NATIONAL AGRICULTURAL AND FOOD CENTRE
RESEARCH INSTITUTE FOR ANIMAL PRODUCTION NITRA

Slovak J. Anim. Sci., 50, 2017 (3): 128–133

ISSN 1337-9984

POSSIBILITIES OF CATTLE OVARIAN TISSUE CONSERVATION: A MINI-REVIEW

A. MAKAREVICH[1]*, M. FOLDESIOVA[1], L. OLEXIKOVA[1], E. KUBOVICOVA[1], P. CHRENEK[1,2]

[1]NPPC – Research Institute for Animal Production Nitra, Lužianky, Slovak Republic
[2]Slovak University of Agriculture in Nitra, Slovak Republic

ABSTRACT

Cryopreservation of ovaries, their surface tissues or ovarian follicles represents the main source of female gametes in the future. In case of serious damage to the animal (limb fractures and others), when it is necessary to slaughter the animal, the ovarian tissue, ovarian follicle or the entire ovary can be collected and frozen. After thawing, the biological material may be transplanted or cultured *in vitro* as a source of oocytes for *in vitro* fertilization and *in vitro* embryo production. Ovarian tissue can be cryopreserved in various forms, such as fragments, slices, hemi-ovaries or whole ovaries with a vascular pedicle for future vascular anastomosis. Whole ovaries tend to be more difficult to preserve due to their dense tissue structure, intricate vascular system and diversity of cell types, all of which results in poor heat transfer and uneven cooling rates. An alternative approach to harvesting and vitrifying oocytes would be to cryopreserve ovarian tissues instead of follicles. Dissection of the ovary into ovarian fragments followed by puncture of the follicles is considered an effective technique toobtain a high number of oocytes in excellent conditions. Therefore, attention must be given to preserving this yield of good oocytes. This method of ovarian tissue storage is very promising but insufficiently elaborated yet. Cryopreservation of ovarian surface tissues or ovarian follicles represents the future main source of female gametes. Last year, we have started investigations focusing on cryopreservation of ovarian fragments from Slovakian cattle breeds for the purposes of national gene bank of animal genetic resources.

Key words: cattle; ovary; oocyte; cryopreservation

INTRODUCTION

To preserve genetic diversity, notably for the conservation of endangered species and indigenous farm animal breeds, it is essential to create genome or genetic resource banks (GRBs) of male and female gametes and embryos, made up of a very large number of individual donors in good condition (Wildt, 2000).

Important role in solving the issue of the preservation of animal genetic resources belongs to a cryopreservation and subsequent long-term storage of genetic material from valuable breeds of livestock or genetically valuable individuals. Cryopreservation of ovaries, their surface tissues or ovarian follicles represents a main source of female gametes in future. In case of serious damage of the animal (limb fractures and others), when it is necessary to slaughter the animal, the ovarian tissue, ovarian follicle or the entire ovary can be collected and frozen. After thawing, the biological material may be transplanted or cultured *in vitro* as a source of oocytes for *in vitro* fertilization (IVF) and *in vitro* embryo production (IVP).

The population of females of Pinzgau cattle breeds in Slovakia according to FAO database is around 2000 units, what is considered as a critical state within the animal genetic resources. Moreover, in Slovakia there are no yet any frozen embryos of Pinzgau cattle, which represents history, landscaping and indispensable part of mountainous and mountain foothill areas of Slovakia. One solution to this problem is the cryopreservation of rare biological material, which will play an important role in addressing the issue of preservation of animal

***Correspondence:** E-mail: makarevic@vuzv.sk
Alexander Makarevič, NPPC – Research Institute for Animal Production
Nitra, 951 41 Lužianky, Slovak Republic
Tel.: +421 37 6546 334

Received: September 11, 2017
Accepted: September 25, 2017

genetic resources. Cryopreservation of ovaries, their surface tissues or ovarian follicles represents the future main source of female gametes.

