

SEMEN & EMBRYO HANDLING GUIDELINES.



Proper handling of semen and embryos is critical to the success of *in vitro* fertilization (IVF). Frozen semen and embryos are very sensitive to temperature variations and should always be handled in a time sensitive manner. If semen quality is poor, more units may be needed to complete IVF. In addition, embryos are biological organisms frozen under specific protocols to avoid crystallization. Any time semen and embryos are handled, crystal formation can occur which damages the cells and decreases pregnancy rates. Semen and embryos need to be carefully handled to ensure reproductive success in your herd.

Handling Semen & Embryo Canes

Semen and embryos are stored in aluminum canes with plastic goblets, which are held in liquid nitrogen tanks for preservation. Cane tops can be read by raising canisters into the tank neck while remaining below the frost line—approximately four inches below the tank neck. If you have trouble reading cane tops, lower the canister back into the tank for a few seconds and start over. Tank canisters should not be held in the neck of the tank for more than 8 seconds as time beyond this damages semen or embryo viability. **Never remove and then return a cane or goblet from the tank to read information. DO NOT use fingers under any circumstance** to remove canes or straws from a tank. Not only can liquid nitrogen burn skin, touching straws with skin induces thawing and crystal formation. Tweezers should be used to reach below the frost line and pick out straws of choice. Always avoid exposing semen and embryos to direct sunlight and wind.

When transferring canes between tanks, 2 people should be present to raise and lower canisters to decrease exposure time and complete the move within 2 seconds. Embryos and some semen are frozen in ¼ cc straws, which have higher sensitivity to temperature fluctuations than ½ cc straws. Never remove a straw from a goblet for any period of time and then return to liquid nitrogen. If a cane of semen or embryos needs to be split, both canes need to be submersed in liquid nitrogen, utilizing tweezers to transfer straws below the liquid nitrogen.

Head of Cane



Record Keeping

Keep an accurate inventory of semen and embryo location in the tank to minimize time spent searching for canes. Record the canister number, cane top information and cane tab color to quickly identified semen or embryos. All direct transfer embryos should have yellow cane tabs with a practitioner freeze code (Vytelle's code is 2103), cane code and donor ID/mating.



Tank Maintenance

Liquid nitrogen tanks are designed to maintain semen and embryos at -320°F . The level of liquid nitrogen can be checked on a regular basis with a plastic ruler to measure evaporation. Tanks regularly lose 2 cm per week and should be filled four times per year depending on the size of the tank. If tanks are opened frequently, they may need to be filled more often. Do not let tanks get below 10 cm of liquid nitrogen. Check for vapor and frost line every time the tank is opened. If no vapor is visible or there is frost on the outside of the tank, the seal or vacuum may be damaged and straws should be transferred to a new tank immediately.

Dry Shipper Tanks

All dry shipper tanks should be charged with liquid nitrogen before use. To charge a dry shipper tank, remove the tank from the mushroom, take the lid off and remove the canister. Record empty weight of the tank. Fill the tank full of liquid nitrogen. If the tank is warm, it will bubble and begin to evaporate. Once full, replace the lid and check the tank in 30 minutes. Fill with liquid nitrogen 2 to 3 times over the day to allow it to fully charge. Let the tank sit for 24 hours. After 24 hours, pour excess liquid nitrogen out of the tank and record charged weight. Let sit for another 24 hours and record weight again. Calculate evaporation and hold time. Return canister and place canes in tank for shipment. Shipper tanks should maintain a safe temperature, between -185°C and -130°C , for 7 days. Shippers should be mailed early in the week to avoid sitting in transit over the weekend. Always remove semen and embryos from a shipper immediately upon arrival and store in a full liquid nitrogen tank.

Tank storage

Liquid nitrogen tanks should be stored off floors on a stable platform that will not scratch the bottom of the tank or allow it to tip over. Wooden platforms are better than metal or cement, which accumulate moisture or cause abrasions, respectively. Tank storage rooms should be well lit and ventilated, with low traffic and a safe exit.

Semen and embryo handling is vital for a successful IVF program. Along with management of donors and recipients, embryos must be handled delicately from the time they leave the lab to on farm transfer, aiding successful pregnancy results. Take time to watch the following videos on transferring canes and semen handling to learn more on proper protocols.

Semen Handling Procedure: <https://bit.ly/2WdG6W4>

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