

# Is it Time to Revisit Your Cryotanks amidst the COVID-19 Pandemic? An Embryologist's Perspective

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## ABSTRACT

**Aim:** To present options for management of errors resulting from the cryopreservation of large volumes of semen samples and long freeze-to-thaw intervals.

**Background:** The cryopreservation of gametes is on the rise owing to its established safety and efficiency.

**Case description:** We present a challenging case at our center of a lost semen vial due to a broken cryocane and a systematic approach to locating it.

**Conclusion:** Systematic troubleshooting can help locating misplaced gamete samples in high-volume centers.

**Clinical significance:** New guidelines for containment systems is the need of the hour.

**Keywords:** Case report, Cryocane, Cryopreservation, Cryotanks, Cryovials, Liquid nitrogen.

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## BACKGROUND

The cryopreservation of gametes is on the rise owing to its established safety and efficiency. Nevertheless, errors may occur at multiple levels at high-volume centers, especially due to a large number of semen samples being frozen and long freeze-to-thaw intervals. In addition, major challenges such as specimen loss due to equipment failure have been reported in the literature.<sup>1</sup> We present one such challenging case at our center.

## CASE DESCRIPTION

The husband of a 26-year-old female suffering from carcinoma of the rectum was referred to our center for semen cryopreservation prior to chemoradiotherapy. A total of six vials with mild oligoasthenoteratozoospermia (WHO 2010) were cryopreserved. The wife's fertility parameters were within normal limits. After three years of completion of the gonadotoxic therapy, the couple was counseled for intrauterine insemination. Once follicular rupture was confirmed, the embryologist was instructed to thaw one frozen vial. To our horror, the cryocane containing the vial was amiss from its storage location. Amidst the numerous frozen samples, a systematic re-exploration was performed. Each canister was checked, sleeve by sleeve, to rule out manual errors of placement, but to no respite.

Finally, a decision to empty the entire cryotank was made. After appropriately labeling each canister, they were emptied serially in a big thermocol box filled with liquid nitrogen. Now, we needed to deal with the emptying of the liquid nitrogen of the cryotank without missing the cryocane containing the cryovials in the fog of the liquid nitrogen. We, therefore, created a sequence of emptying the liquid nitrogen from the cryotank to the thermocol box, to a spare cryotank, and then back to the thermocol via a funnel (Fig. 1). At the end of the tank, ice crystals started pouring into the funnel. Once the vapor evaporated and liquid nitrogen drained into the thermocol box, we found our lost semen samples stuck to the ice crystals. One sample was also found in the nozzle of the funnel. On complete inversion and shaking the cryotank to pour out all the ice

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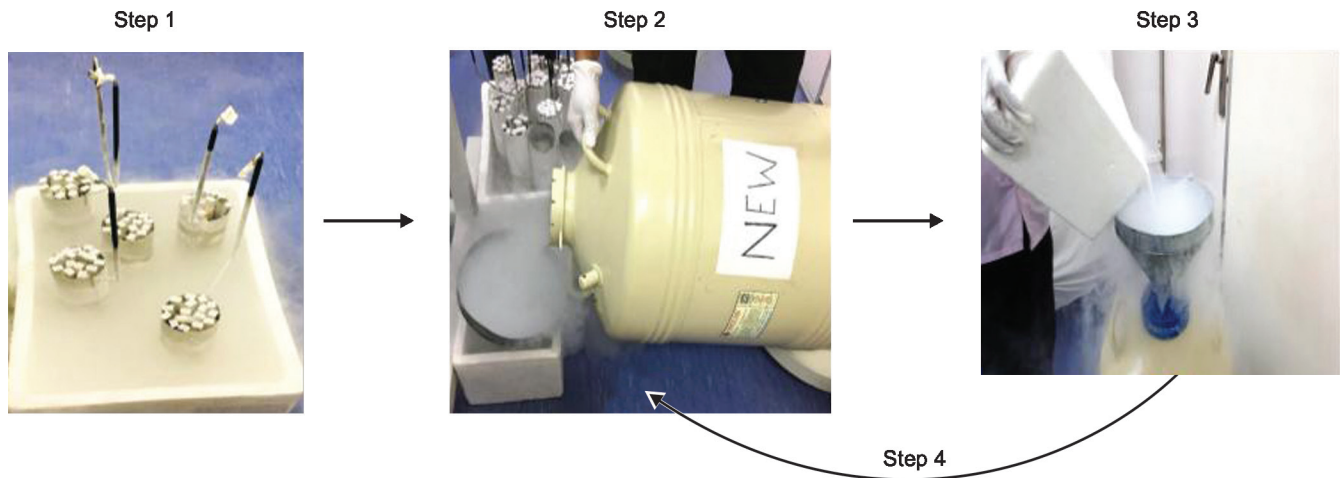
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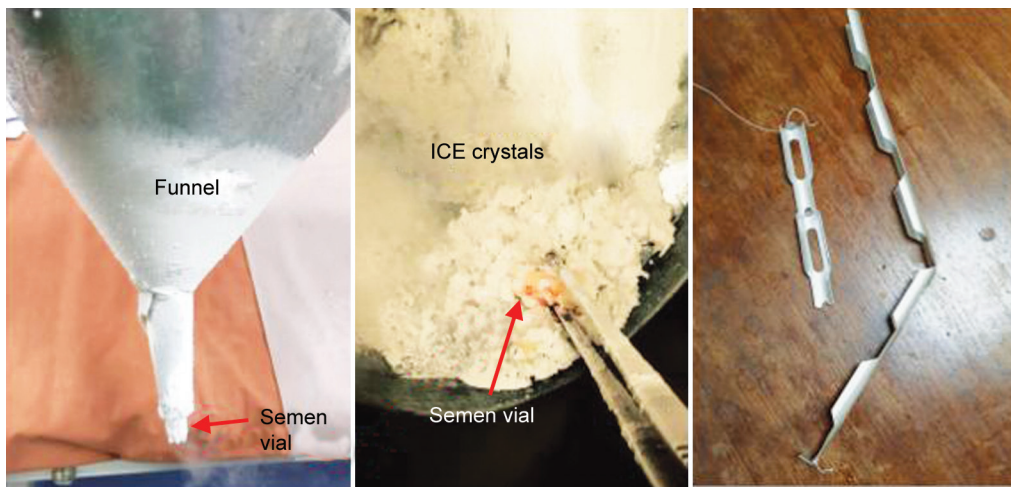
crystals, a rattling sound was heard. We finally found the cryocane that turned out to be bent and broken (Fig. 2). The canisters were transferred without delay from the thermocol box back into their respective place within the cryotank, cross verifying their identity, and the cryotank was refilled with liquid nitrogen.