The ability to preserve oocytes and ovarian tissues in a healthy state for a desired period of time would have tremendous application in the field of biomedical research. Besides the application for the purpose of animal breeding, ovarian tissue banking in humans is also being considered in the hope of restoring fertility to patients who lose ovarian function due to chemo- or radiotherapy during cancer treatment (Newton, 1998; Paynter *et al.*, 1997).

SUCCESS OF CRYOPRESERVATION OF OVARIAN TISSUES AND CELLS

Methods developed for oocyte and ovarian tissue cryopreservation must protect structural and functional viability. To date, several strategies have been suggested to restore fertility using cryopreserved oocyte or ovarian tissue. These strategies describe cryopreservation of oocytes at either an immature (isolated from the ovary) or a mature (after ovulation) state. In regard to ovarian tissue, one strategy involves autologous orthotopic or xenogeneic transplantation of the frozen-thawed ovaries and monitoring the developmental potential and follicular dynamics *in vivo* (Aubard *et al.*, 1998; Gosden *et al.*, 1994a,b; Gunasena *et al*., 1997a,b; Harp *et al*., 1994; Oktay *et al*., 1998b; Weissman *et al*., 1999). The second strategy involves *in vitro* maturation, fertilization and culture of primordial follicles isolated from frozen-thawed ovaries (Carroll *et al*., 1990; Oktay *et al*., 1998a; Sztein *et al.*, 2000).

In most of the studies on human (Huang *et al.*, 2008; Wang *et al.*, 2008; Zhou *et al*., 2010) and cattle (Gandolfi *et al.*, 2006), the success of the ovarian tissue cryopreservation procedures was evaluated using morphological and histological assessment. The use of histology alone is insufficient to assess the freezing-thawing success, as morphological analysis is not often correlated to the viability or developmental competence of the follicle (Santos *et al*., 2007). These results need to be validated by *in vitro* oocyte development and by fertilization. Faheem *et al.* (2011) isolated bovine oocytes for *in vitro* culture using the procedure of dissection of ovarian cortex into small fragments followed by puncture of the follicle from frozen-thawed ovarian tissue. They recovered a great number of good quality oocytes, which were successfully matured *in vitro* (maturation rate 73-80 %), fertilized and developed into *in vitro* produced embryos at morula and blastocyst stages.

The cryopreservation of ovarian cortex containing the primordial follicles would certainly be very useful in the conservation of female gametes. There are two possibilities: the extraction of slices of the ovarian cortex after the puberty onset without disturbing the female reproductive system. Another way is to remove the whole ovaries after the animal's death, to be used later in techniques such as grafting or *in vitro* maturation.

The aim of ovarian tissue cryopreservation is to store primordial follicles located in the ovarian cortex, which represents the oocyte reserve. Cryopreservation of matured (metaphase II) oocytes gives poor results due to problems encountered during fertilization and embryo development. Following thawing, hardening of the *zona pellucida* associated with the exocytosis of premature cortical granules blocks the penetration of the spermatozoon. The freezing procedure can also damage the oocyte cytoskeleton (Vincent and Johnson, 1992). The freezing of germinal vesicle oocytes could be an alternative because there is no risk of aneuploidy, however hardening of the *zona pellucida* and damage to the cytoskeleton is still present (Tucker *et al.*, 1998). Limited success with the above described methods has focused research on cryopreservation of the immature oocytes contained in primordial follicles, which are located in the ovarian cortex. This method is more likely to be successful, because the oocyte is less differentiated, it possesses fewer organelles, the *zona pellucida* is yet absent and the cortical granules do not exist, so the follicle is less prone to ischaemia (Oktay *et al*., 1998).

Usually, to produce oocytes suitable for *in vitro* fertilization, the extended (about two weeks) follicle culture is needed, because the majority of oocytes in ovarian tissue slices are situated within primordial follicles and are, therefore, immature (Cotrvindt *et al.*, 1996b; Hartshorne, 1997). However, a population of antral follicles with good post-thaw survivability exists in these tissues that can provide source of oocytes for *in vitro* maturation (Nayudu and Osborn, 1992). Therefore, by selecting the ovarian fragments containing more antral follicles it is possible to avoid prolonged procedure of ovarian *in vitro* culture, results of which may be unexpected.

