Strategies of vitrification in animals

Background of Vitrification

An important aspect in preserving female gametes for future use, permitting the female genetic material to be stored unfertilized until an appropriate mate is selected is known as cryopreservation of oocytes. [5] In gametes and embryos, cryopreservation has become an essential part of assisted reproductive technologies (ART) [3] as the extensive use of animal reproductive technologies is often dependent on the success of gamete and embryo cryopreservation. [1] Sperm cryopreservation has had a high success rate when used in humans as well as a variety of mammalian species. [3] Embryo cryopreservation allows the full genetic complement of sire and dam conservation, thus having potential for protecting and managing species population cohesion and heterozygosity. [5] Embryo cryopreservation has also been used in clinical ART with a result in healthy baby deliveries, however, there are known setbacks as it requires the male to produce embryos. It has been prohibited in some countries due to ethical, legal, and religious beliefs. [3]

Slow freezing (slow cooling) and vitrification are the two leading methods for cryopreservation. The standard technique which is favored in early procedures and used in various species for oocyte and embryo cryopreservation is known as slow freezing (slow cooling). The new method of vitrification is being widely used as it damages the oocytes and embryos less than slow freezing. Vitrification is known as the ultra-rapid cooling method. [3] *Vajta* states that the “ physical definition of vitrification is the solidification of a solution (glass formation) at low temperatures without ice

crystal formation. The phenomenon can be regarded as an extreme increase of viscosity and requires either rapid cooling rates or the use of cryoprotectant solutions, which depress ice crystal formation and increase viscosity at low temperatures.” [6]

Vitrification in domestic animals [6] (2000)

Vitrification is an important study as it leads to embryo cloning. There was no decrease for further developmental ability in bovine embryos in the pre-compaction stage along with blastomeres being successfully used as donors for nuclear transfer, allowing for a rapid increase in research for the cloning industry. [6] The blastocyst rates were maintained as recipient cytoplasts for successful cryopreserved and full-term development were obtained. [6] There was a breakthrough in cryopreservation of bovine oocytes as the blastocyst rates after fertilization and in vitro culture stated to approach non-cryopreserved control values and live offspring. [6] It has been understood that the success of cryopreservation is dependent on the culture conditions as recent studies in culture methods have improved embryo quality and the traditional equilibrium freezing method may also be successfully applied. [6] A major encounter researchers found was to improve and stabilize the excellent survival rates in vitro and birth of some piglets while permitting large scale porcine embryo transfer as it has not been successful for long. [6] There has also been challenge in establishing a universal standardized vitrification method, that may be successful for cryopreservation for different species in different developmental stages of oocytes and embryos. [6]

Caffeine and Oocyte: Sheep [3] (2018)

There has been extensive research has been done on cryopreservation of metaphase II (MII) oocytes, where oocytes at this stage can disrupt the meiotic spindle, however, could be avoided by freezing the oocytes at the germinal vesicle (GV) stages. [3] There have been advantages in both human and animal reproduction of freezing oocytes. There have also been studies to cryopreserve GV-oocytes in mammalian species with live human and animal births and the blastocyst development rate is low. [3] With advantages, there are also many obstacles. One is the that there is no standard protocol established in both humans and some animals regarding frozen GV-oocytes prior to fertilization while being matured in vitro (IVM). [3] *Moawad et al* state that " oocyte meiotic maturation is controlled by the level of two cytoplasmic protein kinases such as maturation promoting factor (MPF) and mitogen-activated protein kinase (MAPK)." [3] Previous studies show that immature ovine oocytes in vitrification decreased the level of both MPF and MAPK after IVM, where high activities of MPF and MAPK are responsible for the onset of GV breakdown and are essential for oocyte maintenance at MII-stage. [3]

In the study they evaluated the effects of vitrification of ovine GV-oocytes on spindle assembly, MPF/MAP kinases activities, and preimplantation development following IVM and IVF along with elucidating the impact of caffeine supplementation during IVM on the quality and development of vitrified/warmed ovine GV-oocytes. [3] They use caffeine (1, 3, 7 – trimethylxanthine) as a phosphodiesterase inhibitor. [3] The study shows in sheep there was an increase in both MPF and MAPK kinases when treated

with in vitro matured oocytes (IVM) along with improving both the frequencies of nuclear envelope breakdown and chromosome condensation of transferred nuclei, It also increased the total cell numbers while reducing the frequency of apoptotic nuclei in blastocyst embryos produced by somatic cell nuclear transfer. [3] It was also found that aged denuded ovine oocytes had an increase in blastocyst development and decrease in the frequency of polyspermy following IVF when using the caffeine treatment. [3] There was a negative impact of oocyte quality and development after IVM/IVF and embryo culture , when trying to restore the spindle integrities, MPF/MAPK activities and subsequent development of vitrified/warmed oocytes at the GV-stage by supplementing them in vitro maturation. [3] There is still little known about the role of development of vitrified/warmed oocytes.

