Summary

Nowadays human cells and tissue vitrification is usually performed by two different techniques: closed systems vitrification, by which any direct contact between cells/tissue and liquid nitrogen is avoided and open systems vitrification which shows a high efficiency when working with oocytes (1) and ovarian tissue (2). With open systems vitrification, as with the so called single-straw-closed-systems, you can’t totally exclude any risk of contamination of the culture medium which may occur during the warming procedure, when carriers outer surface and liquid nitrogen are in direct contact if, by any means, the latter was accidentally contaminated (3,4). This potential contamination is the real reason as to why it’s absolutely necessary to guarantee liquid nitrogen sterility while carrying out vitrification procedures, especially in Europe where strict European Directives regulate cells and tissues procurement and processing (European Union technical requirements for the donation, procurement and testing of human tissues and cells: 2004/23/EC, 2006/17/EC and 2006/86/EC).

It should be noted that, while most embryologist agree that open carrier vitrification using non-sterile liquid nitrogen is a safe procedure and that pathogens cross-contamination is a negligible risk (5, 6), international regulations demand totally safe procedures for embryos/oocytes/ovarian tissue cryopreservation.

For this very reason the affirmation of an “open systems” aseptic vitrification protocol, which would prevent any hypothetical risk of contamination, could be feasible not only for human oocytes/ovarian tissue but also for other tissues and even whole organs.

With open systems this could be achieved sterilizing the liquid nitrogen used for the vitrification procedure by UV irradiation, and afterwards by cryostoring carriers inside sterile vapor phase LN2-filled hermetical containers (7,8).

It’s been recently demonstrated that this procedure is indeed safe and doesn’t affect oocyte development (4). This finding could induce producers into specific hermetical containers R&D for most carrier type cryostorage.

In this video we’re showing a really simple procedure set up in our labs in order to create hermetrical goblets using disposables usually marketed for human cells cryostorage, which are:
- cryoflex: a polyethylene tube specifically designed for cryopreservation in LN2 (Nunc, Roskilde, Denmark)
- plastic visotubes for cryopreservation (Cryo Bio System, L’Aigle, France)
- stainless steel weights.

Hermetrical goblets have to be prepared in a sterile setup (i.e. IVF hood) following these steps (square brackets indicate video time):
1) [not shown] - cut the Cryoflex to obtain a pipe of 25 cm (10 in) in length (Figure 1);
2) [0’09’’] decontamination; disinfect the out-
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er and the inner (with a cotton swab) surface of Cryoflex by wiping with ethanol for at least 5 minutes (9) (Figure 2);
3) [0’30"] - stainless steel weight; put a sterilized stainless steel weight inside the Cryoflex in order to prevent it from floating during cryostorage (Figure 3);
4) [0’43"] - labelled goblet; put a sterilized coloured visotube (labelled with patient/specimen’s code) inside the Cryoflex [0’55"] - heat seal one end of the Cryoflex with a heat gun, according to manufacturer’s instructions for use (http://www.nuncbrand.com) (Figure 4);
5) [1’18"] - plunge the goblet in sterile LN2; pre-cool for at least 10 min (4) the Cryoflex by submerging it in sterilized LN2, taking care to keep it vertical in order to prevent the infiltration of LN2 (Figure 5);
6) [1’28"] - vitrification; plunge the open carrier in sterile LN2 (Figure 6);
7) [1’44"] - close the open carrier (Figure 7);
8) [1’49"] - enclose the carrier into the goblet; after vitrifying the cells/tissue in sterilized LN2, insert the carriers in the visotube, taking care to keep the strip/palette/hook/hemistraw/single-straw/etc. containing the cells/tissue in the nitrogen vapour phase above the LN2 (4) (Figure 8);
9) [1’52"] - heat seal the goblet; heat seal the remaining open end of the Cryoflex and store this hermetical goblet containing the carriers in a LN2 cryobank, avoiding any potentially dangerous temperature shock (10): the goblet should always be kept below -180°C, which seems to be the safest threshold for vitrified human oocytes (11) (Figure 9);
10) [2’52"] - cryostorage (Figure 10);
11) [3’06"] - warming procedure (Figure 11);
12) [3’24"] - cut the Cryoflex (Figure 12).

We really hope that the procedure for vapor phase-LN₂ hermetical goblets preparation could be useful for our colleagues and that producers will upgrade their carriers for open vitrification to guarantee a safe hermetic storage in the upcoming future.

KEY WORDS: vitrification, embryos, oocytes, sterile liquid nitrogen, hermetical goblets.
Figure 4 - Labelled goblet; put a sterilized coloured viso-tube (labelled with patient/specimen’s code) inside the cryoflex - heat seal one end of the cryoflex with a heat gun, according to manufacturer’s instructions for use (http://www.nuncbrand.com).

Figure 5 - Plunge the goblet in sterile LN2; pre-cool for at least 10 min (4) the cryoflex by submerging it in sterilized LN2, taking care to keep it vertical in order to prevent the infiltration of LN2.

Figure 6 - Vitrification; plunge the open carrier in sterile LN2.

Figure 7 - Close the open carrier.

Figure 8 - Enclose the carrier into the goblet; after vitrifying the cells/tissue in sterilized LN2, insert the carriers in the visotube, taking care to keep the strip/palette/hook/hemistraw/single-straw/etc. containing the cells/tissue in the nitrogen vapour phase above the LN2 (4).

Figure 9 - Heat seal the goblet; heat seal the remaining open end of the cryoflex and store this hermetrical goblet containing the carriers in a LN2 cryobank, avoiding any potentially dangerous temperature shock (10): the goblet should always be kept below -180°C, which seems to be the safest threshold for vitrified human oocytes (11).
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References


