International Journal of Livestock Research, Vol. 14 (12) Dec' 2024
http://www.ijlr.org
eISSN: 2277-1964
NAAS Score:4.31

Review

Ovarian Cortical Tissue Preservation Through Vitrification - An Overview

Khursheed Ahmad Sofi[1*], Beenish Qureshi[2]

[1]Assistant Professor, Division of Veterinary Clinical Complex, F.V.Sc and A.H, Shuhama, SKUAST-Kashmir, Srinagar, INDIA
[2]Junior Research Fellow, Division of Veterinary Clinical Complex, F.V.Sc and A.H, Shuhama, SKUAST-Kashmir, Srinagar, INDIA

*Corresponding Author: drsofi.vet54321@gmail.com

How to cite this paper
Sofi, K. A., & Beenish Qureshi. (2024). Ovarian Cortical Tissue Preservation Through Vitrification - An Overview. *International Journal of Livestock Research*, 14 (12), 1-10.

Received : Jun 12, 2024
Accepted : Dec 12, 2024
Published : Dec 31, 2024

Abstract

Cryopreservation of ovarian tissues is becoming an important option for fertility preservation because of its availability and the storage of many immature oocytes besides minimizing the delay needed for freezing mature oocytes or embryos. Ovarian tissue cryopreservation followed by auto-transplantation is a promising method for fertility preservation undergoing gonadotoxic treatment and could also be useful for patients suffering from progressive ovarian dysfunction. Ovarian tissue cryopreservation also has the advantage of storing many immature oocytes inside early follicles. Therefore, Cryopreservation of ovarian tissue has a great potential for preserving genetic diversity, conserving individuals, breeds, or species and adding fundamental knowledge on reproductive biology. The present review presents various aspects of ovarian cortical tissue preservation through vitrification.

Keywords: Cortical Tissue, Preservation, Ovary, Vitrification.

Introduction

Over the course of the last few decades many hundreds of exotic species and domestic animal breeds have been lost (FAO, 2009; IUCN, 2010). It is crucial to develop and apply rescue strategies at an earliest to animals threatened with extinction in order to ensure their survival for the future (Wildt, 2000). Cryopreservation of primordial follicles enclosed within the ovary which are the main source of female gametes is one option as a safeguard measure for conservation of female germplasm (Cleary et al., 2001). Cryopreservation of primordial follicles offers certain advantages compared to mature oocytes as these are more resistant to cryoinjury and facilitates penetration of cryoprotectants thereby reducing sensitivity to low temperature. Further, oocytes within the primordial follicles are less cryosensitive due to relatively inactive metabolism, lack of meiotic spindle, zona pellucida and cortical granules in the oocytes. These oocytes within the follicles as of now can be cryopreserved in the form of whole ovary, ovarian cortical tissue and isolated follicles using slow freezing or vitrification method of cryopreservation. After cryopreservation, fertilizable oocytes can be harvested from preserved material following in vitro culture or transplantation. As we know that there are thousands to millions of primordial follicles in the ovarian cortex which can remain viable even several hours following death of animal (Silva *et al*., 2000), which can be therefore collected and cryopreserved for future xenotransplantation (Paris *et al*., 2004) or in vitro cultured (Amorim *et al*., 2003a) to obtain mature fertilizable oocytes. Thus, preservation of female gametes in the form of whole ovary, ovarian cortical tissue or isolated follicles could be used to enlarge the gene pool (Donnez *et al*., 2006). Currently, ovarian tissues cryopreservation is being considered as an important option for fertility preservation because of its various advantages like availability and the storage of a large number of immature oocytes in ovarian cortical tissue, reducing the time needed for freezing of mature oocytes or embryos (Loren *et al*., 2013). For patients undergoing gonadotoxic treatments, a promising method for fertility preservation is ovarian tissue cryopreservation followed by auto-transplantation (Andersen *et al*., 2012; Gamzatova *et al*., 2014). This procedure could also be useful for patients suffering from progressive ovarian dysfunction as ovarian tissue cryopreservation is suitable by preserving their immature oocytes in early ovarian follicles. Following ovarian transplantation and follicle growth, early follicles could develop into pre-ovulatory follicles to allow the generation of mature eggs for fertility treatment. Cryopreservation of ovarian tissues allows sufficient time for histological analyses to evaluate the presence of residual follicles in preserved ovarian tissue before opting for transplantation procedure. Although vitrification is now tried as an alternative technique for preservation of ovarian tissues but till now most studies on cryopreservation of ovarian tissue have been performed using conventional freezing procedures. With respect to ovarian tissue cryopreservation, cortical fragments are preferred for cryopreservation because of better post warm survival and due to dense fibrous structure of large mammalian ovaries making cryoprotectant perfusion of the whole organ difficult (Gosden *et al*., 1994; Baird *et al*., 2004; Bordes *et al*., 2005).

Ovarian Tissue Vitrification

Study of ovarian vitrification procedures have mainly been done using murine ovarian tissue due to sponge-like structure rich in pores (Candy *et al*., 1997; Harp *et al*., 1994), which facilitates CPA (Cryoprotective agents) permeation, decreasing the CPA exposure period, and increases cooling and warming rates. These characteristics probably make vitrification methods very successful in murine ovarian tissue. All studies on vitrification of sheep ovarian tissue (Baudot *et al*., 2007; Bordes *et al*., 2005; Courbiere *et al*., 2006, 2009b) have been carried out by Salle's team having extensive experience in cryopreservation and transplantation of ovine ovaries (Massardier *et al*., 2010; Salle *et al*., 1998, 1999, 2002). Vitrification of whole sheep ovaries has been tried as a model for humans (Baudot *et al*., 2007; Courbiere *et al*., 2006, 2009b) following live births obtained with vitrification of half ovaries (Bordes *et al*., 2005). In general, vitrification of ovarian tissue from other animal species has produced promising results. After in-vitro culture, preantral follicles from vitrified monkey ovarian tissue developed similarly to follicles from frozen–thawed ovarian tissue (Yeoman *et al*., 2005). In dogs (Ishijima *et al*., 2006), rats (Kagabu and Umezu, 2000; Sugimoto *et al*., 2000), hamsters, monkeys and rabbits (Kagabu and Umezu, 2000), vitrified follicles were able to develop after xenografting and auto grafting of bovine preantral follicles after vitrification of ovarian tissue also showed follicular development, indicating that such a procedure does not appear to affect follicles compared with fresh ovarian tissue (Kagawa *et al*., 2009).

