

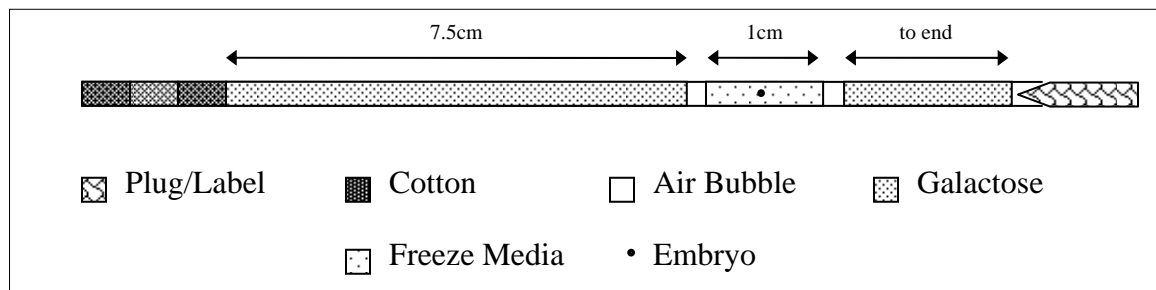
Slow Freezing of Bovine Embryos

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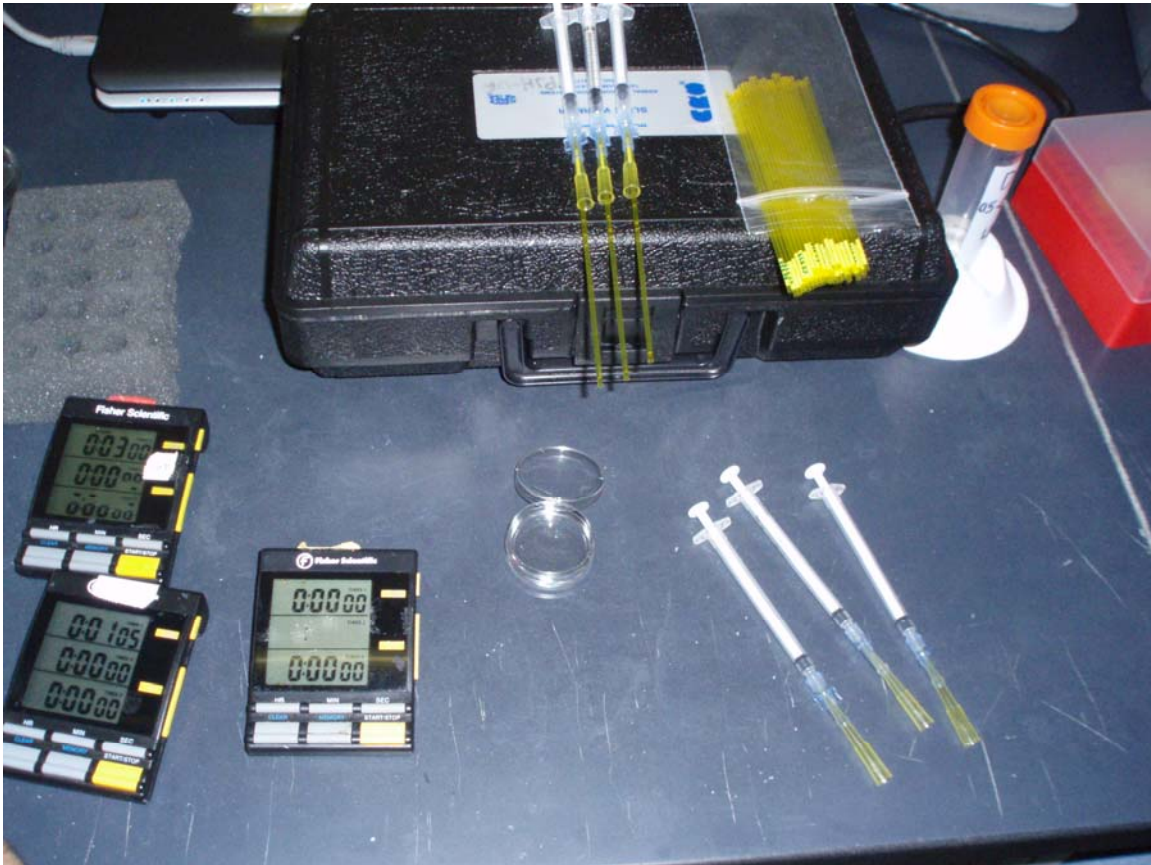
The following protocol was based on procedures from Dr. George E. Seidel, Colorado State University.

- **Media Preparation** (note all media are sterile-filtered)
 - **Medium H (base medium):** Syngro® ([Bioniche Animal Health](#); available from [Agtech](#)) + 0.1% (w/v) polyvinyl alcohol
 - **Freezing Medium:** base medium + 1.5 M Glycerol + 0.1 M Sucrose. Add 8.9 ml of base medium to a beaker with a stir bar. Add 1.1 ml glycerol and 0.342 g sucrose to the beaker and stir with mild heat (~35 C) on a stirring plate.
 - **Galactose Medium:** base medium + 0.5 M Galactose. Add 3.6 g galactose and 30 ml H Medium to a beaker with a stir bar. After galactose is dissolved, add base medium to bring volume to 40 ml.
- **Freezing Procedure**
 - Transfer the embryos to 0.5 ml Medium H in a 4-well plate for 10-20 min.
 - Move the embryos to 25 µl microdrops of Freezing Medium covered with mineral oil.
 - Load embryos into 0.25 ml straws as indicated in the diagram below with 7.5 cm Galactose Medium followed by 0.5 cm air, 1 cm of Freezing Medium containing 1-30 embryos, 0.5 cm air, and Galactose Medium 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).*



- After 10 min at room temperature, place the straws into a freezing machine set at -6°C.

- After 2 to 3 min, seed each straw by grasping the straw with a forceps dipped in liquid nitrogen at the air bubble; make sure that the seed forms by checking for ice about a minute later.
 - After 10 min, start the freezing machine with a cooling rate of 0.5 °C/min until straws reach -32 °C.
 - No later than 3 min after straws reach -32 °C, plunge straws into liquid nitrogen.
- **Thawing Procedure**
 - Thaw the straws in air for 8 seconds, and then 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 μM 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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