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Preservation of reproductive cells and tissues by vitrification

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1. INTRODUCTION

On 10 December 2009, the Superior Health Council (SHC) received a request for advice on the preservation of reproductive cells and tissues by vitrification from the Federal Agency for Medicines and Health Products (FAMHP).

The FAMHP wishes to objectivise this choice of procedure during inspections for the accreditation of banks for human body material that are linked to the programmes for assisted human reproduction.

The FAMHP therefore asks the SHC to define the criteria for this type of preservation and to integrate its advice in the quality standards for reproductive cells and tissues.

On 9 August 2010 the SHC received an additional inquiry from the FAMHP concerning the use of “open” systems for the vitrification of human oocytes and embryos: “Is it acceptable for the vitrification method to use non-sterile liquid nitrogen or should additional qualitative criteria apply to the liquid nitrogen?”

These questions were submitted to a sub-group of experts before the subject was treated by the working group “Cells, tissues and organs of human and animal origin”. The sub-working group consisted of experts in the following fields: human body material, reproductive medicine, embryology and cryobiology.

2. RECOMMENDATIONS

The SHC formulates the following answers to the questions from the FAMHP and proposes the provisional recommendations mentioned below.

This advisory report discusses the state of the art of the technology involved in the vitrification of reproductive cells.

1. Embryo and blastocyst vitrification:

In this case, vitrification is a validated and reproducible technique. There is no difference between the efficacy of vitrification in closed and open systems. However, the SHC recommends that, for safety reasons (contamination and cross-contamination), closed systems be used for both the cooling stage and for storage. There are several closed systems commercially available.

2. The vitrification of metaphase II oocytes:

2.1. Oocyte vitrification is more efficient than slow controlled-rate freezing. If the oocytes are to be preserved, the SHC recommends the use of vitrification

techniques. However, these techniques for human oocyte vitrification need to be further optimised.

- 2.2. The data from the literature that are currently available show that closed systems seem to give satisfactory results. However, their efficacy compared to that of the open systems still has to be objectivised. On a theoretical basis, the use of closed systems should be preferred. Still, as there is not enough information available to date about their efficacy, the SHC takes the view that the open systems can continue to be used for the time being.
3. Vitrification of spermatozoa and gonadal tissues:
These techniques aren't up to par yet and slow freezing remains the cryopreservation method recommended.
4. If both open and closed systems are used during vitrification and storage, the open systems should be physically separated from the closed systems during storage.
5. As regards open and closed systems that have both been used and stored in the past, mixed storage is tolerated for a five-year transitional period. If, after these five years, the aim is to continue to preserve these samples in an open system or to donate them for scientific research purposes, they should be physically separated from closed system samples.
6. If the serological data are unknown or unavailable, the vitrification systems should be preserved in quarantine banks or in well-identified systems that guarantee that there will be no cross-contamination (High Security straws).
7. Vitrification and storage in non-sterile liquid nitrogen are allowed within the framework of closed, semi-closed and open vitrification procedures.
8. The advisory report will be revised in two years and will be adapted to take into account any new data from the literature.

3. ELABORATION AND ARGUMENTATION

List of abbreviations used

ASRM	<i>American Society for Reproductive Medicine</i>
CPA	<i>Cryo-Protectant Agent</i>
DMSO	Dimethyl sulfoxide
EG	Ethylene glycol
ESHRE	<i>European Society for Human Reproduction and Embryology</i>
EU	European Union
FAMHP	Federal Agency for Medicines and Health Products
HSV	<i>High security vitrification</i>
ICSI	Intracytoplasmic sperm injection
IVF	In Vitro Fertilisation
PG	Propylene glycol
RCT	<i>Randomised controlled trial</i>
SHC	Superior Health Council

3.1 Methodology

The advisory report is based on an exhaustive review of the recent scientific literature and of the gray literature as well as on the experts' opinion.

3.2 Elaboration

3.2.1 What is currently said by the specific quality standards for reproductive cells and tissues? (SHC 8292)

Paragraph E 2.2.4 of the SHC advisory report No. 8292 (Quality standards for reproductive tissues and cells) mentions the following about cryopreservation in liquid or vapour-phase nitrogen: “preservation and storage by cryopreservation are possible both in liquid and in vapour-phase nitrogen if there is a validated protocol within the bank. As regards donations that present a contamination hazard, security measures must be set up within the bank in order to avoid any contamination (e.g. by means of High Security straws or separate storage).” It is true that all partner donations involve a potential contamination hazard (on account of their being frozen immediately rather than being put into quarantine), so that distinct containers or High Security straws need to be used anyway. The methods used for freezing (slow controlled-rate freezing or vitrification) are not mentioned anywhere.

