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PERSPECTIVE

RECENT ADVANCEMENTS IN VITRIFICATION CRYODEVICES FOR GAMETE AND GONADAL TISSUE

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ABSTRACT

Cryopreservation of gametes and gonadal tissue is nowadays primarily accomplished through vitrification. Variables such as cooling rate, viscosity and volume of vitrification solution are critical in gamete vitrification. In addition, sample size and stepwise exposure are also crucial for gonadal tissue vitrification. Recently a class of cryodevices has been developed to reduce the volume of vitrification solution so as to achieve higher cooling rates. Vitrification devices are classified as "open" or "closed" depending on whether the medium comes into direct contact with liquid nitrogen during the process. Examples of the open cryodevices for gamete vitrification are Cryotop, Cryolock, open pulled straw (OPS), etc., and closed devices are Vitrisafe, CryoTip, and high security vitrification kit. Similarly, for tissue vitrification open cryodevices used are needles, cryovials and closed devices used are Cryotissue, ovarian tissue cryosystem, etc. Among all the gamete cryodevices, Cryotop is unique and the best-selling micro-volume storage device. Use of this device has resulted in the highest number of babies born after embryo or oocyte vitrification. Another novel device, Kitasato vitrification system, is a vitrification solution absorber, which is similar to Cryotop but differs in one way, as it possesses a porous membrane that absorbs extra solution from the gamete. This review provides an update on the recent use of cryodevices for gamete and gonadal tissue vitrification.

Keywords: closed system; cryodevices; CryoLoop; CryoTip; Cryotop; Kitasato vitrification system.

INTRODUCTION

The term vitrification is derived from the Latin word 'vitreum' meaning 'glass'. It describes the process of cryopreservation using high initial concentrations of cryoprotectants and ultra-rapid cooling to solidify the cell into a glass-like state without the formation of ice (1). Since the birth of the first baby from a frozen embryo in 1983 and the first pregnancy from a frozen oocyte in 1986, interest in cryopreservation techniques for gamete and gonadal tissue has grown tremendously. Over the past 30 years, two main techniques of cryopreservation have been used in clinical practice; namely, slow freezing (SF) and vitrification. Due to better success rates, SF has

been replaced by vitrification in most parts of the world for the cryopreservation of embryos, oocytes and, in certain circumstances, for ovarian tissue (2, 3, 4) and testicular tissue (5).

The idea of cryopreserving cells within a volume rendered glassy by rapid cooling is generally credited to Luyet (6). The first cell to be vitrified was a human RBC that was cooled to LN_2 temperature (-196°C) without ice formation (7). Vitrification is based on the partial dehydration principle, effectively suspending biological activity of cells or gonadal tissues whilst preserving their functional status by exposure to liquid nitrogen at -196°C (8). Unlike SF, vitrification permits the cell(s) and extracellular environment to solidify into a glass-like condition without the creation of ice. A reduction in water content and a highly viscous cytoplasm are required for successful vitrification (9). This is achieved by exposing cells to high concentrations of penetrating and non-permeating cryoprotectants, resulting in significant cellular shrinkage. The exposure length is shortened to as little as one minute to reduce the influence of the hyperosmotic stress (10). The major risk associated with high concentrations of a single permeating cryoprotectant are cell lethality (11) or impaired development (12). The risk can be addressed by combining multiple cryoprotectants (13) to reduce individual cryoprotectant toxicity while achieving a highly viscous solution (11).

In practice, vitrification is accomplished by exposing oocytes, embryos or tissues to appropriate concentrations of permeating cryoprotectants for a short period of time (1 min) before loading onto a variety of carriers (CryoTop, Cryoloop, Hemi straw, and Flexipet) in a very small to small (0.1–2.0 mL) volume to facilitate the rapid cooling process. Rapid reduction in temperature (>10,000°C/min) is achieved by immediate exposure to LN_2, in either an open or closed system. Equivalent rapid warming is also required, which is followed by stepwise dilution of cryoprotectant solutions and rehydration.