METHODOLOGY OF OVARIAN TISSUE CRYOPRESERVATION

Cryopreservation of ovarian tissue offers many advantages over mature oocytes or embryos to preserve 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 of 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 amount of lipids (Hovatta, 2005). Nevertheless, cryopreservation of ovarian tissue is still problematic and should be optimized to handle the diversity of cell types and tissue components (oocytes, granulosa cells, extracellular matrix) (Hovatta, 2005). The cryopreservation and transplantation of ovarian tissue is currently being investigated in various large animals, most notably in sheep and pigs (Posillico *et al.*, 2010).

Cryopreservation aims to maintain cell in a viable state for long periods of time. Cells can be cryopreserved using any of two approaches: conventional freezing or vitrification. Using conventional freezing protocols in domestic animals, promising results have been obtained after cryopreservation of ovarian tissue from cats, sheep, goats, cows, pigs, horses and rabbits (Santos *et al*., 2010). In conventional freezing, sophisticated and expensive programmable freezers are required to assist the cooling procedure. However, the use of freezing devices is not always available, especially when endangered animals are found dead in the nature, because transport of such ovaries to laboratories may result in cellular degeneration due to hypoxia caused by the delay between animal death and the time of cryopreservation of the ovarian tissue. Therefore, an alternative for cryopreservation in field conditions may be a vitrification.

Although the conventional slow freezing method using programmable device has been successfully used for a number of years, it has disadvantages; the most noticeable is intracellular ice formation and subsequent cell damage. Opposite to that, vitrification is a rapid cooling cryopreservation method that results in solidification without crystallization, thus avoiding cryodamage resulting from ice formation (Bahchi *et al*., 2008). The first critical step in vitrification is the cooling rate, which must exceed the solution's critical cooling rate (CCR). Water has an extremely high CCR, which makes vitrification of cellular water almost impossible in lab settings. Therefore, water needs to be replaced as much as possible with cryoprotectants (CPA) such as dimethylsulfoxide (DMSO) or ethylene glycol (EG), which have lower CCR. By achieving the adequate cooling rate, that exceeds the CCR of the sample at a given viscosity and dehydration level, cryodamage due to ice formation can be avoided, as the sample transforms immediately from an aqueous to a vitreous state (Courbiere *et al.*, 2006). The high rates of cooling and thawing necessary for vitrification are achieved with classic protocols in which a small volume of the specimen is loaded into a loading device along with the minimum necessary volume of the cryoprotectant. The specimen is either directly exposed to liquid nitrogen or is loaded into a closed, thin-walled device to achieve maximum heat transfer rates (Kuwayama, 2007).

Vitrification presents many advantages for cryobiology and could be a suitable approach for organs vitrification, given the complexity of tissues and vascular system (Lornage and Salle, 2007). Since vitrification aims to avoid ice formation, it should principally provide a better alternative to tissue preservation than slow freezing. However, this procedure requires extremely fast cooling rate and extracellular water replacement by permeating cryoprotectants, which may cause cytotoxic effect (Lornage *et al*., 2006). In order to reduce this deleterious influence, different ways can be used to reduce the CPA concentration, such as a combination of relatively low concentrations of different CPA's in order to obtain a vitrifiable concentration of total solutions and to diminish the specific toxicity (Vajta and Nagy, 2006), or the addition of non-permeable CPA's, like sera, sugars and polymers.

