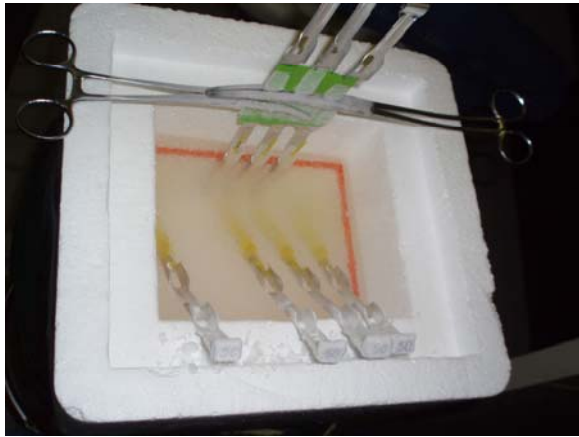
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The following protocol was based on procedures from Dr. George E. Seidel, Colorado State University

- **Media** (note all media are sterile-filtered except V2)
 - **Medium H (base medium):** Syngro® ([Bioniche Animal Health](#)) + 0.1% (w/v) polyvinyl alcohol
 - **Medium V1:** base medium + 5 M Ethylene Glycol. Add 4.2 ml of ethylene glycol to 10.8 ml of base medium.
 - **Medium V2:** base medium + 7 M Ethylene Glycol + 18% (w/v) Ficoll 70 + 0.5 M Galactose. Add 15 ml of base medium to a beaker with a stir bar. Add 19.5 ml ethylene glycol, 9.0 g Ficoll 70 and 4.5 g galactose to the beaker and stir overnight with mild heat (~35 C) on a stirring plate. After galactose is dissolved, add base medium to bring volume to 50 ml.
 - **Medium D:** base medium + 1 M galactose. Add 9.0 g galactose to 35 ml H Medium to a beaker with a stir bar. After galactose is dissolved, add base medium to bring volume to 50 ml.
- **Vitrification Procedure**
 - Transfer the embryos to 0.5 ml Medium H in a 4-well plate.
 - Move the embryos to 0.5 ml Medium V1 in a 4-well plate for 3 min.
 - Move the embryos to a 15 μ L drop (not covered with oil) of Medium V2 for 45 sec (up to 30 embryos per drop). *Don't wait too long or the drop will evaporate.*
 - Prepare a straw by loading the following in sequence (see diagram below): 1 cm Medium D followed by 0.5 cm air, 7cm Medium D, 0.5 cm air, the entire drop of 15 μ L of V2 plus the embryo, 0.5 cm air, and Medium D almost to the end of the straw. A label can be inserted into the open end of the straw. *We have noticed that the long plugs that are purchased from AgTech often contribute to the explosion of the straw and therefore we label with a regular short plug designed for AI straws (also from AgTech).*
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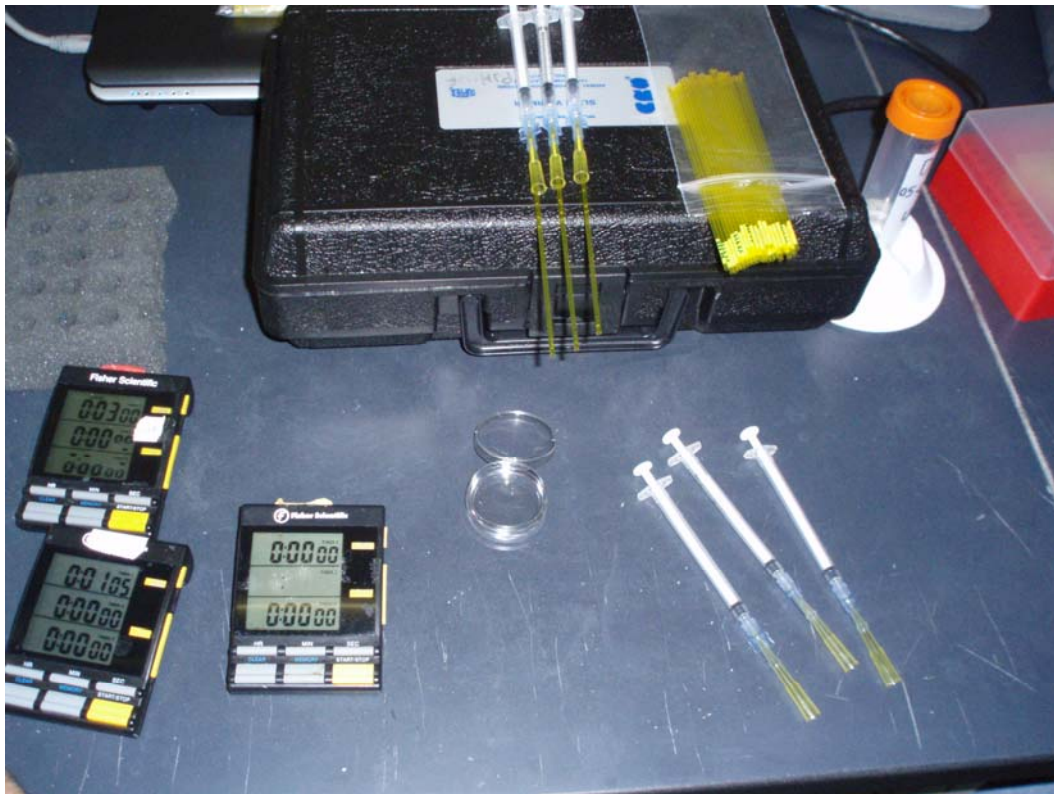
- After 45 sec, place the straw into a plastic goblet (the kind used for semen storage) previously placed into liquid nitrogen so the air in the goblet is cold (there is no liquid nitrogen inside the goblet) – see figure below for one example of a workable setup.
- Held the straws in vapor for 1 min and then plunge into liquid nitrogen.



Example of an apparatus for cooling straws in liquid nitrogen vapor.

- **Thawing Procedure**

- Thaw the straws in air for 8 seconds, and place in a 35-37°C waterbath for 15 sec.
- Hold the straw at the cotton-plugged end and immediately shake 4 times.
- Direct [embryo transfer](#) can be performed within 3 to 10 min (with one or two embryos per straw) or embryos can be deposited into culture medium (wash in [HEPES-TALP](#) 3x and culture in medium containing 10% (v/v) fetal bovine serum and 50 μ M dithiothreitol)



Some supplies used for embryo freezing. Shown from left to right are timers, a beaker with medium, and several examples of devices to place media in straws. The devices are constructed of a 1 cc syringe and pipette tip. Note that the small end of the pipette tip is inserted in the syringe and the straw is inserted into the wide end of the pipette tip for aspiration of media into the straw.

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