Ovarian tissue cryopreservation studies in bovine, caprine and ovine due to their similarities in size, fibrotic tissue structure and folliculogenesis length to humans have become important experimental models for human, (Baird *et al*., 2004; Gandolfi *et al*., 2006; Santos *et al*., 2009). However, most studies on ovarian tissue cryopreservation have been performed in sheep and live births were obtained with auto transplanted frozen ovarian cortical pieces (Gosden

et al., 1994; Baird *et al*., 2004), frozen ovarian hemi-cortex (Salle *et al*., 2003) and vitrified ovarian hemi-cortex (Bordes *et al*., 2005) with long-term ovarian function up to 2 years also reported (Salle *et al*., 2003; Baird *et al*., 2004). Cryopreservation of ovarian cortical fragments in sheep yielded metaphase II oocytes after in vitro culture of follicles isolated from tissue after freezing (Cecconi *et al*., 2004) or after IVM (*in vitro* maturation) of COCs (cumulus oocyte complexes) recovered from tissue after vitrification (Al-Aghbari and Menino, 2002). Recovery of gonadal function and complete follicular development resulted in cryopreserved caprine ovarian tissue after autotransplantation (Santos *et al*., 2009) and survival of follicles after vitrification and in vitro culture (Santos *et al*., 2007a). In cows, follicular morphology and viability of ovarian tissue was preserved after slow freezing (Lucci *et al*., 2004; Celestino *et al*., 2008) or vitrification (Gandolfi *et al*., 2006). Similar results were reported after vitrification and subsequent allotransplantation of the ovarian tissue (Kagawa *et al*., 2009).

Sample Size

The size of ovarian tissue pieces also plays an important role in the success of the vitrification procedure as longer equilibration period is required to allow penetration of the vitrification solution to the inner parts of the tissue when large fragments are used for vitrification. This longer equilibration period as required in large fragments lead to overexposure of surface cells to CPA, thereby results in injuries caused by the toxicity of high CPA concentrations. Thus, cell survival is very likely higher in smaller ovarian fragments and would allow faster cooling rates, which are necessary to decrease the likelihood of chilling injury during the critical temperature zone that ranges from 15 to 5°C (Liebermann *et al*., 2002). When samples are plunged into liquid nitrogen, there is extensive evaporation on their surface, which creates a vapour coat around them, isolate the samples from the liquid nitrogen and lead to decrease in cooling rate (Liebermann *et al*., 2002; Vajta, 2007). Therefore, the size of tissue fragments to be preserved through vitrification can be reduced in order to minimize the duration of vapour coat formation and hence increasing the cooling rate. Probably taking into account the importance of sample size, all studies on vitrification of ovarian tissue from humans and animals have used small fragments of less than 10 mm^2 with thickness of less than 1mm (Gandolfi *et al* 2006; Ishijima *et al* 2006; Kagawa et al., 2009; Amorim *et al*., 2012; Lunardi *et al*., 2012; Tian *et al*., 2015). The only exception were studies in sheep by Salle's group, which aimed to vitrify half (Bordes *et al*., 2005) and whole ovaries (Baudot *et al*., 2007; Courbiere *et al*., 2005, 2006, 2009b; Lornage *et al*., 2006). The results were very promising after vitrification of half ovaries, with the birth of lambs (Bordes *et al*., 2005) and recovery of ovarian endocrine function (Courbiere *et al*., 2009b). However, when whole ovaries were vitrified, the authors reported ice formation (Baudot *et al*., 2007), a lower percentage of viable follicles (Lornage *et al*., 2006), poor follicular survival rates after vitrification and grafting and a high incidence of pedicle thrombosis (Courbiere *et al*., 2009b). Recovery of normal blood flow and endocrine function has been reported after auto transplantation of slow frozen (Bedaiwy *et al*., 2003; Arav *et al*., 2005; Imhof *et al*., 2006) or vitrified (Courbière *et al*., 2009) whole ovaries in sheep. Production of 8-cell embryo after parthenogenetic activation of oocytes retrieved from the slow frozen and grafted ovary (Arav *et al*., 2005) and even live births after conventional freezing and transplantation of whole ovaries have been reported in sheep (Imhof *et al*., 2006) and rats (Wang *et al*., 2002). In addition, differently from the short lifespan of the grafted ovarian fragment, recently Arav *et al*. (2010) have reported the long-term ovarian function (6 years) of frozen–thawed and auto transplanted ovine whole ovary, from which follicular development was obtained, followed by oocyte harvesting and in vitro maturation.