Al-Aghbari and Menino (2002) reported in their study on ovine ovarian tissue that vitrification of ovarian tissue using an ultra-rapid cooling technique (Dinnyes *et al*., 2000) appears to be a viable alternative to cryopreserving sheep oocytes by conventional freezing or other vitrifying methods. Their study confirmed that dropping ovarian tissues, equilibrated in a vitrification solution, directly onto solid surface cooled by liquid nitrogen, can be used for oocyte cryopreservation. *In vitro* survival of oocytes obtained from vitrified ovarian tissues was similar to the non-vitrified control, suggesting that the vitrification process and subsequent thawing did not obviously induce damage to the oocytes. Cryopreservation rates for vitrified oocytes and oocytes obtained from vitrified ovarian tissues in the mentioned study were higher compared to conventional freezing and vitrification techniques using slower cooling rates (Martino *et al*., 1996; Saunders and Parks, 1999).

This success in cryopreservation procedure may be attributed to several new steps used in the procedure of Dinnyes *et al.* (2000). First, a higher cooling rate was reached by direct contact with metal surface chilled with liquid nitrogen. Second, ovarian tissues were thawed quickly by dropping the vitrified samples directly into warm thawing solutions. Third, ovarian tissues were relatively small (0.5 cm × 0.5 cm) which allowed rapid temperature exchange during cooling-thawing procedures, as well as rapid flushing out of a cryoprotectant.

The oocyte has always been the most challenging specimen to cryopreserve because of its high sensitivity and intolerance to cryopreservation due to large cytoplasmic volume and intricate cellular structure (Kim, 2006). Primordial follicles (PMF), which comprise about 90 % of the follicular population of each ovary, are less cryosensitive than mature oocytes, embryos and maturing follicles, primarily due to their morphology - less cytoplasm and cytoplasmic components, no *zona pellucida*, fewer granulosa cells - as well as the low

metabolic rate associated with their inactivity (Amorim *et al*., 2003; Courbiere *et al*., 2009). Therefore, PMF represents a better chance of surviving cryopreservation than any growing stage follicles (Huang *et al*., 2008). Until successful *in vitro* maturation procedure can be developed, the PMF can be stored *in situ* in the ovarian tissue (Amorim *et al*., 2003; Jewgenow and Paris, 2006).

Ovarian tissue can be cryopreserved in various forms, such as fragments, slices, hemi-ovaries or whole ovaries with a vascular pedicle for future vascular anastomosis. The smaller forms - fragments, slices and isolated follicles may have a higher probability of being viable after cryopreservation, because the cryoprotectants can more entirely permeate the tissue and prevent damages due to ice formation. Whole ovaries tend to be more difficult to preserve due to their dense tissue structure, intricate vascular system and diversity of cell types, all of which results in poor heat transfer and uneven cooling rates (Courbiere *et al*., 2009; Possilico *et al*., 2010).

An alternative approach to harvesting and vitrifying oocytes would be to cryopreserve ovarian tissues instead of follicles. Dissection of the ovary into ovarian fragments followed by puncture of the follicles is considered an effective technique to get a high number of oocytes in excellent conditions (Faheem *et al.*, 2011). Therefore, attention must be given to preserve this yield of good oocytes.

In most of the studies on human (Huang *et al.*, 2008; Wang *et al*., 2008; Zhou *et al.*, 2010) and cattle (Celestino *et al*., 2008; Gandolfi *et al*., 2006), the success of the ovarian tissue cryopreservation procedures was evaluated using morphological and histological assessment. The use of histology alone is insufficient to assess the freezing-thawing success, as morphological analysis is not often correlated to the viability or developmental competence of the follicle (Santos *et al*., 2007). These results need to be validated by *in vitro* oocyte development and by fertilization.

Faheem *et al.* (2011) isolated bovine oocytes for *in vitro* culture using the procedure of dissection of ovarian cortex into small fragments followed by puncture of the follicle from frozen-thawed ovarian tissue. They recovered a great number of good quality oocytes, which were successfully *in vitro* matured (maturation rate 73-80 %), fertilized and developed into *in vitro* produced embryos of morula and blastocyst stage.