The main factors that influence the probability for vitrification (P_{Vit}) to occur are:

1. sample volume - the lower the volume, the better heat transfer, higher cooling rates and greater the chance of vitrification;
2. cooling rate - as the cooling rate increases, the chances for ice to grow into large crystals decreases;
3. sample viscosity - the higher the viscosity of the sample, the greater the likelihood of avoiding ice crystallization (14). Thus,

$$P_{Vit} = \frac{\text{Cooling rate x Viscosity}}{\text{Volume}}$$

Additionally for tissue vitrification, important factors that affect successful vitrification are sample size, stepwise addition of the vitrification solution, and the temperature of exposure to the vitrification solution (15).

Cryodevices are designed to maximise the likelihood of vitrification by accounting for the dynamic interaction between cooling rate, cryoprotectant viscosity, tissue permeability and sample volume.

Cryodevices can be divided into two types of systems: open and closed (Table 1). These can be separated into five available groups of vitrification tools (16). Group one is open (G-1) and the other four are closed (G-2, G-3, G-4, G-5). In G-1, G-2 and G-3 there are the chances of contamination of samples during the cryopreservation procedure. Using G-4, contamination may be through the use of warm water for rewarming and during the opening of devices. Only G-5, which is a relatively rarely used cryodevice, is regarded as safe from liquid nitrogen-mediated contamination.

In the open method, embryos and oocytes are directly exposed to LN_2 to increase the cooling/ warming rates and thereby improve the efficiency of the procedure (16). However, the open technique raises concern about embryos and oocytes being exposed to further interaction and possible cross-contamination during storage. Closed devices have been developed as an option to avoid direct contact of the samples with LN_2, during vitrification and storage (16). However, all closed systems available commercially are not completely free of any possible sources of contamination (16). High microbial and fungal contamination levels in vitrification carriers immersed in LN_2 have been demonstrated (17). However, no reports of actual cross contamination of cryopreserved embryos have been published when open containers are used (18). Although microbial contamination of LN_2 has been reported, there is no evidence that an embryo was contaminated by direct contact with LN_2 (19). Nonetheless, to ensure biosafety during cryopreservation, it is always advisable to use sterile approaches provided there is no compromise on adequate cooling and particularly warming rates. Furthermore, in closed systems, a

Table 1. Classification of cryodevices, with examples.

Cryodevice type	Parameters		Examples
	Cooling/ Storage	Cooling & warming rate	
Fully open system, G-1.	Direct contact with liquid nitrogen.	Extremely high.	OPS, Cryotop, CryoTech, Cryolock, Cryoleaf, Vitri-Onga with unsealed protective straw; and CryoLoop in cryotubes.
Open cooling & closed storage, G-2.	Direct contact with LN_2 during cooling but not during storage.	Extremely high.	OPS, Cryotop, CryoTech, Cryolock, Cryoleaf, Vitri-Inga with unsealed protective straw & CryoLoop in cryotubes.
Semi-closed cooling, G-3.	No direct contact with LN_2 during cooling. But presence of nitrogen vapour is unavoidable.	Cooling rates high on metal surface but compromised in vapour cooling. Warming rate high.	(Cryohook; CryoLogic) and solid surface vitrification.
Closed thin walled narrow capillaries, G-4.	No direct contact with LN_2 because device is heat sealed before cooling and opened only after warming.	Decreased cooling and warming rates.	CryoTip, Cryopette.
Carrier tools sealed into container, G-5.	Devices sealed into container and remain separated during cooling, storage, warming.	Compromised cooling rates.	VitriSafe, High Security Vitrification (HSV) kit.

slower cooling/warming rate can affect survival and success rates.

In this review we describe a wide range of cryodevices and reveal their clinical evaluation (outcomes) for various gamete and gonadal tissue systems (Tables 2 and 3).

CRYODEVICES COMPENDIUM

CryoLoop™

CryoLoop is a small nylon loop (0.7-1.0 mm diameter) mounted on a stainless steel handle. The sample is placed directly on the loop, which is coated with a thin layer of cryoprotectant solution film and then immersed in LN_2. Specimens on the CryoLoop are placed into a cryovial, which is submerged and filled with liquid nitrogen before being sealed, enabling very rapid cooling (20).