Vitrification Solutions

The vitrification solutions used currently usually consist of a combination of permeable cryoprotectants like ethylene glycol, DMSO (Dimethyl Sulphoxide), polyvinylpyrollidone and a non-permeable cryoprotectant like sucrose, trehalose in various concentrations. Various approaches like using combination of permeable cryoprotectants were used as a consequence of the high concentrations required for vitrification thereby reducing their toxic and osmotic damage. In addition, various other permeable components including propylene glycol, acetamide, glycerol were tested in various combinations (Dela Pena *et al*., 2001; Kasai and Mukaida, 2004), with currently the most acknowledged choice as mixture of ethylene glycol (EG) and DMSO (Ishimori *et al*., 1992a, b) because of less toxic effects of each cryoprotectant and higher permeability of the mixture than that of the individual components (Vicente and Garcia-Ximenez, 1994). Similarly, mono- and disaccharides including sucrose, trehalose, glucose and galactose are the primary candidates as non-permeable cryoprotectants (Kasai, 1997; Wright *et al*., 2004) with sucrose being the most common choice although it was for a long time challenged by trehalose. However, it is interesting to observe that most protocols yielding successful results use solutions containing a mixture of DMSO and EG (Huang *et al*., 2008; Kagawa *et al*., 2009; Keros *et al*., 2009; Wang *et al*., 2008; Xiao *et al*., 2010;

Zhou *et al*., 2010).

Table 1: Vitrification of ovaries using different sample sizes in different species

Species	Ovarian tissue	Reference
Rat	Whole ovary	Sugimoto *et al.*, 1996
Sheep	Half ovary	Bordes *et al.*, 2005
	Whole ovary	Torre *et al*., 2012.
	Ovarian fragments (3x3x1: 9mm^3)	Lunardi *et al*., 2012.
Dog	1mm^3; 3mm^3; 5mm^3	Ishijima *et al.*, 2006
Pig & Cow	1.0 mm^3	Gandolfi *et al*., 2006
Cow	10mm× 10mm ×1mm	Kagawa *et al*., 2009; Faheem *et al*., 2011
Humans	Ovarian fragments (1x1-2x5-8 mm)	Keros *et al*., 2009.
	1mm^3 cortical tissue	Zhou *et al*., 2010
	2-3 mm^3cortical tissue	Xiao *et al*., 2010
	Ovarian fragments (0.25 cm)	Oktem *et al*., 2011
	Ovarian fragments (1.0x1.0x1.0mm)	Amorim *et al*., 2012
	Ovarian fragments (1–1.5 mm3)	Salehnia et al., 2012
	1.4-5.2mm wide & 0.4-1.2mm thick	Tian *et al*., 2015
Cat	Ovarian fragments (2.0x1.0x0.2mm)	Martins *et al*., 2018

Prolonged exposure and overdose of commonly used cryoprotectants for ovarian tissue vitrification like DMSO and ethylene glycol can cause cytotoxicity and impair the viability of tissue. However, cryotoxicity of cell is determined by the concentration of vitrification solution used, time and temperature of exposure with lower temperatures requiring longer time of exposure which in turn would increase the likelihood of osmotic injury (Newton *et al*., 1998). According to Pegg (2005), it is probably less harmful for tissues to raise the temperature of the vitrification solution to allow faster penetration of cryoprotectant solution than lowering the temperature and increasing the duration of contact of tissues with the cryoprotectant solution. Some studies tested exposure time and concentration of cryoprotective agents, but there is no standard regimen to serve as reference for the validation. So far studies have shown that combined use of cryoprotectants and antifreeze polymers may decrease the toxicity and maintains the quality of cryopreservation in ovaries (Silva et al., 2021)

Carrier Systems

Different carrier systems such as plastic straws (Amorim *et al*., 2011; Isachenko *et al*., 2002; Nagano *et al*., 2007; Rahimi *et al*., 2009), copper grids (Choi *et al*., 2007a,b; Isachenko *et al*., 2003), cryovials (Hemadi *et al*., 2009; Isachenko *et al*., 2006), handcut straws (Keros *et al*., 2009), metal strips (Kagawa *et al*., 2009), cryotops (Bao *et al*., 2010; Kagawa *et al*., 2007; Moniruzzaman *et al*., 2009; Trapphoff *et al*., 2010), glass tubes (Sugimoto *et al*., 1996, 2000), needles (Wang *et al*., 2008; Xiao *et al*., 2010) and cryosupport (Hashimoto *et al*., 2010) have been applied in ovarian tissue vitrification in order to decrease the volume of vitrification solution so that extremely high cooling and warming rates are achieved. However, most of the carriers' devices are made of non-conductive materials (e.g., plastic) with thick walls and large working dimensions which may negatively affect the cooling rate. Some authors have therefore applied alternative carrier less systems, such as solid surface vitrification (SSV: Santos *et al*., 2007; Huang *et al*., 2008; Amorim *et al*., 2011) and minimum drop size (MDS: Yeoman *et al*., 2005; Wang *et al*., 2008; Rahimi *et al*., 2010; Amorim *et al*., 2011). Santos *et al*. (2007) reported higher follicular viability with a carrier less system (SSV) compared a carrier approach (plastic straw) in vitrified caprine ovarian tissue. Similarly, Amorim *et al*. (2011) reported that a carrier less system (MDS) offered better results than plastic straws to vitrify human ovarian tissue. Percentage of morphologically normal primordial follicles was found similar using needles or MDS for human ovarian tissue vitrification, although ultra-structurally, preantral follicles and ovarian stroma were better preserved when needles were used (Wang *et al*., 2008). The amount of vitrification solution around the tissue in the carrier less system might have decreased the cooling rate due to the formation of a nitrogen vapour coat. One can also summarize that the superior results obtained with the needle procedure were due to a lower concentration of CPA in the vitrification solution. Before plunging into liquid nitrogen, the vitrification solution is removed from the ovarian tissue using absorbent gauze, minimizing its thermal mass and hence reducing vapour coat formation.