CONCLUSION

Cryopreservation of bovine ovaries or ovarian follicles represents the main source of female gametes in future. In case of serious damage to a cow (limb fractures and others), when it is necessary to slaughter the animal, the entire ovary or the ovarian cortical tissue which usually contains a plenty of ovarian preantral or antral follicles can be collected and frozen. After thawing in relevant laboratory conditions, the biological material may be processed *in vitro* as a source of oocytes for *in vitro* fertilization and subsequent *in vitro* embryo production. This strategy could enable accumulation and long-term storage (at ultra-low temperature) of preimplantation stage embryos from animals which are not alive anymore. Such embryos may form basis for establishment of the animal gene bank. Currently, successful cryopreservation of animal ovarian tissue is still a challenge and protocols should be optimized.

ACKNOWLEDGEMENT

This paper was created through the realization of the project of the Slovak Research and Development Agency Bratislava: the grant APVV-15-0196.

REFERENCES

AL-AGHBARI, A. M. – MENINO, A. R. 2002. Survival of oocytes recovered from vitrified sheep ovarian tissues. *Animal Reproduction Science*, vol. 71, 2002, p. 101–110.

AMORIM, C. A. – GONCALVES, P. B. – FIGUEIREDO, J. R. 2003. Cryopreservation of oocytes from preantral follicles. *Human Reproduction Update*, vol. 9, 2003, p. 119–129.

AUBARD, Y. – NEWTON, H. – SCHEFFER, G. – GOSDEN, R. 1998. Conservation of the follicular population in irradiated rats by the cryopreservation and orthotopic autografting of ovarian tissue. *European Journal of Obstetrics, Gynaecology and Reproductive Biology*, vol. 79, 1998, p. 83–87.

BAGCHI, A. – WOODS, E. J. – CRITSER, J. K. 2008. Cryopreservation and vitrification: recent advances in fertility preservation technologies. *Expert Review of Medical Devices*, vol. 5, 2008, p. 359–370.

CARROLL, J. – WHITTINGHAM, D. G. – WOOD, M. J. – TELFER, E. – GOSDEN, R. G. 1990. Extra-ovarian production of mature viable mouse oocytes from frozen primary follicles. *Journal of Reproduction and Fertility*, vol. 90, 1990, p. 321–327.

CELESTINO, J. J. – dos SANTOS, R. R. – LOPES, C. A. – MARTINS, F. S. – MATOS, M. H. – MELO, M. A. – BAO, S. N. – RODRIGUES, A. P. – SILVA, J. R. – de FIGUEIREDO, J. R. 2008. Preservation of bovine preantral follicle viability and ultrastructure after cooling and freezing of ovarian tissue. *Animal Reproduction Science*, vol. 108, 2008, p. 309–318.

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*, vol. 42, 2001, p. 121–133.

COTRVINDT, R. – SMITZ, J. – Van STEIRTEGHEM, A. 1996. *In vitro* maturation, fertilization and embryo development of immature oocytes from early pre-antral follicles from prepubertal mice in a simplified culture system. *Human Reproduction*, vol. 11, 1996, p. 2656–2666.

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. *Fertility and Sterility*, vol. 91, 2009, p. 2697–2706.

COURBIERE, B. – ODAGESCU, V. – BAUDOT, A. – MASSARDIER, J. – MAZOYER, C. – SALLE, B. – LORNAGE, J. 2006. Cryopreservation of the ovary to slow-cooling protocols. *Fertility and Sterility*, vol. 86, 2006, p. 1243–1251.

DINNYES, A. – DAI, Y. – JIANG, S. – YANG, X. 2000. High developmental rates of vitrified bovine oocytes following parthenogenetic activation, *in vitro* fertilization and somatic cell nuclear transfer. *Biology of Reproduction*, vol. 63, 2000, p. 513–518.

JEWGENOW, K. – PARIS, M. C. 2006. Preservation of female germ cells from ovaries of cat species. *Theriogenology*, vol. 66, 2006, p. 93–100.

FAHEEM, M. S. – CARVALHAIS, I. – CHAVEIRO, A. – MOREIRA DA SILVA, F. 2011. *In vitro* oocyte fertilization and subsequent embryonic development after cryopreservation of bovine ovarian tissue, using an effective approach for oocyte collection. *Animal Reproduction Science*, vol. 125, 2011, p. 49–55.