Vitrification of oocytes using the CryoLoop has advantages over traditional vitrification processes in that the open system, which lacks any type of thermo insulating layer, combined with the tiny volume of less than 1 µL, allows for rapid and uniform heat exchange during cooling (21). This approach can produce cooling rates of up to 700,000°C/min with a loading volume of 0.1 µL (20). Another benefit is the chance to inspect the gamete or embryo at each stage of the procedure (22). The CryoLoop device is used for the vitrification of sperm, oocytes, and embryos. Studies demonstrate that both mouse and human blastocysts can be successfully vitrified by suspension on a nylon loop and immersing directly into LN_2 (23). Tests on rabbit oocytes showed a good survival rate (approximately of 80%) for four different protocols. Studies have shown 74% fertilization using the CryoLoop system (21).

Cryotop

The Cryotop system, which was developed in Japan, by Kuwayama in the Advanced Medical Research Institute of Kato Ladies Clinic was widely distributed and quickly became the best-selling micro-volume storage device (24). The Cryotop concept is based on the idea that reducing the volume of the vitrification solution accelerates cooling and warming rates while lowering the risk of ice crystal nucleation and growth. Cryotop is a unique vitrification container that uses a narrow, thin film strip coupled to a hard plastic handle to achieve high

cooling (23,000°C/min) and warming (42,100°C/min) rates with minimal volume cooling. The sample (<0.1 µL) is loaded with a glass capillary on top of the film strip. Then, the CPA is removed, leaving behind a thin layer adequate to cover the cells to be vitrified. The Cryotop vitrification method has been applied successfully in various areas of animal cell technology. It is also the process that has resulted in the highest number of babies born after vitrification of human embryos and after cryopreservation of human oocytes worldwide (23).

O***pen pulled straw (OPS)***

Open pulled straw (OPS) was designed to ensure ultra-quick freezing without the growth of ice crystals. The technique, developed by Vajta (25), is based on the concept that by reducing the conventional straw diameter, the volume of solution to vitrify is reduced as well, resulting in a faster cooling rate. A mini straw is heat softened and pulled to decrease the inner diameter from 1.7 mm to 0.8 mm and wall diameter from 1.7 mm to 0.8 mm. Several OPS have been developed reducing the diameter of standard straws to a half, increasing the cooling rate by 10 times, and reducing by 30% the concentration of

Table 2. Evaluation of various cryodevices for the successful cryopreservation of disparate tissue systems: chronology and outcomes.

Device	Species	Application	Outcome	Year (ref)
	Mouse.	Blastocyst vitrification.	Re-expansion rate was 100%, hatching rate was 95.5% and attachment rate was 85.9%.	1999 (67)
CryoLoop.	Human.	Blastocyst vitrification.	Re-expansion rate was 83.3%, hatching rate was 73.3% and attachment rate was 60%.	1999 (67)
	Human.	Blastocyst vitrification.	Survival rate was 79% and pregnancy rate was 36%.	2003 (68)
Cryotop.	Mouse.	Oocyte vitrification.	Fertilization rate of in vitro matured M-II oocytes was 44.1% as compared to 50% for ovulated mice oocytes.	2015 (69)
	Humans.	Oocyte vitrification.	Survival rate was 99.4%, fertilization rate was 92.9%, pregnancy rate was 32.5% and implantation rate per embryo was 13.2%.	2007 (70)
OPS.	Bovine.	Oocyte vitrification.	Blastocyst development rate was 25%.	1998 (22)
CryoTech.	Bovine.	In vitro produced blastocyst vitrification.	Survival rate was 100% and pregnancy rate of in vitro produced blastocysts was 46.8%, similar to that obtained with fresh in vitro produced blastocysts.	2013 (71)
Cryolock.	Human.	In vitro produced blastocyst vitrification.	Blastocyst survival rate after warming was 95% (38/40); 36 embryos were transferred, leading to a pregnancy rate of 80% and implantation rate of 53%.	2007 (72)
McGill CryoLeaf.	Human.	Oocyte vitrification.	Survival rate (88.5%) and intact cell rate (74.5%) was higher than by slow freezing (74.5% and 64%, respectively).	2012 (73)
Vitri-Inga.	Human.	Oocyte vitrification.	Survival rate of vitrified oocytes was 84.9%. Fertilization, pregnancy and implantation rates were 80.8%, 45.7%, and 14.9%, respectively, and showed no significant differences between the fresh and vitrified/ thawed groups. Only the number of blastomeres was lower in the vitrified-thawed group than in the fresh oocyte group.	2010 (74)

Table 2 (continued). Evaluation of various cryodevices for the successful cryopreservation of disparate tissue systems: chronology and outcomes.