Furthermore, use of a support to keep the tissue immersed in the liquid nitrogen prevents it from floating, which would also reduce formation of a vapour coat and increase the cooling rate. Based on the SSV system, Lindemans *et al*. (2004) developed the cryological vitrification method (CVM) in which tissue is first vitrified in the metal box and then inserted into a straw with an in-built sealant before plunging into liquid nitrogen and was also tested by Aerts *et al*. (2008) to vitrify mouse ovarian tissue. This CVM avoids contact between ovarian tissue and liquid nitrogen during and after vitrification. Indeed, when different carrier systems to vitrify human ovarian tissue were analyzed, a significant difference in follicular morphology was observed after incubation compared with fresh ovarian tissue (Amorim *et al*., 2011). Similarly, Zhou *et al*. (2010) demonstrated the advantages of using direct cover vitrification developed by Chen *et al*. (2006) to cryopreserve human ovarian tissue. This technique has the benefit of requiring a lower concentration of CPA than other vitrification solutions, a higher percentage of morphologically and ultra-structurally normal follicles after 24 days of xenografting compared to a minimum droplet-size method and a lower incidence of apoptotic cells in direct cover vitrification.

Advantages and Challenges of Ovarian Tissue Cryopreservation

Cryopreservation of ovarian tissue offers many advantages over mature oocytes or embryos to preserve the female germline of endangered animals. Firstly, the ovary contains a large pool of oocytes enclosed in primordial follicles. Secondly, ovarian tissue can be collected from animals at almost all developmental age (adult, prepubertal and foetus) and status (alive or dead) (Cleary *et al*., 2001). Thirdly, primordial follicles are more resistant to cryodamage, because their oocytes have a relatively inactive metabolism, lack of metaphase spindle, zona pellucida and cortical granules, and low amounts of lipids (Hovatta, 2005). In addition, larger tissues are very likely to be subjected to non-uniform cooling, which can cause fractures, with devastating consequences for whole organs. Indeed, Courbiere *et al*. (2005) observed pedicle fracture in whole ovaries after vitrification. Such mechanical destruction of the tissue may preclude organ transplantation. Finally, it can also be difficult to warm vitrified organs rapidly enough to avoid devitrification (Taylor *et al*., 2004). Therefore, vitrification of whole ovaries or large pieces of tissue (half ovaries) remains a considerable challenge. Also, it would be prudent to have an incubation period, as proposed by Hovatta *et al*. (1996) and Keros *et al*. (2009), before fixing or preparing the tissue for further analysis, because it would probably allow the detection of changes in organelles after cryopreservation.

Conclusion

It seems inevitable that many species and breeds will be extinct soon. For this reason, it is necessary to consider how it would be possible to take advantage of additional 'emerging tools' in the reproductive sciences. Somatic tissue from endangered species and breeds has already been stored in tissue banks. Mammalian ovarian tissue contains a large pool of follicle-enclosed oocytes, thus providing a rich potential source of female gametes. Cryopreservation of ovarian tissue, in the form of whole ovary, ovarian cortical pieces or isolated follicles, followed by xenotransplantation or in vitro culture, will certainly play a direct role in preserving genetic diversity, conserving individuals, breeds or species, as well as be used for adding fundamental knowledge on reproductive biology. These techniques are highly complex and their efficacy is still very limited. The biological diversity of the ovarian tissue among species is the most challenging obstacle, requiring the development of species-specific protocols. New cryopreservation techniques such as cryopreservation of whole ovaries and isolated follicles are being currently developed. Different standardized cryopreservation protocols need to be developed and evaluated with the aim of applying them to related species.

Acknowledgement

The authors gratefully acknowledge **SERB-DST India** for funding the project on vitrification of oocytes **(CRG-2018-003354)** which facilitated the preparation of this manuscript.

Contribution by Authors

Each co-author contributes equally.

Conflict of Interests

There is no conflict of interest.

Publisher Disclaimer

IJLR remains neutral concerning jurisdictional claims in published institutional affiliation.