3.2.2 Context

All forms of cryopreservation in reproductive medicine pursue the same goal, viz. stopping the biological clock. One of the main advantages of cryopreserving oocytes for in vitro fertilisation (IVF) are the better chances of obtaining results with donor oocytes. First of all, optimal cryopreservation makes it possible to provide a certain margin for the preservation of donor oocytes in the form of oocyte banks and for possible subsequent donor screening for health hazards such as hepatitis B contamination. Next, the use of cryopreserved donor oocytes also means that the donor’s and the recipient’s cycles no longer need to be synchronised. Moreover, oocyte cryopreservation can also take place in the event of there being no spermatozoa during IVF/ICSI treatment or prior to treatment that is potentially harmful to the ovaries (e.g. chemo- or radiotherapy). Cryopreservation can also contribute considerably to reducing the number of surplus embryos in current IVF practice. Finally, the possibility of oocyte cryopreservation reduces the moral/ethical load for those who object in principle against the creation of surplus embryos. For all of these reasons, highly efficient techniques for human oocyte cryopreservation are of the utmost importance.

Because of their size and chill sensitivity, the freezing of metaphase II human oocytes by means of traditional procedures for slow controlled-rate freezing and thawing remains particularly difficult. The literature clearly shows that slow controlled-rate oocyte freezing is less than optimal and involves risks (Oktay et al., 2006).

Since the introduction of IVF, embryo cryopreservation has been an integral part of any sterility treatment. A broad range of embryos can be frozen: one-celled embryos (zygote) on day 1 of the oocyte collection cycle, cleavage stage embryos on days 2 and 3 and blastocysts on days 5 and 6. Cryopreservation makes it possible to increase the chances of fertilisation on the basis of a single egg recovery procedure, thanks to the possibility it offers of carrying out one, or even several additional delayed transfers. Thus, surplus embryos can be thawed later, if the transfer of fresh embryos has proved unsuccessful. Moreover, since 2003, only single embryos have been transferred in well-defined conditions in Belgium in order to avoid an iatrogenic epidemic of twins and triplets (Kingdom of Belgium, 2003). After the transfer of the thawed embryos and blastocysts, an attempt should be made to avoid the procreation of twins and triplets. To this end, it would be preferable for a single thawed embryo to be transferred. Cryopreservation therefore provides an excellent means of avoiding multiple IVF pregnancies. Clearly, such strategies require that efficient cryopreservation programmes be available.

As regards the embryos and blastocysts, it should be noted that, after over 20 years of practice with slow controlled-rate freezing techniques and notwithstanding extensive cryogenic expertise, the chance of healthy child being born amounts to 3 to 7 % per frozen and thawed embryo and 5 to 10 % per transferred embryo, depending on the procedure used as well as the morphological quality and the stage of development of the embryo prior to freezing. Conversely the average chances of implantation after transferring fresh embryos amount to 20 % in Belgium (Belgian Register for Assisted Procreation; BELRAP). It sometimes turns out to be difficult to retrieve fully intact human embryos immediately after thawing. In addition, implantation is less likely for

embryos with cell damage than for intact embryos. As it turns out, controlled-rate freezing procedures that appear to be highly successful for embryos and blastocysts from other mammals cannot be applied quite as simply to human embryos and blastocysts. Recently, a vitrification method (minimum volume vitrification) has been introduced with very promising results for human oocytes as well as embryos and blastocysts (Kuwayama et al., 2005).

3.2.3 Why resort to vitrification ?

Two factors play an important part in the appearance of damage due to the cryopreservation of oocytes and embryos: (1) the formation of intracellular ice and (2) damage caused by the dehydration process (osmotic damage). Vitrification is a freezing method which, unlike slow freezing, does not entail the formation of ice crystals. It consists in a solution being solidified at a very low temperature. Therefore, from a theoretical point of view, vitrification causes less damage to the embryo/oocyte, because the mechanical stress, chill sensitivity and osmotic damage due to ice-crystal formation are countered. As a result, vitrifying oocytes and embryos may be expected to make cryopreservation more efficient. The methods for the vitrification of reproductive cells will then replace slow controlled-rate methods.

The cryobiology of vitrification

Vitrification is a process whereby a solution is completely transformed to a glassy, ice-crystal free state by cooling it to e.g. -196°C . This intra- and extracellular amorphous or glassy state is obtained thanks to high concentrations of cryoprotectant agents in conjunction with an extremely rapid cooling rate. Vitrifying oocytes and embryos is usually done by means of a solution of permeating and non-permeating cryoprotectant agents. The permeating cryoprotectant agents (dimethyl sulfoxide – DMSO, propylene glycol– PG, ethylene glycol – EG) protect the cells both internally and externally, whereas non-permeating cryoprotectant agents (sucrose, galactose) protect the cell through osmotic dehydration. In order for vitrification to be successful and safe, various parameters must be set correctly: the cryoprotectant concentration as well as the length and temperature of exposure, the cooling and warming rates and the carrier system used (i.e. the system used as an embryo container) (Pegg, 2005; Vajta & Nagy, 2006; Vajta & Kuwayama, 2006; Yavin & Arav, 2007). In 2005, Paynter also explained that the cryopreservation protocols to be worked out should be highly reproducible, mainly in order to avoid any variability in the results between centres and within the centres themselves. This is especially important during the vitrification of oocytes and embryos.