GANDOLFI, F. – PAFFONI, A. – BRAMBILLA, E. P. – BONETTI S. – BREVINI, T. A. L. – RAGNI, G. 2006. Efficiency of equilibrium cooling and vitrification procedures for the cryopreservation of ovarian tissue: comparative analysis between human and animal models. *Fertility and Sterility*, vol. 85, 2006, p. 1150–1156.

GOSDEN, R. G. – BOULTON, M. I. – GRANT, K. – WEBB, R. 1994a. Follicular development from ovarian xenografts in SCID mice. *Journal of Reproduction and Fertility*, vol. 101, 1994, p. 619–623.

GOSDEN, R. G. – BAIRD, D. T. – WADE, J. C. – WEBB, R. 1994b. Restoration of fertility to oophorectomized sheep by ovarian autografts stored at -196 °C. *Human Reproduction*, vol. 9, 1994, p. 597–603.

GUNASENA, K. T. – VILLINES, P. M. – CRITSER, E. S. – CRITSER, J. K. 1997a. Live births after autologous transplant of cryopreserved mouse ovaries. *Human Reproduction*, vol. 12 (1), 1997, p. 101–106.

GUNASENA, K. T. – LAKEY, J. R. – VILLINES, P. M. – CRITSER, E. S. – CRITSER, J. K. 1997b. Allogeneic and xenogeneic transplantation of cryopreserved ovarian tissue to athymic mice. *Biology of Reproduction*, vol. 57, 1997, p. 226–231.

HARP, R. – LEIBACH, J. – BLACK, J. – KELDAHL, C. – KAROW, A. 1994. Cryopreservation of murine ovarian tissue. *Cryobiology*, vol. 31, 1994, p. 336–343.

HARTSHORNE, G. M. 1997. *In vitro* culture of ovarian follicles. *Reviews in Reproduction*, vol. 2, 1997, p. 94–104.

HOVATTA, O. 2005. Methods for cryopreservation of human ovarian tissue. *Reproductive BioMedicine Online*, vol. 10, 2005, p. 729–734.

HUANG, L. – MO, Y. – WANG, W. – LI, Y. – ZHANG, Q. – YANG, D. 2008. Cryopreservation of human ovarian tissue by solid-surface vitrification. European *Journal of Obstetrics & Gynecology and Reproductive Biology*, vol. 139, 2008, p. 193–198.

KIM, S. S. 2006. Fertility preservation in female cancer patients: current developments and future directions. *Fertility and Sterility*, vol. 85, 2006, p. 1–11.

KUWAYAMA, M. 2007. Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method. *Theriogenology*, vol. 67, 2007, p. 73–80.

LORNAGE, J. – COURBIERE, 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. *Gynécologie Obstétrique & Fertilite*, vol. 34, 2006, p.746–753.

LORNAGE, J. – SALLE, B. 2007. Ovarian and oocyte cryopreservation. *Current Opinions in Obstetrics and Gynecology*, vol. 19, 2007, p. 390–394.

MARTINO, A. – POLLARD, J. W. – LEIBO, S. P. 1996. Effect of chilling bovine oocytes on their developmental competence. *Molecular Reproduction and Development*, vol. 45, 1996, p. 503–512.

NAYUDU, P. L. – OSBORN, S. M. 1992. Factors influencing the rate of preantral and antral growth of mouse ovarian follicles *in vitro*. *Journal of Reproduction and Fertility*, vol. 95, 1992, p. 349–362.

NEWTON, H. 1998. The cryopreservation of ovarian tissue as a strategy for preserving the fertility of cancer patients. *Human Reproduction Update*, vol. 4, 1998, p. 237–247.

OKTAY, K. – NEWTON, H. – AUBARD, Y. – SALHA, O. – GOSDEN, R. G. 1998a. Cryopreservation of immature human oocytes and ovarian tissue: an emerging technology? *Fertility and Sterility*, vol. 69, 1998, p. 1–7.