References

1. Aerts, J.M., De Clercq, J.B., Andries, S., Leroy, J.L., Van Aelst, S., Bols, P.E. 2008. Follicle survival and growth to antral stages in short term murine ovarian cortical transplants after cryologic solid surface vitrification or slow-rate freezing. Cryobiology, 57: 163–169.
2. Al-aghbari, A.M. and Menino, A.R., 2002. Survival of oocytes recovered from vitrified sheep ovarian tissues. Anim. Reprod. Sci. 71: 101–110.
3. Amorim CA, Dolmans MM, David A, Jaeger J, Vanacker J, Camboni A, Donnez J, Van Langendonckt A. 2012. Vitrification and xenografting of human ovarian tissue. Fertil Steril. 98:1291-8.e1-2.
4. Amorim, C. A., David, A., Van Langendonckt, A., Dolmans, M. M., Donnez, J. 2011. Vitrification of human ovarian tissue: effect of different solutions and procedures. Fertility and Sterility, 95: 1094-1097.
5. Amorim, C.A., Gonc¸ alves, P.B., Figueiredo, J.R., 2003a. Cryopreservation of oocytes from pre-antral follicles. Hum. Reprod. Update, 9: 119–129.
6. Andersen CY, Kristensen SG, Greve T, Schmidt KT. 2012. Cryopreservation of ovarian tissue for fertility preservation in young female oncological patients. Future Oncol., 8:595– 608.
7. Arav, A., Gavish, Z., Elami, A., Natan, Y., Revel, A., Silber, S., Gosden, R.G., Patrizio, P., 2010. Ovarian function 6 years after cryopreservation and transplantation of whole sheep ovaries. Reprod. Biomed. Online 20, 48–52.
8. Arav, A., Revel, A., Nathan, Y., Bor, A., Gacitua, H., Yavin, S., Gavish, Z., Uri, M., Elami, A., 2005. Oocyte recovery, embryo development and ovarian function after cryopreservation and transplantation of whole sheep ovary. Hum. Reprod. 12: 3554–3559
9. Baird, D.T., Campbell, B., Souza, C., Telfer, E., 2004. Long-term ovarian function in sheep ovariectomy and auto transplantation of cryopreserved cortical strips. Eur. J. Obstet. Gynecol. Reprod. Biol. 1, 55–59
10. Bao, R.M., Yamasaka, E., Moniruzzaman, M., Hamawaki, A., Yoshikawa, M., Miyano, T. 2010. Development of vitrified bovine secondary and primordial follicles in xenografts. Theriogenology, 74(5):817-27.
11. Baudot, A., Courbiere, B., Odagescu, V., Salle, B., Mazoyer, C., Massardier, J., Lornage, J., 2007. Towards whole sheep ovary cryopreservation. Cryobiology 55, 236–248.
12. Bedaiwy, M.A., Jeremias, E., Gurunluoglu, R., Hussein, M.R., Siemianow, M., Biscotti, C., Falcone, T., 2003. Restoration of ovarian function after autotransplantation of intact frozen–thawed sheep ovaries with microvascular anastomosis. Fertil. Steril. 79, 594–602
13. Bordes, A., Lornage, J., Demirci, B., Franck, M., Courbiere, B., Guerin, J.F., Salle, B., 2005. Normal gestations and live births after orthotopic autograft of vitrified-warmed hemi-ovaries into ewes. Hum. Reprod. 20, 2745–2748
14. Candy, C.J., Wood, M.J., Whittingham, D.G., 1997. Effect of cryoprotectants on the survival of follicles in frozen mouse ovaries. J. Reprod. Fertil. 110, 11–19.
15. Cecconi, S., Capacchietti, G., Russo, V., Berardinelli, P., Mattioli, M., Barboni, B., 2004. In vitro growth of preantral follicles isolated from cryopreserved ovine ovarian tissue. Biol. Reprod. 70, 12–17.
16. Celestino, J.J., Santos, R.R., Lopes, C.A., Martins, F.S., Matos, M.H., Melo, M.A., Báo, S.N., Rodrigues, A.P., Silva, J.R., Figueiredo, J.R., 2008. Preservation of bovine preantral follicle viability and ultra-structure after cooling and freezing of ovarian tissue. Anim. Reprod. Sci. 108, 309–318
17. Chen, S. U., Chien, C. L., Wu, M. Y., Chen, T. H., Lai, S. M., Lin, C. W. and Yang, Y. S. 2006. Novel direct cover vitrification for cryopreservation of ovarian tissue increases follicles viability and pregnancy capability in mice. *Human Reproduction* **21(11)**: 2794–2800
18. Choi, J., Lee, J.Y., Lee, E., Yoon, B.K., Bae, D., Choi, D., 2007a. Cryopreservation of the mouse ovary inhibits the onset of primordial follicle development. Cryobiology 54, 55–62.
19. Choi, W.J., Yeo, H.J., Shin, J.K., Lee, S.A., Lee, J.H., Paik, W.Y., 2007b. Effect of vitrification method on survivability, follicular growth and ovulation of preantral follicles in mice. J. Obstet. Gynaecol. Res. 33, 128–133.
20. Cleary, M., Snow, M., Paris, M., Shaw, J., Cox, S.L., Jenkin, G., 2001. Cryopreservation of mouse ovarian tissue following prolonged exposure to an ischemic environment. Cryobiology 42, 121–133).
21. Courbiere B, Baudot A, Odagescu V, Salle B, Mazoyer C, Massardier J, Lornage J. 2006. Physical