The term “equilibrium” vitrification is used when very high concentrations of cryoprotectant agents are used, mainly over 50 % (v/v). In equilibrium vitrification, the solidification is relatively independent of the cooling and warming rates and fairly large volumes of samples may be vitrified. However, the concentrations of cryoprotectant agents (CPA – Cryo-Protectant Agent) used are so high as to be toxic for the reproductive cells. Therefore, this vitrification technique is not being used for human gametes and embryos.

In case of non-equilibrium vitrification, the CPA concentration is reduced to non-toxic levels (30 % v/v). In these cases, very high cooling rates are absolutely essential to obtain vitrification. The higher the cooling rate, the lower the CPA concentration will be that is necessary for vitrification. The challenge posed by IVF vitrification consists in finding this equilibrium between the least toxic CPA concentrations that guarantee (extracellular and intracellular) vitrification at possible cooling and warming rates. The simplest means for obtaining realistically high cooling and warming rates is therefore to use very small volumes and plunging the sample directly into liquid nitrogen. This technique was applied for the first time by Vajta et al. (1997). He developed an Open Pulled Straw system in which samples of less than 1 μl (30 % CPA) containing oocytes or embryos could be loaded. The direct contact of the vitrification solution with the liquid nitrogen enabled him to achieve cooling and warming rates over $20,000^{\circ}\text{C}/\text{min}$, resulting in an apparent vitrification of the drop. The success of the vitrification method was also based on the correct combined action of sufficient penetration by the permeating CPAs (EG, PG, DMSO or mixtures) and sufficient dehydration by a non-permeating CPA (sucrose, trehalose, galactose). It should be noted that, because of the lower CPA concentration in the vitrification solution, both during cooling and warming, the vitrification solution has to go through a risk zone in which (intracellular and

extracellular) ice crystals may form. This “minimum volume approach” is currently the norm as regards IVF vitrification methods.

3.3 Issue

Vitrification (mainly used for oocytes and embryos) is a special freezing technique where it is possible to work in open systems (open pulled straw, Cryoloop, Cryotop, Electron Microscope grid) or in more recent closed systems (High security vitrification – (HSV) straws, Cryo-Tip™, Cryopette™, Rapid VITi™). By working in closed rather than open systems, the norms of the SHC advisory report No. 8292 are fully met. When using open systems, there may be direct contact between the tissue or the cells and the nitrogen, which can result in potential microbial and/or viral contamination (Bielanski et al., 2000; Bielanski et al., 2003). This is one of the reasons why the European directive requires that the samples be physically separated.

The controversy over open and closed vitrification systems results from contradictory views in the literature. For some, there is nothing like the (old) open systems, but in many countries, closed systems are increasingly being turned to because of concern about (cross-) contamination. In the closed vitrification methods, both the vitrification stage as well as the storage stage are closed (straw-in-straw method, Kuleshova, 2009).

Apart from the issue of the potential contamination of the open systems, the controversy over the closed and open systems is also fed by the following statements:

- without direct contact between the sample and the liquid nitrogen, the cooling rate is affected, so that vitrification is compromised (Vajta et al., 2009)
- the warming rate plays a more dominant role than the cooling rate, so that vitrification in a closed system is possible as long as warming occurs in open carriers (Seki & Mazur, 2008);
- the cooling stage can be separated from the storage stage (Vajta et al., 2009).

Conclusions

If both open and closed systems are used during vitrification and storage, the open systems should be physically separated from the closed systems during storage.

As regards open and closed systems that have both been used and stored in the past, mixed storage is tolerated for a five-year transitional period. If, after these five years, the aim is to continue to preserve these samples in an open system or to donate them for scientific research purposes, they should be physically separated from closed system samples.

If the serological data are unknown or unavailable, the vitrification systems should be preserved in quarantine banks or in well-identified systems that guarantee that there will be no cross-contamination (High Security straws).

Vitrification and storage in non-sterile liquid nitrogen are allowed within the framework of closed, semi-closed and open vitrification procedures.

3.4 What does the literature say about the efficacy of vitrification as compared with slow freezing and about the efficacy of the open and closed vitrification systems for human oocytes, embryos and blastocysts?

3.4.1 Embryos and blastocysts

In a randomised and controlled trial (RCT) as well as in various meta-analyses and reviews of the literature, it has recently been shown that the vitrification of embryos and blastocysts yields better results than slow freezing (Balaban et al., 2008; Kolibianakis et al., 2009; AbdelHafez et al., 2010).