Device	Species	Application	Outcome	Year (Ref)
Kitazato vitrification system.	Mouse.	Blastocyst vitrification.	Re-expansion and hatching rate of vitrified-warmed blastocysts was 100% and 91.8%, respectively. Pregnancy rate was 100%.	2017 (30)
Solid surface vitrification.	Ovine.	Oocyte vitrification.	Recovery rate was 75.5%, viability rate was 74.8%. IVM rate obtained by SSV was 39.9% as compared to 64.7% in the control.	2012 (75)
CryoTip.	Human.	Vitrification of oocytes, pronuclear (PN) zygotes and blastocysts.	Survival rate for human oocytes, PN zygotes and blastocysts were 90%, 100% and 92%, respectively. Pregnancy rate after transfer of vitrified thawed blastocysts was 52%.	2005 (37)
Isachenko method.	Human.	Pronuclear oocyte vitrification.	Oocyte development up to expanded blastocyst stage after in vitro culture was 14% compared to 29% in the control group.	2005 (38)
Sareh.	Bovine.	Zygote vitrification.	Cleavage rate obtained was 54% and blastocyst rate was 9%.	2018 (46)
		Oocyte vitrification.	Cleavage rate obtained was 73% and blastocyst rate was 7%.	
Cryosupport	Porcine.	Ovarian cortex vitrification.	Proportions of morphologically normal follicles at the primordial, intermediary and primary stages were 92.2%, 84.5% and 77.2% respectively.	2013 (48)
CryoTissue	Bovine.	Ovarian cortex vitrification.	No difference was found in oocyte viability between fresh and vitrified tissue.	2009 (50)
Direct cover vitrification.	Mice.	Ovarian cortex vitrification.	Viability of follicles, i.e. primordial, primary and secondary follicles, was significantly higher (92.3%, 85.2%, 69.4%) with DCV than conventional vitrification (59.6%, 42.1%, 27.6%) and slow freezing (77.8%, 70.5%, 38.8%).	2006 (52)
Ovarian tissue cryosystem.	Canine.	Ovarian cortex vitrification.	Proportion of morphologically normal follicles at pre-antral stage was 52.1%.	2021 (76)
	Caprine.	Tissue fragment, hemi-ovary or whole ovary vitrification.	Normal follicles was 68.1% in control, 58.1% in tissue fragment, 54.5% in hemi-ovary and 54.3 in whole ovary.	2013 (51)

cryoprotectant solution needed for vitrification and the time of exposure. Loading volumes of 1-2 µL are used and the samples are loaded into the straw by capillary action by touching the open end of the straw to the vitrification solution containing the samples. The cooling and warming rates achieved with this method are estimated to be 16,700℃/min and 13,900 ℃/min respectively. This approach reduces the possibility of freezing injury and toxicity and osmotic damage (25).

CryoTech

A minimal volume approach, introduced by Dr. Kuwayama is the latest method of importance for oocyte and embryo vitrification. Using the CryoTech method of vitrification, oocyte survival

