CFAS Guidance document on cryo-storage in assisted reproduction

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Members of the cryo-storage sub-committee

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Introduction

This guidance document relates to the cryo storage of assisted reproductive material including gametes, embryos, and ovarian and testicular tissue. It is not intended to replace existing standards such as those published by the Canadian Standards Association (CSA) as well as standards and regulations enforced by Health Canada and Provincial medical colleges. This document does not cover related subjects such as cryo sample identification or infectious disease control in cryo storage, which are well described elsewhere.

All clinics should carry out risk assessments within their clinic for all procedures related to cryo storage and the handling of cryo stored materials in order to minimize the potential of cryo storage failures. However, it is important to note that even if best practices are adhered to the occurrence of the catastrophic failure of a liquid nitrogen storage tank cannot be totally prevented.

1.0 Personnel safety

1.1 Any area containing cryo storage tanks and liquid nitrogen must have appropriate signage according to WHMIS/GDS regulations.

1.2 All staff working within an area containing liquid nitrogen and/or handling liquid nitrogen and cryopreserved samples stored in liquid nitrogen must be adequately trained in the associated dangers that liquid nitrogen can present.

1.3 No individual should work alone within an area containing liquid nitrogen.

1.4 Appropriate safety equipment including cryo-gloves, goggles or face masks, and aprons must be supplied and used at all times. Open-toed footwear must not be worn when working with liquid nitrogen.

1.5 An oxygen depletion sensor must be installed in all areas with the presence of liquid nitrogen at a maximum of 4-ft above the floor. The oxygen sensor must be calibrated taking into account the altitude of the cryobank (since the pO_2 decreases at higher

altitudes) and maintained by the manufacturer or designated supplier. The oxygen sensor should have both audible and visual annunciators both inside and outside the room.

1.6 In spaces with significant volumes of LN_2 a system of extraction ventilation must be installed at floor level since cold nitrogen vapor is heavier than air.

2.0 Alarm systems

2.1 All cryo storage tanks must have at least one sensor – either for temperature or nitrogen level and it is preferable that both temperature and nitrogen level sensors should be used simultaneously.

2.2 Temperature sensors should be located so that in the event of a temperature increase, the alarm is triggered before the samples are compromised. For example, sensors should be placed above the sample level and set to alarm at -160°C or warmer giving time to react to an alarm.

2.3 Alarm systems must be connected to an external call out system. This system may include the central alarm monitoring company or a direct call-out system. The call-out system should not use one of the clinics exchange or VOIP telephone lines and must be a direct telephone company line to ensure permanent call-out ability.

2.4 Alarm sensors must be checked on a regular basis with alarm conditions initiated to ensure that the system functions as expected.

2.5 Users must always be aware of the manufacturer's instructions related the use of cryo alarms.

2.6 A spare tank is recommended in the event that a storage tank fails. In the absence of a spare tank, sufficient space in other tanks must be available to accommodate the samples from the failed tank. A spare tank must be empty of specimens and be maintained with liquid nitrogen however it should not be full of LN_2 to facilitate the quick transfer of canisters from the failed reservoir without the need to empty excess LN_2 from the spare tank.

3.0 Dewar maintenance

3.1 Liquid nitrogen storage tanks should be refilled at least once a week however users should be aware of the static holding time of their tanks and create refill schedules based on this information. Refilling tanks too often can limit the ability to monitor the LN_2 usage of those tanks.

3.2 Dewars must be monitored for LN_2 usage and these measurements used to create a control chart which can be used to identify the degrading performance of a tank. Any

tank that demonstrates degrading performance should be removed from service and replaced.

3.3 Tanks must be inspected regularly for signs of damage and potential indicators of imminent failure; for example, condensation around the neck of the unit indicating potential issues with the vacuum seal. Any tank demonstrating such signs should be removed from service and replaced.

3.4 Tanks need to be appropriately handled to minimize the risk of damage which could compromise their function. Staff should be aware of the manufacturer's recommendations in terms of the maintenance and handling of dewars.

3.5 Tanks that are self-filling need to be verified on a regular basis to ensure that the self-fill function is fully operational and that any built-in alarm systems are functional.

3.6 In terms of the lifespan of liquid nitrogen tanks, users should be able to demonstrate the continued normal performance of a tank. A carefully monitored tank which demonstrates normal performance should be suitable for use and its age being a secondary factor. Users should also be aware of manufacturer's warranties however these do not indicate the working lifespan of a tank. The correct maintenance and performance assessment of a tank is more important than relying on age alone. Users should also be aware of any insurance imposed limitations on tank age that may apply in your facility.

4.0 Storage practices

4.1 Cryo stored specimens must be maintained without fail below -132°C; the ice transition point of water. At temperatures higher than this, irreversible ice crystal growth will occur even within still frozen aqueous material (Meryman, 1957).

4.2 Clinics should perform risk assessment to establish whether regular auditing of cryo stored samples is appropriate. There are significant risks to the integrity of the cryopreserved material associated with physical auditing of cryopreserved samples. Clinics may decide to only audit their storage tanks if indicated by evidence of a significant discrepancy between the cryobank records and the specimens in storage (for example, if there is more than a predetermined number of discrepancies in their storage records within a given period of time).

References

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Mortimer D, Björndahl L, Barratt CLR, Castilla JA, Menkveld R, Kvist U, Alvarez JG, Haugen TB. *A Practical Guide to Basic Laboratory Andrology, 2nd edition.* Cambridge University Press, Cambridge (UK); in preparation, expected publication late 2021.