In 2005, Kuwayama et al. compared the open Cryotop™ system with the closed Cryo-Tip™ system for blastocysts and found that the two systems provided equally good results. This is shown clearly by the following table taken from this publication:

Table 1. Survival, pregnancy and delivery rates after single embryo transfer of human blastocysts vitrified with either the Cryotop or the CryoTip method

	Cryotop™ (open)	Cryotip™ (closed)
<i>Survived/vitrified rate (%)</i>	221/227 (97)	82/88 (93)
<i>Pregnancy/transfer rate (%)</i>	131/221 (59)	42/82 (51)
<i>Delivery/transfer rate (%)</i>	113/221 (51)	39/82 (48)

No significant differences between corresponding values were found.

In a recent review, Kader et al. show very clearly that excellent results can be obtained for blastocysts with both closed and open systems (Kader et al., 2009). Below can be found a comparative table taken from this publication:

Table 2: Comparison of survival, implantation and pregnancy rates according to loading device

	Loading Device	Sample Size	Survival Rate	Implantation Rate	Pregnancy Rate
Mukaida et al., 2001	Cryoloop (open)	N = 60	63 %	-	31 %
Cho, 2002 et al	EM grid (open)	N = 21	83 %	-	34 %
Reed et al., 2002	Cryoloop (open)	N = 54	100 %	15 %	-
Mukaida et al., 2003	Cryoloop (open)	N = 725	80 %	20 %	37 %
Osada et al., 2003	Cryotop (open)	N = 580	99 %	-	56 %
Stehlik et al., 2005	Cryotop (open)	N = 41	100 %	-	50 %
Takahashi et al., 2005	Cryoloop (open)	N = 1.129	86 %	29 %	44 %
Kuwayama et al., 2005	Cryotip (closed)	N = 5.695	90 %	-	53 %
Liebermann et al., 2006	Cryotop (open)	N = 547	97 %	31 %	46 %
Mukaida et al., 2008	Cryoloop (open)	N = 5.412	92 %	36 %	49 %

The study by Kuwayama et al. (2005) is the only one to use a closed system in this survey. It is, however, a very extensive study (n = 5,695).

A recent survey presented during the congress of the American Society for Reproductive Medicine (ASRM) in Atlanta in 2009 (Desai et al., 2009) compared an open system with two closed systems for embryos and blastocysts and came to the conclusion that the open system (Cryoloop™) and one of the closed systems (HSV straws™) were the best and that both yielded superior results to the closed Cryo-Tip™ system.

The more recent literature about **blastocysts and closed systems** (Stachecki et al., 2008; Van der Zwalmen et al., 2010a; Liebermann, 2009) confirms Kuwayama et al.'s (2005) results and observations.

Liebermann, 2009

Implantation/Blastocyst replaced: 166/543 (30,6 %)

Implantation/Blastocyst warmed: 166/563 (29,6 %)

Vander Zwalmen et al., 2010a

Implantation/Blastocyst replaced: 60/231 (25,9 %)

Implantation/Blastocyst warmed: 60/348 17,2 %)

Stachecki et al., 2008

Implantation/Blastocyst replaced: 37/80 (46,2 %)

Implantation/Blastocyst warmed: 37/93 (39,7 %)

Wilding et al. (2010) also described excellent results for the vitrification of human zygotes and embryos in closed systems.

A recent survey presented at the congress of the European Society for Human Reproduction and Embryology (ESHRE) in Rome in 2010 (Portmann et al., 2010) compares a new closed system (Cryopette™) with an existing closed system (Cryo-TIP™) for the vitrification of human oocytes, embryos and blastocysts. For all these stages, the use of Cryopette™ seems to yield results comparable to those of Cryo-TIP™.

In a survey presented during the ESHRE congress in Barcelona in 2008, Balaban et al., (2008) showed the results of 8-cell human embryo vitrification by means of a closed RapidVit™ system commercialised by Vitrolife. Over 250 embryos were warmed, 72.1 % of which remained fully intact. An implantation rate per transferred embryo of 29.7 % was obtained. In 2010 the firm Vitrolife presented reassuring additional clinical data (online) for both human embryos and blastocysts using the closed RapidVit™ system.

As regards all these different closed systems, it should, nonetheless, be emphasised that, under the influence of extreme temperatures and pressure, not all the types of plastic used in the carriers are impervious to hazardous viruses and other contaminants. Carelessly sealed carriers can, in certain cases, pose a hazard, especially if they are made of low-quality plastic (Vajta et al., 2009).

Conclusion

For human embryos and blastocysts, vitrification is a validated and reproducible technique. There is no difference between the efficacy of vitrification in closed and open systems. However, the SHC recommends that, for safety reasons (contamination and cross-contamination), closed systems be used for both the cooling stage and for storage. There are several closed systems commercially available.