experimental studies to improve a vitrification procedure in whole sheep ovaries permeated with cryoprotectant agents. Hum Reprod. 21: i107.
22. Courbiere B, Massardier J, Salle B, Mazoyer C, Guerin JF, Lornage J. 2005. Follicular viability and histological assessment after cryopreservation of whole sheep ovaries with vascular pedicle by vitrification. Fertil Steril. 84: 1065-71.
23. Courbiere, B., Caquant, L., Mazoyer, C., Franck, M., Lornage, J., Salle, B., 2009b. Difficulties improving ovarian functional recovery by microvascular transplantation and whole ovary vitrification. Fertil. Steril. 91, 2697–2706.
24. Courbiere, B., Caquant, L., Mazoyer, C., Franck, M., Lornage, J., Salle, B., 2009. Difficulties improving ovarian functional recovery by microvascular transplantation and whole ovary vitrification. Fertil. Steril. 91, 2697–2706
25. Dela Pena, E.C., Takahashi, Y., Katagiri, S., Atabay, E.C., Nagano, M., 2002. Birth of pups after transfer of mouse embryos derived from vitrified preantral follicles. Reproduction 123, 593–600.
26. Donnez, J., Martinez-Madrid, B., Jadoul, P., Van Langendonckt, A., Demylle, D., Dolmans, M.M., 2006. Ovarian tissue cryopreservation and transplantation: a review. Hum. Reprod. Update 12, 519–535.
27. Faheem, M.S., Carvalhais, I., Chaveiro, A. & Moreirada Silva, F. 2011. In vitro oocyte fertilization and subsequent embryonic development after cryopreservation of bovine ovarian tissue, using an effective approach for oocyte collection. *Anim. Reprod. Sci.* 125, 49–55.
28. FAO, 2009. Domestic animal genetic diversity. Available from: http:// www.fao.org/biodiversity/geneticresources/bio-domesticanimals/en/.
29. Gamzatova Z, Komlichenko E, Kostareva A, Galagudza M, Ulrikh E, Zubareva T, Sheveleva T, Nezhentseva E, Kalinina E. 2014. Autotransplantation of cryopreserved ovarian tissue—effective method of fertility preservation in cancer patients. Gynecol Endocrinol, 30 (Suppl 1): 43–47.
30. Gandolfi, F., Paffoni, A., Papasso Brambilla, E., Bonetti, S., Brevini, T.A., Ragni, G., 2006. Efficiency of equilibrium cooling and vitrification procedures for the cryopreservation of ovarian tissue: comparative analysis between human and animal models. Fertil. Steril. 85, 1150–1156.
31. Gosden, R.G., Baird, D.T., Wade, J.C., Webb, R., 1994. Restoration of fertility to oophorectomized sheep by ovarian autografts stored at −196 degrees C. Hum. Reprod. 9, 597–603.
32. Harp, R., Leibach, J., Black, J., Keldahl, C., Karow, A., 1994. Cryopreservation of murine ovarian tissue. Cryobiology 31, 336–343
33. Hashimoto, S., Suzuki, N., Yamanaka, M., Hosoi, Y., Ishizuka, B., Morimoto, Y., 2010. Effects of vitrification solutions and equilibration times on the morphology of cynomolgus ovarian tissues. Reprod. Biomed. Online 21, 501–509.
34. Hemadi, M., Abolhassani, F., Akbari, M. , Sobhani, A., Pasbakhsh, P., Ahrlund-Richter, L. b , Modaresi, M.H., Salehnia, M. 2009. Melatonin promotes the cumulus–oocyte complexes quality of vitrified–thawed murine ovaries; with increased mean number of follicles survival and ovary size following heterotopic transplantation. *European Journal of Pharmacology*, **618**: 84–90.
35. Hovatta, O., 2005. Methods for cryopreservation of human ovarian tissue. Reprod. Biomed. Online 10, 729–734).
36. Hovatta, O., Siye, R., Krausz, T., Abir, R., Margara, R., Trew, G., Lass, A., Winston, R.M.L., 1996. Cryopreservation of human ovarian tissue using dimethylsulphoxide and propanediol-sucrose as cryoprotectants. Hum. Reprod. 11, 1268–1272.
37. Huang, L., Mo, Y., Wang, W., Li, Y., Zhang, Q., Yang, D., 2008. Cryopreservation of human ovarian tissue by solid-surface vitrification. Eur. J. Obstet. Gynecol. Reprod. Biol. 139, 193–198.
38. Imhof, M., Bergmeister, H., Lipovac, M., Rudas, M., Hofstetter, G., Huber, J., 2006. Orthotopic microvascular reanastomosis of whole cryopreserved ovine ovaries resulting in pregnancy and live birth. Fertil. Steril. 1, 1208–1215.
39. Isachenko, E., Isachenko, V., Rahimi, G., Nawroth, F., 2003. Cryopreservation of human ovarian tissue by direct plunging into liquid nitrogen. Eur. J. Obstet. Gynecol. Reprod. Biol. 108, 186–193.
40. Isachenko, V., Isachenko, E., Rahimi, G., Krivokharchenko, A., Alabart, J.L., Nawroth, F., 2002. Cryopreservation of human ovarian tissue by direct plunging into liquid nitrogen: negative effect of disaccharides in vitrification solution. Cryoletters 23, 333–344.
41. Isachenko, V., Montag, M., Isachenko, E., van der Ven, K., Dorn, C., Roesing, B., Braun, F., Sadek, F., van der Ven, H., 2006. Effective method for in-vitro culture of cryopreserved human ovarian tissue. Reprod. Biomed. Online 13, 228–234.
42. Isachenko E, Isachenko V, Weiss JM, et al. 2008. Acrosomal status and mitochondrial activity of human