Table 3. Comparison of various cryodevices in different species

Species	Specimen	Devices compared	Outcome	Year (Ref)
Human	Blastomere vitrification	CryoTip	Survival rate was 85.41%	2011 (77)
		CryoTop	Survival rate was 38.46%	
Human	Ovarian cortical tissue	Conventional vitrification (CV)	The percentages of normal morphology of primordial, primary and secondary follicles in the CV group were (83%, 76.8%, 51.7%) & Viability of follicles with CV primordial, primary & secondary follicles was (59.6%, 42.1%, 27.6%)	2006 (52)
		DCV	The percentages of normal morphology of primordial, primary and secondary follicles in the DCV group were (95.1%, 88.8%, 74.3%) & Viability of follicles i.e. primordial, primary & secondary follicles was significantly higher (92.3%, 85.2%, 69.4%) with DCV	
Bovine	Oocytes	CryoTop	Cryotop showed 46.6% fertilization rate and 60.2% normal spindle formation	2008 (78)
		OPS	OPS showed 31.5% fertilization rate and 37.8% normal spindle formation	
		Conventional straw	Cleavage rate was 31% and the rate oocytes developing into blastocyst stage was 16%	
Ovine	Oocytes	OPS	Cleavage rate was 67% and the rate oocytes developing into blastocyst stage was 49%	2016 (79)
		CryoLoop	Cleavage rate was 81% and the rate oocytes developing into blastocyst stage was 55%	
Ovine	Oocytes	OPS	Recovery of normal oocytes after vitrification was higher in OPS (89.2%), viability was 69.11% , abnormal percentage was 8.6%.	2021 (80)
		Conventional straw	Recovery of normal oocytes after vitrification in CS was 73.6%, viability was 54.5% , abnormal percentage was 16.8%.	

of 97.1 % (26) and embryo survival of 97.3 % have been achieved (27). For secure handling and storage, this carrier device comprises a plastic handle coupled to a polypropylene strip and a protective cap. It can be used for closed cooling after sealing or for open cooling before sealing.

Cryolock

Cryolock is a square stick-shaped device, with four flat surfaces. At room temperature and at low cryogenic temperatures, both the cap and the body have the same coefficient of expansion, ensuring a secure connection. Gaps on the extremes of the body and cap allow for easy grip with forceps during manipulation. It has a concave tip, where the oocytes or embryos are placed, protecting the sample from contact with other surfaces and preventing specimen loss or damage. Its unique design allows a hermetic seal which is created by a tapered surface where the Cryolock body and cap fit in a perfect seal creating a closure system that is able to keep the

tip isolated from liquid nitrogen (LN_2) once the Cryolock is submerged into the LN_2. While Cryolock is under LN_2 the cap shrinks over the sealing area due to the low temperatures. While specimens are stored, LN_2 cannot recirculate inside the cap towards the tip or vice versa. When Cryolock is used as a Closed System, the only time the specimens are in direct contact with LN_2 is during the capping step under LN_2. It has been used for porcine oocyte maturation (28).

CryoLeaf

The McGill CryoLeaf™ is a vitrification and storage device designed for easy handling. The system is open to achieve maximum cooling rate. Safety during storage has been improved, as the vitrified cells are protected from mechanical stress and contamination by a closed cover system. CryoLeaf is an open device for embryos and oocytes vitrification and storage. It was developed by Dr. Chian and Prof. Tan at McGill University, Montreal. The recommended maximum load of the McGill CryoLeaf is 2-3 oocytes or embryos (23). The outer cover of the McGill CryoLeaf is plunged into the liquid nitrogen bath, allowing the air to come out. Vitrified oocytes or embryos are quickly loaded into the McGill CryoLeaf using a suitable pipette. The excess of medium, that must be less than 1 μL, has to be removed. The McGill CryoLeaf is inserted, with oocytes or embryos, directly into liquid nitrogen. Then, the CryoLeaf is blocked, sliding the protective sleeve over the tip (29).

Vitra-Inga

This is an apparatus that consists of a fine, very thin polypropylene strip (0.7 mm thick) with a specially designed round tip, in which there is a minute hole to receive cells. The strip is connected to a hard and thicker plastic handle. It is made from 0.5 mL midi straw having a cut in the middle. The total time from when the oocyte is placed into the vitrification solution until its submersion into LN_2 is between 50 and 60 seconds (23).