3.4.2 Oocytes

A meta-analysis of cohort studies and non-randomised comparative trials has shown that vitrification allows for better morphological survival, more clinical pregnancies and live births per transfer (Oktay et al., 2006).

According to Vajta et al. (2009), very high cooling rates are of vital importance for the vitrification of human oocytes because of their size. He takes the view that no closed system would offer a sufficient guarantee for successful oocyte vitrification because the cooling rate is lower in closed systems than in open systems. Furthermore, he also entirely rules out cooling by means of “pre-chilled solid surfaces”, as they are looked upon as unreliable. So far, the literature seems to prove him right. Various authors reporting on successful oocyte vitrification all make use of open systems (Antinori et al., 2007; Kuwayama et al., 2005; Cobo et al., 2010; Nagy et al., 2009; Rienzi et al., 2010). They all show an over 90% morphological survival rate and an over 90% fertilisation rate as well as blastocyst formation and a live birth rate that are comparable to those found with fresh oocytes. Nevertheless, it is a fact that, in these publications, the vitrified oocytes are mainly donor oocytes. By definition, these are oocytes from younger women, which means they are of optimal quality. One question remains, namely that of knowing whether vitrifying oocytes for infertile patients will yield the same results.

Vajta’s claim has recently been challenged. Thus, it has been claimed that the warming rate plays a more dominant role than the cooling rate in post-vitrification survival. A high warming rate can

prevent microscopically small and inoffensive ice crystals which appeared during the vitrification stage from becoming harmful during the warming phase (Seki & Mazur, 2008). Two studies have been published in which oocytes undergo vitrification in closed systems and which guarantee high warming rates (Smith et al., 2010; Van der Zwalmen et al., 2010a). Both studies show that oocyte vitrification in closed systems can work but that it is less effective than the vitrification in Cryo-TOP open systems described in the literature. Recently, the closed Cryo-TIP™ system has also been compared to the open Cryo-TOP system. This study clearly shows that the Cryo-TOP system yields better results in terms of its efficacy (Paffoni et al., 2010). The UZ Brussels hospital has launched a randomised prospective trial that compares an open with a closed system for donor oocyte vitrification. The trial has been randomised with regard to morphological survival. No difference in morphological survival has been shown between a closed system (92 %, 73 oocytes) and an open system (83 %, 64 oocytes). In this trial, vitrification in a closed system has resulted in 6 pregnancies after 12 embryo transfer procedures. Although the value of this trial resides in its observations on morphological survival, the pregnancy results show that human oocyte vitrification in a closed system can work. This trial is still in progress and has therefore not been published yet.

Kuleshova (2009) suggested that any carrier, no matter whether open or closed, can work efficiently, provided that the vitrification procedure (type of CPA and concentration, addition and dilution of CPAs) be optimised for each of the stages of development. Van der Zwalmen et al. (2010b) explain that it is advisable to increase the CPA concentration to compensate for the slower cooling rate.

Another method that has been suggested is to separate the cooling stage (the vitrification) from the storage stage (semi-closed vitrification methods) (Vajta et al., 2009 ; Bielanski & Vajta, 2009 ; Kuleshova, 2009). The reasoning goes as follows: the contact of the liquid (and the oocyte) with the liquid nitrogen is crucial for safe intra- and extracellular vitrification. After the cooling stage, the vitrified carrier is inserted in a High Security straw, sealed (closed) and stored in the non-sterile liquid nitrogen. During warming, one of the ends of the straw may be cut off in order to allow open warming.

The method still has the disadvantage that there can be contamination during the cooling stage. However, there can be no cross-contamination during storage as a result of the use of High Security external carriers. The cooling stage can take place in non-sterile liquid nitrogen (Vajta et al., 2009).

Yet, it has been suggested that sterile liquid nitrogen should be used during the cooling stage (Parmegiani et al., 2010). Sterile liquid nitrogen can be obtained by using a 0.2-µm filter or through UV radiation (Vajta et al., 2009 ; Bielanski & Vajta, 2009 ; Parmegiani et al., 2010). Sterile nitrogen remains sterile under fully sterile conditions. As a result, sterile vitrification is particularly complex to carry out on a daily basis. For the moment, storage in sterile liquid nitrogen is not even technically realistic. In open, semi-closed and closed systems, metal tanks intended to contain liquid nitrogen should be carefully and systematically disinfected (patient after patient). It is also important to point out that there have never been any cases of cross-contamination and contamination of patients and children reported in the IVF environment (Vajta et al., 2007; Pomeroy et al., 2010).