Non-Vitrified vs Vitrified in vitro-derived embryos are transferred in cattle [1] (2018)

Do et al [1] states that in the cattle industry, the slow freezing method is used more when producing embryos of superovulation in cryopreservation. When comparing slow freezing and vitrification of in vitro0derived embryos transferred to cattle, there was lower survival rates in slow freezing procedures. [1] This was based on their capacity to re-expand and to hatch after being thawed or warmed and cultured in vitro making it an effective method used in cryopreservation in human oocytes along with showing promising results in bovine oocytes and embryos. [1] There is no vigorous procedure for cattle embryos which optimize pregnancy through invitro-derived embryos being transferred into females. [1]

Re-vitrification or storage in liquid nitrogen with pig embryos [4] (2018)

An improved vitrification procedure, The open pulled straw (OPS) method is an improved vitrification procedure which provides high rate in vitro using post warming embryo hopefully leading to higher rates in pregnancy and farrowing in porcine embryos after surgical ET without any prior treatments. [4] There were similar survival rates after 24 h in culture between vitrified and fresh embryos. [4] Embryo transfer (ET) technology is on high demand in the swine industry. [4] There was a loss of interest in this technology for decades due the need of surgical procedures for embryo collection along with the difficulties arising when trying to use embryo cryopreservation in swine. [4] But thanks to new developments and safer non-surgical ET, vitrification is becoming the only effective method for porcine embryos when trying to preserve them for long periods as it avoids the formation of intracellular ice crystals. [4]

As re-vitrification and air embryo shipment in the vapor phase of liquid nitrogen is a new method in cryopreservation, there are many unsolved factors. [4] As *Nohalez et al* states, “ the number of warmed embryos exceed the number of embryos necessary to be transferred to the recipients or when some recipients cannot receive embryos due to health problems or difficulties during the insertion of the non-surgical ET catheter, a number of unexpected supernumerary embryos could have been warmed.” [4] They believe they could use re-vitrification as a way to restore extra warm embryos for future ET as it is has more flexibility for transfer allowing for an

increase in number of transferred embryo per recipient. [4] However the survival rates for post-warming embryos in pigs have not yet been discover for the effects of re-vitrification. [4] But through this study, further information was found to help explain the re-vitrification process. [4] The results were detrimental to a significant proportion of embryos as more than 60% of porcine blastocyst derived from vitrified and warmed CCMs (cavitating morulae) and UBLs (unhatched blastocyst) survive re-vitrification and re-warming. [4] The embryos were either accidentally warmed or warmed embryos were not transferred into recipients due to special circumstances and may be re-vitrified and used in future ETs. [4]

Vitrification of buffalo oocyte and embryos [5] (2016)

In bovines, most methods of reproductive biotechnology have been applied, however, are not as efficient. [5] Buffaloes also have been reported to have poor response to superovulation treatments, exhibiting relatively low yield of in vivo-derived embryos. [5] For buffalo embryo cryopreservation both slow freezing and vitrification techniques are used. [5] In the future, vitrification will become the most suitable method of cryopreservation of any cells and tissues. Especially for buffalo oocytes as the extremely large single cells contain an excess amount of intracellular lipid making them sensitive to cryopreservation. [5]

Conclusion

Compared to slow-freezing (slow-cooling) method, vitrification technologies applied on oocytes and embryos has become more successful along with MII-stage oocytes rather than GV stage for their higher membrane stability during freezing. [5] Although there has been success, a major obstacle in vitrification is associated with chilling injury, osmotic stress, CPA toxicity, and ice crystallization. [5] More studies need to be done to further understand and identify key aspects in a protocol which will increase the GV-stage oocytes maturation rate, fertilization and blastocyst rate, as well as the survival and hatching rate of vitrified-thawed embryos. [5]

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