OKTAY, K. – NEWTON, H. – MULLAN, J. – GOSDEN, R. G. 1998b. Development of human primordial follicles to antral stages in SCID/hpg mice stimulated with follicle stimulating hormone. *Human Reproduction*, vol. 13, 1998, p. 1133–1138.

PAYNTER, S. J. – FULLER, B. J. – SHAW R. W.

1997. Temperature dependence of mature mouse oocyte membrane permeabilities in the presence of cryoprotectant. *Cryobiology*, vol. 34, 1997, p. 122–130.

POSILLICO, S. – KADER, A. – FALCONE, T. – AGARWAL, A. 2010. Ovarian tissue vitrification: Modalities, challenges and potentials. *Current Women's Health Reviews*, vol. 6, 2010, p. 352–366.

SANTOS, R. R. – VAN DEN HURK, R. – RODRIGUES, A. P. – COSTA, S. H. – MARTINS, F. S. – MATOS, M. H. – CELESTINO, J. J. – FIGUEIREDO, J. R. 2007. Effect of cryopreservation on viability, activation and growth of *in situ* and isolated ovine early-stage follicles. *Animal Reproduction Science*, vol. 99, 2007, p. 53–64.

SANTOS, R. R. – AMORIM, C. – CECCONI, S. – FASSBENDER, M. – IMHOF, M. – LORNAGE, J. – PARIS, M. – SCHOENFELDT, V. – MARTINEZ-MADRID, B. 2010. Cryopreservation of ovarian tissue: an emerging technology for female germline preservation of endangered species and breeds. *Animal Reproduction Science*, vol. 122, 2010, p. 151–163.

SAUNDERS, K. M. – PARKS, J. E. 1999. Effects of cryopreservation procedures on the cytology and fertilization rate of *in vitro* matured bovine oocytes. *Biology of Reproduction*, vol. 61, 1999, p. 178–187.

SZTEIN, J. M. – O'BRIEN, M. J. – FARLEY, J. S. – MOBRAATEN, L. E. – EPPIG, J. J. 2000. Rescue of oocytes from antral follicles of cryopreserved mouse ovaries: competence to undergo maturation, embryogenesis and development to term. *Human Reproduction*, vol. 15, 2000, p. 567–571.

TUCKER, M. J. – WRIGHT, G. – MORTON, P. C. – MASSEY, J. B. 1998. Birth after cryopreservation of immature oocytes with subsequent *in vitro* maturation. *Fertility and Sterility*, vol. 70, 1998, p. 578–579.

VAJTA, G. – NAGY, Z. P. 2006. Are programmable freezers still needed in the embryo laboratory? Review on vitrification. *Reproductive BioMedicine Online*, vol.12, 2006, p. 779–796.

VINCENT, C. – JOHNSON, M. H. 1992. Cooling, cryoprotectants and the cytoskeleton of the mammalian oocyte. *Oxford Reviews in Reproductive Biology*, vol. 14, 1992, p. 72–100.

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. *Human Reproduction*, vol. 23, 2008, p. 2256–2265.

WEISSMAN, A. – GOTLIEB, L. – COLGAN, T. – JURISICOVA, A. – GREENBLATT, E. M. – CASPER, R. F. 1999. Preliminary experience with subcutaneous human ovarian cortex transplantation in the NOD-SCID mouse. *Biology of Reproduction*, vol. 60, 1999, p. 1462–1467.

WILDT, D. E. 2000. Genome resource banking for wildlife research, management and conservation. *ILAR Journal*, vol. 41, 2000, p. 228–234.

ZHOU, X. H. – WU, Y. J. – SHI, J. – XIA, Y. X. – ZHENG, S. S. 2010. Cryopreservation of human ovarian tissue: comparison of novel direct cover vitrification and conventional vitrification. *Cryobiology*, vol. 60, p. 101–105.