The issue of open systems and (cross-) contamination during storage could be solved by storing in vapour-phase nitrogen (Cobo et al., 2010). Yet, it seems that there is no guarantee that there will be no contamination if the storage occurs in vapour-phase nitrogen in larger and more sophisticated tanks (Grout & Morris, 2009 ; Pomeroy et al., 2010). In addition, there should be an absolute guarantee as regards the amplitude of the temperature gradients in the vapour-phase system (Pomeroy et al., 2010). Small repeated temperature variations can, in the long run, cause irreversible damage to the oocyte. In addition, the risk of spontaneous devitrification (on account of the particularly small volumes) is much higher in vapour-phase than in liquid nitrogen.

As far as human oocytes are concerned, the ASRM takes the view that the vitrification method is still experimental. Further studies are required to arrive at a reproducible vitrification procedure that is safe and abides by the European Union (EU) directive.

It can be expected that in 2011 a greater number of data will be made available concerning human oocytes and closed vitrification systems.

Conclusions

Oocyte vitrification is more efficient than slow controlled-rate freezing.

If the oocytes are to be preserved, it is advisable to use vitrification techniques. However, these techniques need to be further optimised for human oocyte vitrification.

The data from the literature that are currently available show that closed systems seem to give satisfactory results. However, their efficacy compared to that of the open systems still has to be objectivised. The use of closed systems should be preferred on a theoretical basis but, on account of the current lack of sufficient information about their efficacy, the SHC takes the view that open systems can continue to be used for the time being.

3.4.3 Ovarian and testicular tissue

The cryopreservation of ovarian and testicular tissue by means of vitrification is still in its infancy and is considered to be purely experimental. Two studies report on the use of vitrification techniques for human ovarian tissue (Xiao et al., 2010 ; Keros et al., 2009). For the time being, slow controlled-rate freezing remains the norm. Also the vitrification of testicular tissue and spermatozoa has only just seen the light of day.

Conclusion

These techniques aren't up to par yet and slow freezing remains the cryopreservation method recommended for ovarian and testicular tissue.

4. REFERENCES

- AbdelHafez F, Desai N, Abou-setta A, Falcone T, Goldfarb J. Slow freezing, vitrification and ultra-rapid freezing of human embryos: a systematic review and meta-analysis. *RBM Online* 2010;20:209-22.
- Antinori M, Licata E, Dani G, Cerusico F, Versaci C, Antinori S. Cryotop vitrification of human oocytes results in high survival rate and healthy deliveries. *RBM Online* 2007;14:72-9.
- Balaban B, Urman B, Ata B, Isiklar A, Larman M, Hamilton R, et al. A randomized controlled study of human day 3 embryo cryopreservation by slow freezing or vitrification: vitrification is associated with higher survival, metabolism, and blastocyst formation. *Hum Reprod* 2008;23:1976-82.
- Bielanski A, Bergeron H, Lau P, Devenish J. Microbial contamination of embryos and semen during long term banking in liquid nitrogen. *Cryobiology* 2003;46:146-52.
- Bielanski A, Nadin-Davis S, Sapp T, Lutze-Wallace C. Viral contamination of embryos cryopreserved in liquid nitrogen. *Cryobiology* 2000;40:110-16.
- Bielanski A, Vajta G. Risk of contamination of germplasm during cryopreservation and cryobanking in IVF units. *Hum Reprod* 2009;24(10):2457-67.
- Cobo A, Meseguer M, Remohi J, Pellicer A. Use of cryo-banked oocytes in an ovum donation programme: a prospective, randomized, controlled, clinical trial. *Hum Reprod* 2010;25:2239-46.
- Cobo A, Romero J, Perez S, de los Santos M, Meseguer M, Remohi J. Storage of human oocytes in the vapor phase of nitrogen. *Fertil Steril* 2010;94:1903-7.
- Desai N, Xu J, AbdelHafez F. Vitrification carriers and DNA damage: open versus closed systems for cryopreservation of cleavage and blastocyst stage embryos. *Fertil Steril* 2009;92(3):349 Suppl 2009.
- Grout B., Morris J. Contaminated liquid nitrogen vapour as a risk factor in pathogen transfer. *Theriogenology* 2009;71:1079-82.
- HGR - Hoge Gezondheidsraad. Specifieke kwaliteitsnormen voor reproductieve weefsels en cellen Brussel: HGR;2009. Advies nr 8292.
- Kader A, Choi A, Orief Y, Argawal A. Factors affecting the outcome of human blastocyst vitrification. *Reprod Biol Endocrinol* 2009;16(7):99.
- Keros V, Xella S, Hultenby K, Petterson K, Sheiki M, Volpe A, et al. Vitrification versus controlled-rate freezing in cryopreservation of ovarian tissue. *Hum Reprod* 2009;24:1670-83.
- Kolibianakis E, Venetis C, Tarlatzis B. Cryopreservation of human embryos by vitrification or slow freezing: which one is better? *Current Opinion in OB Gyn* 2009;21:270-4.
- Kuleshova L. Fundamentals and current practice of vitrification. In: Borini A, Coticchio G, editors. *Preservation of human oocytes: from cryobiological science to clinical applications: Informa Healthcare United Kingdom;2009. p. 63-1.*
- Kuwayama M, Vajta G, Kato O, Leibo S. Highly efficient vitrification method for cryopreservation of human oocytes. *Reprod Biomed Online* 2005;11:300-8.
- Kuwayama M, Vajta G, Leda S, Kato O. Comparison of open and closed methods for vitrification of human embryos and the elimination of potential contamination. *Reprod Biomed Online* 2005;11(5):608-15.
- Liebermann J. Vitrification of human blastocysts: an update. *RBM Online* 2009;19suppl4:4328.
- Nagy Z, Chang C, Shapiro D, Bernal D, Elsner C, Mitchel-Leef D, et al. Clinical evaluation of the efficiency of an oocyte donation program using egg cryo-banking. *Fertil Steril* 2009;92:520-6.
- Oktay K, Cil A, Bang H. Efficiency of oocyte cryopreservation: a meta-analysis. *Fertil Steril* 2006;86:70-80.
- Paffoni A, Guarneri C, Ferrari S, Liliana R, Nicolosi A, Claudia S, et al. Effects of two vitrification protocols on the developmental potential of human mature oocytes. *RBM*

