Workshop II: Preservation of IVP embryos

Cryopreservation of IVP bovine embryos

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The number of bovine *in vitro* produced embryos (IVP) has increased year by year. In 2016, a total of 666,215 IVP embryos were produced, exceeding for the first time, the volume of embryos generated *in vivo* (Perry G. Embryo Technology Newsletter, 35:1-46, 2017). Also, 121,490 frozen embryos and 326,623 fresh embryos were transferred. In the same year, 60,723 frozen embryos were transferred from the OPU-IVP bovine embryo in Brazil, while in the USA this number was 48,533. In this context, the total embryo production is sometimes higher than the number of embryos transferred, so the cryopreservation methods are a good alternative. However, differences have been reported regarding the freezability of Bos taurus vs Bos indicus IVP embryos. Studies have reported quantitatively higher lipid content rates in the *indicus* embryos rather than taurine counterparts. Despite the IVP advantages, cryopreservation represents a challenge for commercial laboratories and adaptations are necessary for each practice. The cryopreservation technique predominantly used for IVP embryos is vitrification. This ultra-rapid freezing process reduces cell damage caused by the formation of ice crystals but requires a high concentration of cryoprotectants. Moreover, it is necessary to be trained to perform a morphological evaluation of embryo quality before the loading process. Thereby, the process of thawing and the direct transfer (DT) of embryos to cows make the slow freezing protocol - method previously described for *in vivo* embryos - more efficient for IVP commercial use. The low concentrations of cryoprotectants are the main advantage of this technique since high concentrations are toxic to embryos. Also, the DT strategy has recently been performed by commercial laboratories, providing good embryo viability after thawing. A study conducted in Brazil with female Girolando donors (1/2 Gir and 1/2 Holstein) (Sanches, B.V. Theriogenology, 85: 1147-1151, 2016), compared pregnancy rates for fresh, vitrified or frozen IVP embryos. The conception rates obtained were 51.35 ± 1.87% (133/259) for the fresh embryos, 35.89 ± 3.87% (84/234) for the vitrified embryos and 40.19 ± 4.65% (125/311) for the frozen directly transferred embryos. These data showed that IVP embryos with sexed semen could be directly transferred with similar conception rates to vitrified embryos. Thus, by facilitating logistics, this technique may become a more practical approach for the transfer of cryopreserved embryos in the field. Due to the promising results, the DT strategy has been applied in large-scale operations, mostly in the US and Brazil. Finally, shortly, while other companies will start using DT, it is likely to reach the point where most IVP embryos will be frozen, as is the case of semen industry.

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The challenge of vitrifying *in vitro*-produced porcine embryos

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Significant progress in pig embryo cryopreservation has been achieved since the development of vitrification as an alternative to slow-freezing, which has resulted ineffective in this species. Currently, high *in vitro* survival rates and promising reproductive performance after ET can be reached with vitrified *in vivo*-derived porcine morulae and blastocysts. Although piglets have been obtained from vitrified *in vitro*-produced (IVP) embryos, their vitrification ability is still far behind that of their *in vivo*-derived counterparts. The development of successful vitrification procedures for IVP porcine embryos would be relevant not only for the livestock industry, but also for banking genetically modified embryos from swine models of human diseases, which are of great importance for biomedical research. It is well known that some factors affect vitrification outcomes regardless the origin of the embryo (*in vivo* or *in vitro*) such as the embryonic stage, the concentration and type of cryoprotectants, the vitrification device or the equilibration temperatures. However, two main aspects make the IVP embryos particularly sensitive to vitrification: A higher lipid content and a much poorer quality compared to *in vivo*-derived ones. Several studies have focused on the importance of embryo lipid content and its relation to vitrification tolerance. In this regard, piglets have been obtained after transfer of parthenogenetic and IVM/IVF embryos subjected to delipidation by micromanipulation. However, protocols that involve zona pellucida disruption should be avoided for sanitary reasons. Thus, stimulation of lipolysis with chemicals agents such as forskolin, has been proposed as an interesting and efficient tool to decrease the lipid content that hinder vitrification, while maintaining the zona pellucida integrity. With respect to the quality of embryos, many supplements have been added during *in vitro* embryo production in order to improve embryo quality and therefore the embryo vitrificability. Among them, those compounds protecting IVP embryos against oxidative stress during *in vitro* culturing and vitrification-warming procedures, such as L-ascorbic acid, seem to be key to improving vitrification efficiency. Also, the length of the *in vitro* culture, which is related to the embryonic stage, influences the quality of IVP embryos and, consequently, the vitrification efficiency. Finally, the high incidence of polyspermic penetrations in porcine IVF, which is still an unsolved problem, is a factor that should be taken into account. In this respect, the selection of monospermic embryos would be advisable when vitrification is to be performed. In this workshop we will discuss all these aspects related to the vitrification of IVP porcine embryos based on our results and also on those from other researchers working in this field.

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Effects of (cryo)preservation on the quality of *in vitro* produced embryos

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Numerous studies have shown *in vitro* produced embryos to differ from their *in vivo* produced counterparts in terms of timing of development and morphology, metabolism, cell number, gene expression patterns and also their chilling sensitivity and their ability to survive cryopreservation.
Cryopreservation of gametes and embryos is a crucial step for the widespread application and conservation of animal genetic resources. Differences between cryopreservation methods can be seen for the first time after cryopreservation in re-expansion and hatching rates. Therefore, comparisons between slow cooling and vitrification methodologies have mainly been drawn at a morphological level. However, the effect of cryopreservation on the quality of *in vitro* produced embryos is also clearly reflected at the molecular level. Over the last two decades it was possible to increase survival rates after thawing/warming by a multiple number of improvements. However, results for post-preservation survival vary immensely. Efforts to improve survival rates were mainly restricted to altering the IVC media or to modifications of the cryopreservation method itself. Additionally, an effect of the age and developmental stage of the embryo has been determined. As vitrification alters fewer transcripts than conventional cryopreservation, it seems to be the more favourable method for cryopreservation of embryos produced in a SOF-based culture system. Comparing vitrification media supplemented with or without DMSO, it was shown that DMSO-containing media result in an increased number of surviving bovine IVP embryos compared to DMSO-free media. Nevertheless, these embryos show a reduced quality at the molecular level. Due to lower pregnancy rates after transfer of biopsied and cryopreserved embryos especially when they are generated via *in vitro* production (IVP), an alternative preservation method also needs to be developed. Liquid preservation employing FBS might fulfill the requirements as shown recently.
Despite all the refinements, fundamental research is still needed to improve the results mainly with *in vitro* produced embryos, especially after biopsy, and oocytes.
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