spermatozoa vitrified with sucrose. *Reproduction*; 136(2):167–173.
43. Ishijima, T., Kobayashi, Y., Lee, D.S., Ueta, Y.Y., Matsui, M., Lee, J.Y., Suwa, Y., Miyahara, K., Suzuki, H., 2006. Cryopreservation of canine ovaries by vitrification. J. Reprod. Dev. 52, 293–299.
44. Ishimori H, Takahashi Y, Kanagawa H., 1992a. Viability of vitrified mouse embryos using various cryoprotectant mixtures. *Theriogenology* **37**, 481–487.
45. Ishimori H, Takahashi Y, Kanagawa H. 1992b. Factors affecting survival of mouse blastocysts vitrified by a mixture of ethylene glycol and dimethylsulfoxide. *Theriogenology* **38**, 1175–1185.
46. IUCN Species Survival Commission, 2010. IUCN Red List Categories and Criteria, Version 3. 1. IUCN, Gland, Switzerland and Cambridge, UK.
47. Kasai, M. and Mukaida, T. 2004. Cryopreservation of animal and human embryos by vitrification. Reproductive BioMedicine Online. 9. 164–170.
48. Kasai M. 1997. Vitrification: refined strategy for the cryopreservation of mammalian embryos. *Journal of Mammalian Ova Research* **14**, 17–28.
49. Kagabu, S. and Umezu, M., 2000. Transplantation of cryopreserved mouse, Chinese hamster, rabbit, Japanese monkey and rat ovaries into rat recipients. Exp. Anim. 49, 17–21.
50. Kagawa, N., Kuwayama, M., Nakata, K., Vajta, G., Silber, S., Manabe, N., Kato, O., 2007. Production of the first offspring from oocytes derived from fresh and cryopreserved pre-antral follicles of adult mice. Reprod. Biomed. Online 14, 693–699.
51. Kagawa, N., Silber, S., Kuwayama, M., 2009. Successful vitrification of bovine and human ovarian tissue. Reprod. Biomed. Online 18, 568–577.
52. Keros, V., Xella, S., Hultenby, K., Pettersson, K., Sheikhi, M., Volpe, A., Hreinsson, J., Hovatta, O. 2009. Vitrification versus controlled-rate freezing in cryopreservation of human ovarian tissue, *Human Reproduction*, 24(7):1670–1683.
53. Liebermann, J., Nawroth, F., Isachenko, V., Isachenko, E., Rahimi, G., Tucker, M.J., 2002. Potential importance of vitrification in reproductive medicine. Biol. Reprod. 67, 1671–1680.
54. Lindemans, W., Sangalli, L., Kick, A., Earl, C.R., Earl, R.C., Fry, R.C., 2004. Vitrification of bovine embryos using the CLV method. Reprod. Fertil. Dev. 16, 174.
55. Loren AW, Mangu PB, Beck LN, Brennan L, Magdalinski AJ, Partridge AH, Quinn G, Wallace WH, Oktay K. 2013. Fertility preservation for patients with cancer: American Society of Clinical Oncology clinical practice guideline update. J Clin Oncol., 31: 2500 – 2510.
56. Lornage, J., Courbière, B., Mazoyer, C., Odagescu, V., Baudot, A., Bordes, A., Poirel, M.T., Franck, M., Salle, B., 2006. Ovarian tissue vitrification: cortex and whole ovary in sheep. Gynecol. Obstet. Fertil. 34, 746–753
57. Lucci, C.M., Kacinskis, M.A., Lopes, L.H., Rumpf, R., Báo, S.N., 2004. Effect of different cryoprotectants on the structural preservation of follicles in frozen zebu bovine (Bos indicus) ovarian tissue. *Theriogenology* 61, 1101–1114
58. Lunardi FO, Araujo VR, Faustino LR, Carvalho AA, Goncalves RFB, Bass CS, Bao SN, Name KPO, Campello CC, de Figueiredo JR, Rodrigues APR. 2012. Morphologic, viability and ultrastructural analysis of vitrified sheep preantral follicles enclosed in ovarian tissue. Small Rumin Res., 107: 121-30.
59. Martins, J.L.A, Lopes, M.D., de Souza, F.F., Possebon, F.S., Wibbelt, G., Jewgenow, K. 2018. Cat preantral follicle survival after prolonged cooled storage followed by vitrification. *Cryobiology*, **81**: 94-100.
60. Massardier, J., Courbiere, B., Lornage, J., Mazover, C., Poirel, M.T., Martinot, S., Franck, M., Salle, B., 2010. Technical aspects of laparoscopic ovarian autograft in ewes after cryopreservation by slow-cooling protocol. Reprod. Domest. Anim. 45, 8–12.
61. Moniruzzaman, M. Bao, H. Taketsuru, T. Miyano 2009.Development of vitrified porcine primordial follicles in xenografts. *Theriogenology*.**72(2):** 280-288
62. Nagano, M., Atabay, E.P., Atabay, E.C., Hishinuma, M., Katagiri, S., Takahashi, Y., 2007. Effects of isolation method and pre-treatment with ethylene glycol or raffinose before vitrification on in vitro viability of mouse preantral follicles. Biomed. Res. 28, 153–160
63. Newton, H., Fisher, J., Arnold, J. R. P., Pegg, D. E., Faddy, M. J., and Gosden, R. G. 1998. Permeation of human ovarian tissue with cryoprotective agents in preparation for cryopreservation. *Human Reproduction*. **13(2):** 376–380.
64. Oktem O, Alper E, Balaban B, Palaoglu E, Peker K, Karakaya C, Urman B. 2011. Vitrified human ovaries have fewer primordial follicles and produce less antimullerian hormone than slow-frozen ovaries. Fertil Steril. 95:2661-4.e1
65. Paris, M.C., Snow, M., Cox, S.L., Shaw, J.M., 2004. Xenotransplantation: a tool for reproductive biology and