- Online:doi:10.1016/j.rbmo. 2010. Internet: [http://www.rbmojournal.com/article/S1472-6483\(10\)00722-4/abstract](http://www.rbmojournal.com/article/S1472-6483(10)00722-4/abstract)
- Parmegiani L, Accorsi A, Cognigni G, Bernardi S, Troilo E, Filicori M. Sterilization of liquid nitrogen with ultraviolet irradiation for safe vitrification of human oocytes or embryos. . Fertil Steril 2010;94:1525-28. Internet: accessed.
 - Pegg D. The role of vitrification techniques of cryopreservation in reproductive medicine. 5. Hum Fertil 2005;8:231-9.
 - Pomeroy K, Harris S, Conaghan J, Papadakis M, Centola G, Basuray R, et al. Storage of cryopreserved tissues: evidence that cross-contamination of infectious agents is a negligible risk. Fertil Steril 2010;94:1181-8.
 - Portmann M, Nagy Z, Behr B. Evaluation of blastocyst survival following vitrification/warming using to different closed carrier systems. Hum Reprod Suppl, in press 2010.
 - Rienzi L, Romano S, Albricci L, Magiulli R, Capalbo A, Baroni E, et al. Embryo development of fresh versus vitrified metaphase II oocytes after ICSI: a prospective randomized sibling-oocyte study. Hum Reprod 2010;25(66-73).
 - Royaume de Belgique. Arrêté royal du 4 juin 2003, annexe 4 – annexe 15 relatif aux modalités pour le règlement relatif à la reproduction médicalement assistée. M.B. du 16 juin 2003. p. 32127
 - Seki S, Mazur P. Effect of warming rate on the survival of vitrified Mouse oocytes and the recrystallization of intracellular ice. Biol Reprod 2008;79:727-37.
 - Seki S, Mazur P. The dominance of warming rate over cooling rate in the survival of Mouse oocytes subjected to a vitrification procedure. Cryobiology 2009;59:75-82.
 - Smith G, Serafini P, Fioravanti J, Yadid I, Coslovsky M, Hassun P, et al. Prospective randomized comparison of human oocyte cryopreservation with slow-rate freezing or vittification. Fertil Steril 2010;94:2088-95.
 - Stachecki J, Garrisi J, Sabino S, Caetano J, Wiemer K, Cohen J. A new safe, simple ans successful vitrification method for bovine and human blastocysts. RBM Online 2008;17:360-7.
 - Vajta G, Kuwayama M. Improving cryopreservation systems. Theriogenology 2006;65:236-44.
 - Vajta G, Holm P, Greve T, Callesen H. Vitrification of porcine embryos using the open-pulled straw (OPS) method. Acta Vet Scand 1997;38:349-52.
 - Vajta G, Kuwayama M, Van der Zwalmen P. Disadvantages and benefits of vitrification. . In: Tucker M, Liebermann J, editors. Vitrification in assisted reproduction: a user's manual and trouble shooting guide: Informa Healthcare United Kingdom;2007. p. 33-44.
 - Vajtan G, Nagy Z. Are programmable freezers still needed in the embryo laboratory? Review on vitrification. RBM Online 2006;12:779-96.
 - Van der Zwalmen P, Zech N, Lejeune B, Wirtleiner B, Zech M, Ectors F. Vitrification and the use of high concentrations of cryoprotectants: is it a justified argument to prefer slow freezing? Gyn Ob Fertil 2010;38(9):536-40.
 - Van der Zwalmen P, Zech N, Prapas Y, Panagiotidis Y, Papatheodorou A, Lejeune B, et al. Closed carrier device: a reality to vitrify ovocytes and embryos in aseptic condition. Gyn OB Fertil 2010;38(9):541-6.
 - Wilding M., Capobianco C., Montanaro N., Kabili G., Di Matteo L., Fusco E., Dale . Human cleavage-stage embryo vitrification is comparable to slow-rate cryopreservation in cycles of assisted reproduction. J Assist Reprod Genet 27:549-554, 2010.
 - Xiao Z, Wang Y, Lei L, Lou S, Shan-wei L. Needle immersed vitrification can lower the concentration of cryoprotectant in human ovarian tissue cryopreservation. Fertil Steril 2010;94:2323-8.
 - Yavin S, Arav A. Measurement of essential physical properties of vitrification solutions. Theriogenology 2007;67:81-9.