Kitasato vitrification system (KVS)

The innovative feature of KVS is that it is a vitrification solution absorber. It consists of a porous membrane that is placed on the polyethylene terephthalate film (30). The excess solution is absorbed spontaneously by the porous membrane in contrast to Cryotop, where excess solution is removed by pipetting. Cryotop's solution is 30-50 nL based on microscopic imaging, but KVS is only 1.3 nL. The cooling and warming rates of KVS are 683,000ºC and 612,000ºC/min, respectively (31)

Solid surface vitrification (SSV)

Solid surface vitrification was developed at the Department of Animal Science, University of Connecticut, USA. The method was designed for the effective cryopreservation of bovine oocytes, for research and practice of parthenogenetic activation, in vitro fertilization and nuclear transfer. A metal cube covered with aluminium foil is partially submersed into LN_2, such that the surface reaches about -150°C. Micro drops of vitrification solution, containing the oocytes, are dropped onto the cold upper surface of the metal cube and are instantaneously vitrified. The vitrified micro drops are then stored in liquid nitrogen (32, 33, 34, 35, 36).

CryoTip

CryoTip consists of a plastic straw with a thin part (250 μm inner diameter, 20 μm wall thickness and 3 cm length) connected to a thick part (2000 μm inner diameter and 150 μm wall thickness, 4.5 cm length) and equipped with a movable protective metal sleeve (37). Embryos are loaded in approximately 1 μL solution into the narrow part of the CryoTip without any air bubbles by aspiration of medium. Subsequently, the straw is heat-sealed at both ends, the protective sleeve is pulled over the narrow part and the device is plunged into LN_2. The time required for loading, sealing, adjustment of the sleeve and plunging should not exceed 90 seconds. The use of the closed CryoTip system eliminates the potential for embryo contamination during cryopreservation and storage without compromising survival and developmental rates in vitro and in vivo (37).

Isachenko method / straw in straw vitrification

In this method embryos are located inside an open-pulled straws (OPS). The OPS is placed inside a sterile insemination straw (indicative size 90-mm), manufactured from standard 0.5-mL insemination straws. One end of sterile insemination straw is previously sealed using a hand-held sealer. The open end is hermetically closed by a metal ball and this container (OPS and sterile insemination straw) is plunged into LN_2 ("straw in straw" vitrification). The Isachenko Method, applied to biopsied mouse pronuclear embryos has been found to be as efficient as

conventional vitrification, guaranteeing a complete isolation of embryos from LN_2 and avoiding potential contamination by pathogenic microorganisms (38).

Minimum drop size vitrification

This technique was developed by Arav Amir in which (0.1–0.5 µL) volumes of vitrification solution are used. The MDS technique has been successfully used to vitrify porcine (39) and bovine (40) oocytes, and bovine and sheep embryos (41). The MDS method was recently modified by depositing 0.1–0.5 µL droplets on glass coverslips followed by immersion into LN_2 or nitrogen slush. This technique was shown to maximize the cooling rates up to 130,000°C/min, allowing up to a 50% reduction in CPA concentrations compared with conventional vitrification protocols. (33, 42, 43, 44)

CryoLogic vitrification method

This is a simple and reliable process used to vitrify and thaw specimens. It has three component parts; the CVM block, and a fibreplug with a hook and a cap. The CVM™ kit overcomes many of the problems usually encountered with vitrification. It has been specially designed to provide ultra-rapid cooling of specimens without their immersion in, or direct contact with, LN_2. Specimens are collected into small droplets and each droplet is transferred onto a hook at the end of a custom designed fibre called a Fibreplug™. The droplets are vitrified by touching the hook of the Fibreplug™ to the surface of a chilled block. The CryoLogic vitrification method not only provides ultra-rapid cooling of specimens, but more importantly, ultra-rapid warming of specimens as well (45).

Sareh

This is the fully automated device for vitrification and warming of gonadal tissue slices, both ovarian and testicular, in addition to oocytes and embryos. Sareh was developed by Fertile Safe Ltd of Israel and has a simple operating LCD screen. It can vitrify/warm simultaneously up to 30 embryos/oocytes (mini straws) and up to 18 ovarian slices (maxi straws). In this device there is automatic stepwise exposure to equilibration and vitrification solutions, automatic cooling into LN_2 or LN slush, automatic warming by exposure to warming solution, automatic stepwise dilutions and washings. This device has a rotating plate with vitrification solution and liquid nitrogen and a robotic handle with straws that moves up and down (46).