## DISCUSSION

The liquid nitrogen cryotank is most commonly used in every reproductive clinic for cryopreservation also known as Dewar after its inventor, James Dewar (1892). The cryopreserved gametes are suspended inside the tank, the walls of which are insulated to contain liquid nitrogen at  $-196^{\circ}\text{C}$ . Small capacity tanks are made of aluminum, while large ones are constructed of steel. Cryostorage failures can be overcome by regular inspections done by experienced embryologists. A full-proof system must also be kept in place by clinics concerned to help avoid mishaps into their systems.<sup>2</sup> For this purpose, the exact storage location and maintenance of records in terms of tank name and number, canister number and location (column/row/level), and unique can ID are mandatory. Optimum quality control is essential for any quality maintenance system that must be supervised by senior staff within the system. A strict adherence to a standard operating



**Fig. 1:** Step 1: Labeled canisters placed inside the thermocol box filled with liquid nitrogen; Step 2: Emptying of liquid nitrogen from the cryotank into the thermocol box via a funnel; Step 3: Pouring of collected liquid nitrogen from the thermocol box into an empty cryotank using a funnel; Step 4: Repeating Step 2 and Step 3 until the lost samples were found



**Fig. 2:** Found semen samples at the funnel nozzle and embedded within the ice crystals along with broken and bent cryocane

procedure based on individual center variation to verify entries like date, name, and location of storage is necessary. In addition, an internal audit enables regular checks on ongoing systems with improvements of preexistent flaws.<sup>3</sup> Certain weak links in the system include the containment system (cryocane and cryovials/straws) that needs to be addressed. High-quality cryostorage canes are made from lightweight aluminum and are designed with slots to firmly hold cryovials in place. Liquid nitrogen transmits infective agents from one sample to the other if they are not sealed properly. Closed systems can, therefore, prevent the former. Accidents like breaking or bending of cryocanes could also be caused by mishandling of Dewar while in the routine process of re-filling of liquid nitrogen. To prevent these, access should be limited to only a few authorized and qualified members of staff within the clinic. Published evidence reveals that cross-contamination through accidental lid opening of seropositive cryopreserved samples can be prevented through isolation of these samples in dedicated cryotanks.<sup>3</sup> Aluminum canes have been time-tested for their use in cryogenic conditions.<sup>4</sup> However,

we hypothesize that company manufacturing flaws for certain pieces of cryocane increase in brittleness over time, and inability to bear the load with prolonged storage could be potential reasons for such an adverse event. No guideline for containment systems is in place which forms the limitation of this case report.

### CONCLUSION

Challenges with cryostorage are commonly faced but under-reported by most fertility laboratories. Of these, broken and bent cryocanes have not been published till date as per the author's knowledge. The risk management of storage devices is an important area to be addressed in terms of sample loss. A systematic approach while finding a lost semen vial can guide embryologists and clinicians in such situations.

### CLINICAL SIGNIFICANCE

Even though guidelines describing cryotank failures and disasters are in place, the containment system is entirely overlooked. Hence,

high-load centers must revisit their cryocanes from time to time under supervision.

## ACKNOWLEDGMENT

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## REFERENCES

1. Alikani M. Cryostorage of human gametes and embryos: a reckoning. *Reprod Biomed Online* 2018;37(1):1–3. DOI: 10.1016/j.rbmo.2018.05.004.
2. Pomeroy KO, Reed ML, LoManto B, et al. Cryostorage tank failures: temperature and volume loss over time after induced failure by removal of insulative vacuum. *J Assist Reprod Genet* 2019;36(11):2271–2278. DOI: 10.1007/s10815-019-01597-5.
3. Schiewe MC, Freeman M, Whitney JB, et al. Comprehensive assessment of cryogenic storage risk and quality management concerns: best practice guidelines for ART labs. *J Assist Reprod Genet* 2019;36(1):5–14. DOI: 10.1007/s10815-018-1310-6.
4. Kotyk M, Boroński D, Maćkowiak P. The influence of cryogenic conditions on the process of AA2519 aluminum alloy cracking. *Materials* 2020;13(7):1555. DOI: 10.3390/ma13071555.