animal conservation. *Theriogenology* 61, 277–291
66. Pegg, D. E. 2005. The role of vitrification techniques of cryopreservation in reproductive medicine. *Human Fertility*. **8(4):** 231–239.
67. Rahimi, G., Isachenko, V., Keienberg, R., Sauer, H., Todorov, P., Tawadros, S., Mallmann, P., Nawroth, F., Isachenko, E., 2010. Re-vascularization in human ovarian tissue after conventional freezing or vitrification and xenotransplantation. Eur. J. Obstet. Gynecol. Reprod. Biol. 49, 63–67
68. Rahimi, G., Isachenko, V., Todorov, P., Tawadros, S., Mallmann, P., Nawroth, F., Isachenko, E., 2009. Apoptosis in human ovarian tissue after conventional freezing or vitrification and xenotransplantation. Cryoletters 30, 300–309.
69. Salehnia M, Sheikhi M, Pourbeiranvand S, Lundqvist M. 2012. Apoptosis of human ovarian tissue is not increased by either vitrification or rapid cooling. Reprod Biomed Online. 25:492-9.
70. Salle, B., Demirci, B., Franck, M., Berthollet, C., Lornage, J., 2003. Long term follow-up of cryopreserved hemi-ovary autografts in ewes: pregnancies, births, and histologic assessment. Fertil. Steril 80, 172–177
71. Salle, B., Demirci, B., Franck, M., Rudigoz, R.C., Guerin, J.F., Lornage, J., 2002. Normal pregnancies and live births after autograft of frozen-thawed hemi-ovaries into ewes. Fertil. Steril. 77, 403–408.
72. Salle, B., Lornage, J., Demirci, B., Vaudoyer, F., Poirel, M.T., Franck, M., Rudigoz, R.C., Guerin, J.F., 1999. Restoration of ovarian steroid secretion and histologic assessment after freezing, thawing, and autograft of a hemi-ovary in sheep. Fertil. Steril. 72, 366–370.
73. Salle, B., Lornage, J., Franck, M., Isoard, L., Rudigoz, R.C., Guerin, J.F., 1998. Freezing, thawing, and autograft of ovarian fragments in sheep: preliminary experiments and histologic assessment. Fertil. Steril. 70, 124–128.
74. Santos, R. R., Tharasanit, T., Haeften, T. V., Figueiredo, J. R., Silva, J. R. V. and Van den Hurk, R. 2007. Vitrification of goat preantral follicles enclosed in ovarian tissue by using conventional and solid surface vitrification. *Cell Tissue Res.* 327(1): 167-176
75. Santos, R.R., Knijn, H.M., Vos, P.L., Oei, C.H., Van Loon, T., Colenbrander, B., Gadella, B.M., Van den Hurk, R., Roelen, B.A., 2009. Complete follicular development and recovery of ovarian function or frozen–thawed, autotransplanted caprine ovarian cortex. Fertil. Steril. 91, 1455–1458
76. Santos, R.R., Tharasanit, T., Van Haeften, T., Figueiredo, J.R., Silva, J.R., Van den Hurk, R., 2007a. Vitrification of goat preantral follicles enclosed in ovarian tissue by using conventional and solid-surface vitrification methods. Cell Tissue Res. 327, 167–176
77. Silva, J.R., Lucci, C.M., Carvalho, F.C., Báo, S.N., Costa, S.H., Santos, R.R., Figueiredo, J.R., 2000. Effect of coconut water and Braun-Collins solutions at different temperatures and incubation times on the morphology of goat preantral follicles preserved in vitro. Theriogenology 15, 809–822.
78. Silva, T. E., Nunes, M., Casalechi, M., Comim, F. V., Rodrigues J. K. and Reis, F. M. 2021. Cryoprotectants agent for ovarian tissue vitrification: systemic review. *Cryobiology.* ISSN 0011-2240.
79. Sugimoto, M., Maeda, S., Manabe, N., Miyamoto, H., 2000. Development of infantile rat ovaries autotransplanted after cryopreservation by vitrification. *Theriogenology* 53, 1093–1103.
80. Sugimoto, M., Miyamoto, H., Kabasawa, T., Manabe, N., 1996. Follicle survival in neonatal rat ovaries cryopreserved by vitrification. Cryoletters 17, 93–98.
81. Taylor, M.J., Song, Y.C., Brockbank, K.G.M., 2004. Vitrification in tissue preservation: new developments. In: Fuller, B.J., Lane, N.J., Benson, E.E. (Eds.), Life in the Frozen State. CRC Press, Boca Raton, USA, pp. 603–641.
82. Tian, T., Zhao, G., Han, D., Zhu, K., Chen, D., Zhang, Z., Wei, Z., Cao, Y., Zhou, P., 2015. Effect of vitrification cryopreservation on follicular morphology and stress relaxation behaviours of human ovarian tissue: sucrose versus trehalose as non-permeable cryoprotective agent. Human reproduction 30:877-883
83. Torre A, Momier M, Mazoyer C, Selva J, Salle B, Lornage J. Validation of a new metabolic marker to assess the vascular viability of vitrified whole sheep ovaries. Hum Reprod. 2012; 27:1811-21
84. Trapphoff, T., El Haji, N., Zechner, U., Haaf, T., Eichenlaub-Ritter, U., 2010. DNA integrity, growth pattern, spindle formation, chromosomal constitution and imprinting patterns of mouse oocytes from vitrified pre-antral follicles. Hum. Reprod. 25(12):3025-42
85. Vajta, G., 2007. Vitrification in animal reproduction: vitrification of embryos using open puller straws. In: Tucker, M.J., Liebermann, J. (Eds.), Vitrification in Assisted Reproduction – A User's Manuel and Trouble-shooting Guide. Informa Healthcare, London, UK, pp. 65–74.
86. Vicente JS and Garcia-Ximenez F. 1994. Osmotic and cryoprotective effects of a mixture of DMSO and ethylene glycol on rabbit morulae. *Theriogenology* **42**, 1205–1215.
87. Wang X, H. Chen, H. Yin, S.S. Kim, S.L. Tan, R.G. Gosden. 2002. Fertility after intact ovary transplantation,

Nature 415; 385. https://doi.org/10.1038/415385a
88. Wang, Y., Xiao, Z., Li, L., Fan, W., Li, S.W., 2008. Novel needle immersed vitrification: a practical and convenient method with potential advantages in mouse and human ovarian tissue cryopreservation. Hum. Reprod. 23, 2256–2265.
89. Wildt, D.E., 2000. Genome resource banking for wildlife research, management, and conservation. *ILAR J*. 41: 228–234.
90. Wright DL, Eroglu A, Toner M *et al*. 2004. Use of sugars in cryopreserving human oocytes. *Reproductive BioMedicine Online*: **9**, 179–186.
91. Xiao, Z., Wang, Y., Li, L., Luo, S., Li, S. W., 2010. Needle immersion vitrification can lower the concentration of cryoprotectant. *Fertility and sterility*. 94: 2323-2328.
92. Yeoman, R.R., Wolf, D.P., Lee, D.M., 2005. Coculture of monkey ovarian tissue increases survival after vitrification and slow-rate freezing. Fertil. Steril. 83, 1248–1254.
93. Zhou, X. H., Wub, Y. J., Shi, J., Ya-xian xia. and Zheng, S. S. 2010. Cryopreservation of human ovarian tissue: Comparison of novel direct cover vitrification and conventional vitrification. *Cryobiology* **60(2)**: 101-105.

****