TISSUE CRYODEVICES

Vitrification is now the technique of choice for oocyte and embryo cryopreservation and it is slowly gaining popularity for gonadal tissue as well. Recently, different devices such as Cryosupport, Cryotissue kit for ovarian tissue cryosystem (OTC) have been developed for gonadal tissue (47).

Cryosupport

The cryosupport consists of four fine stainless needles (length: 15 mm; diameter: 0.45 mm) and a cryogenic vial (32815, BD; Falcon, NJ, USA) (48). The tissue is loaded onto these needles which are then immersed and closed in the cryogenic vials.

Needle immersion vitrification (NIV)

A special carrier ‘acupuncture needle’ (Cloud & Dragon Medical Device Co. Ltd, China) is used in this NIV method. The acupuncture needle consists of two parts: a stainless steel filiform (thread-like) needle and a handle. The long needle can hold several ovarian tissue samples in a row and thus all the samples can be exposed to cryoprotectants and then immersed into LN_2 under the same conditions. This can maximize the cooling rate and simplify the vitrification process (49).

Cryotissue

Kagawa et al developed a vitrification device for ovarian tissue cryopreservation (Cryotissue® Kit; Kitazato BioPharma Co., Ltd., Shizuoka, Japan). It is a thin metal strip with holes. It has also a template for getting a tissue section. Transplantation of vitrified-thawed ovarian tissue was first achieved in March 2011, with high survival rate for oocytes (50).

Ovarian tissue cryosystem (OTC)

Developed by Carvello for caprine ovary vitrification, the OTC is a cylindrical structure made of stainless steel and composed of three pieces that can be hermetically closed, thus avoiding contact of the tissue with LN_2 during vitrification. OTC is a cylindrical structure composed of three structures: (i) a basal (2.1 cm height; 2.8 cm diameter and 0.2 cm thickness), in which the samples are placed; (ii) an insert

(2.8 cm height; 2.3 cm diameter and 0.1 cm thickness) containing 20 perforations to allow exposure and removal of VS, and speed up the ultra-rapid cooling which is necessary in vitrification; (iii) a cover (2.0 cm height; 2.8 cm diameter and 0.2 cm thickness) to close the device hermetically. The OTC can support temperatures lower than −196 °C and higher than 200 °C under high pressure, allowing it to be sterilized and reused (51).

***Direct cover vitrification** (DCV)*

DCV is a new cooling method based on the minimum use of concentrated cryoprotectants and direct application of LN_2 to the ovarian tissue. This way, the potential toxicity of cryoprotectants is reduced and the ice crystal injury is prevented. The ovary is put in a 1.8 mL plastic standard cryovial, placed on a piece of gauze to remove the surrounding vitrification medium. Liquid nitrogen is directly applied onto the ovary for vitrification (52). The cap of the cryovial is closed. The lid does not have a hole. The vial is then placed into a LN_2 tank. DCV vitrification of mouse ovarian tissue was shown to be highly efficient at increasing morphologically normal and viable follicles from cryopreserved ovarian tissue, when compared with slow freezing and conventional vitrification (52).

CONCLUSION

The vitrification devices rely on the achievement of very high cooling (and warming) rates for small volumes of vitrification medium that ensure a highly efficient vitrification process and better post-vitrification recovery of gametes, embryos and tissues. To attain the highest temperature conductivity between the sample containing medium and the cooling or the warming agent, the wall between the sample and the agent has been eliminated, but the chances of contamination are greater. Alternatively, devices such as VitriSafe, offer high security vitrification and limited cross contamination, but with a compromised cooling rate. In devices such as the Kitazato vitrification system, volume reduction ensures more efficient vitrification. Whilst there are a wide range of techniques and devices for ovarian tissue vitrification, there is still a need to develop more efficient and effective closed devices that can achieve faster cooling rates without LN_2 contamination. The design of various other devices for ovarian tissue vitrification – such as plastic straws (33, 53, 54, 55), cryogrids (56, 57), cryovials (58, 59), handcut straws (60), metal strips (50), Cryotops (61, 62, 63, 64) and glass tubes (65, 66) is further evidence of the ongoing evolution of cryodevices directed towards efficient, ultra-rapid vitrification of cells.

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