5. COMPOSITION OF THE WORKING GROUP

All experts joined the working group *in a private capacity*. The names of the members and experts of the Superior Health Council are indicated with an asterisk*.

The following experts took part in drawing up the advisory report:

DE SUTTER Petra*	gynaecologist, reproductive medicine	UZ Gent
THONON Fabienne*	reproductive medicine, embryology	CHR de la Citadelle de Liège
VANSTEENBRUGGE Anne*	embryology	CHR Namur
VAN DEN ABBEEL Etienne*	embryology	UZ Gent

The sub-working group was chaired by Petra DE SUTTER, the scientific secretary was Muriel BALTES.

The following experts read and approved the advisory report:

ANGENON Elyane*	nursing, transplant coordination	ULB
BAUDOUX Etienne*	medicine, cell therapy	ULg
BEELE Hilde*	medicine, dermatology	UZ Gent
DELFORGE Alain*	medicine, cell therapy	ULB
DELLOYE Christian*	medicine, orthopaedic surgery	UCL
GUNS Johan*	medical-social sciences	UZ Brussel
MUYLLE Ludo*	medicine, clinical biology	AFMPS Vigilance - UZA
PIRNAY Jean-Paul*	medical sciences	LabMCT HCB-KA
VAN GEYT Caroline*	medical-social sciences	UZ Gent
VAN RIET Ivan*	medicine, cell therapy	UZ Brussel
VANDERKELEN Alain*	medicine, general surgery	EHB
VERBEKEN Gilbert*	biology, QA/QC/RA	LabMCT HCB-KA

The Administration was represented by:

BONTEZ Walter	Coordination Blood, Cells, Tissues and Organs	FAMHP
VANTHUYNE Karen	Coordination Blood, Cells, Tissues and Organs	FAMHP

The working group was chaired by Hilde BEELE, the scientific secretary was Muriel BALTES.

About the Superior Health Council (SHC)

The Superior Health Council is a federal body that is part of the Federal Public Service Health, Food Chain Safety and Environment. It was founded in 1849 and provides scientific advisory reports on public health issues to the Ministers of Public Health and the Environment, their administration, and a few agencies. These advisory reports are drawn up on request or on the SHC's own initiative. The SHC takes no decisions on the policies to follow, nor does it implement them. It does, however, aim at giving guidance to political decision-makers on public health matters. It does this on the basis of the most recent scientific knowledge

Apart from its 25-member internal secretariat, the Council draws upon a vast network of over 500 experts (university professors, members of scientific institutions), 200 of whom are appointed experts of the Council. These experts meet in multidisciplinary working groups in order to write the advisory reports.

As an official body, the Superior Health Council takes the view that it is of key importance to guarantee that the scientific advisory reports it issues are neutral and impartial. In order to do so, it has provided itself with a structure, rules and procedures with which these requirements can be met efficiently at each stage of the coming into being of the advisory reports. The key stages in the latter process are: 1) the preliminary analysis of the request, 2) the appointing of the experts within the working groups, 3) the implementation of the procedures for managing potential conflicts of interest (based on the declaration of interest, the analysis of possible conflicts of interest, and a referring committee) and 4) the final endorsement of the advisory reports by the Board (ultimate decision-making body). This coherent set of procedures aims at allowing the SHC to issue advisory reports based on the highest level of scientific expertise available whilst maintaining all possible impartiality.

The advisory reports drawn up by the working groups are submitted to the Board. Once they have been endorsed, they are sent to those who requested them as well as to the Minister of Public Health and are subsequently published on the SHC website (www.css-hgr.be), except as regards confidential advisory reports. Some of them are also communicated to the press and to target groups among healthcare professionals.

The SHC is also an active partner in developing the EuSANH network (European Science Advisory Network for Health), which aims at drawing up advisory reports at the European level.

In order to receive notification about the activities and publications of the SHC, you can subscribe to the mailing-list and/or an RSS-feed via the following link:

<http://www.css-hgr.be/rss>.