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In-vitro development of vitrified–warmed bovine oocytes after activation may be predicted based on mathematical modelling of cooling and warming rates during vitrification, storage and sample removal

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KEY MESSAGE

Survival and development of parthenogentically activated bovine oocytes after vitrification, storage, and warming may be predicted based on mathematical modelling.

A B S T R A C T

Heat transfer during cooling and warming is difficult to measure in cryo-devices; mathematical modelling is an alternative method that can describe these processes. In this study, we tested the validity of one such model by assessing in-vitro development of vitrified and warmed bovine oocytes after parthenogenetic activation and culture. The viability of oocytes vitrified in four different cryo-devices was assessed. Consistent with modelling predictions, oocytes vitrified using cryo-devices with the highest modelled cooling rates had significantly ($P < 0.05$) better cleavage and blastocyst formation rates. We then evaluated a two-step sample removal process, in which oocytes were held in nitrogen vapour for 15 s to simulate sample identification during clinical application, before being removed completely and warmed. Oocytes exposed to this procedure showed reduced developmental potential, according to the model, owing to thermodynamic instability and devitrification at relatively low temperatures. These findings suggest that cryo-device selection and handling, including method of removal from nitrogen storage, are critical to survival of vitrified oocytes. Limitations of the study

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include use of parthenogenetically activated rather than fertilized ova and lack of physical measurement of recrystallization. We suggest mathematical modelling could be used to predict the effect of critical steps in cryopreservation.

Introduction

Current advances in cryopreservation of human gametes have led to near-complete replacement of traditional slow cooling protocols by ultra-fast cooling methods (Edgar and Gook, 2012; Kuleshova et al., 1999). Vitrification, described by Luyet (1937), refers to the solidification of a sample into an amorphous, glassy-state without intracellular and extracellular ice crystal formation. When applied to embryology, this process requires high concentrations of cryoprotectants, extremely rapid cooling rates and minimal handling volume (Jain and Paulson, 2006; Rall and Fahy, 1985).

Description of thermodynamic events during cryopreservation is of great importance to elucidate what type of phenomenon is occurring: freezing, cooling, vitrification, thawing of ice in the sample or devitrification (Mazur, 1984). Devitrification events have been previously defined (Shaw and Jones, 2003; Yavin and Arav, 2007) as ice nuclei or crystals forming during warming of a vitrified solution. Therefore, measurement of temperature histories is necessary to establish whether vitrification or phase change transition (ice formation) takes place during cooling, and whether a vitrified sample undergoes devitrification during warming.

Direct temperature measurement in cryo-devices is often difficult (Choi and Bischof, 2010). Both the minimal volumes and the minute size of the cryo-devices for loading, plunging and storage of mammalian oocytes and embryos present a challenge. Although experimental observations through modified cryostereoscopes of sample crystallization during cooling and recrystallization fractures during warming have been reported (Yavin and Arav, 2007), direct observation and measurement of these events is not feasible for most laboratories. A useful alternative to predict time-temperature curves is the use of numerical modelling of the heat-transfer process. In the past decade, an increasing number of engineers have collaborated with researchers in the biomedical, embryology or life sciences fields to solve complex problems related to thermodynamic processes and, ultimately, to be able to predict heat and mass transfer processes and other kinetic-driven phenomena (Lunardini, 1981; Arce et al., 1983; Pham and Ruffin, 1992; Alexiades and Solomon, 1993; Diller, 1997; Baudot and Odagescu, 2004; Choi and Bischof, 2010). This numerical simulation approach is useful for predicting temperature histories and other important heat-transfer parameters, such as surface heat-transfer coefficients (Cleland et al., 1984; Sansinena et al., 2010, 2011, 2012; Santos et al., 2012), but it also contributes to the validation of experimental results and reduces the number and scope of experiments needed when assessing cryopreservation protocols (Choi and Bischof, 2010).

Previously, our laboratory applied the finite element method, a numerical solution to heat conduction problems, for the prediction of heat-transfer performance of cryo-devices used in oocyte and embryo vitrification (Sansinena et al., 2011). This mathematical approach was also used to describe thermodynamic conditions during storage and warming of vitrified oocytes and embryos in relation to their glass transition rubbery state and to predict sample devitrification thresholds (Sansinena et al., 2014). To validate these numerical models, however, it is important to relate numerical predictions with cell survival and embryo development.

Therefore, the objective of this study was to find the relationship between previously reported numerical simulations with in-vitro performance during cooling and warming of samples in cryo-devices. The modelled cooling and warming conditions (Sansinena et al., 2011, 2014) were replicated *in vitro*, followed by analysis of morphological oocyte survival and in-vitro embryo development after parthenogenetic activation in a bovine model.

Materials and methods

Selection of experimental conditions based on previous numerical models

The experimental conditions for oocyte vitrification, sample storage and warming were selected on the basis of conditions previously modelled in theoretical analyses (Sansinena et al., 2011, 2014) for several cryo-devices, including Cryoloop, Cryotop, Miniflex and Open Pulled-Straw (OPS).

Experiment: in-vitro oocyte survival and embryo development after vitrification in cryo-devices previously simulated by numerical model

Oocyte in-vitro maturation and vitrification. Bovine ovaries were recovered from selected abattoirs classified as 'Exportation Accredited' by Animal Health National Service (Servicio Nacional de Seguridad Animal, SENASA, Argentina), with all the regulatory, animal health and slaughter practice that such accreditation entails.

Ovaries were collected and transported to the laboratory at 30°C in saline solution supplemented with antibiotics within 2 h of slaughter (Blondin and Sirard, 1995); cumulus–oocyte complexes were aspirated by follicular puncture. Classification of cumulus–oocyte complex quality was conducted following International Embryo Technology Society guidelines (Grade 1 [excellent] to Grade 4 [poor]). Only those oocytes classified as grades 1 and 2 were selected for in-vitro maturation. Cumulus–oocyte complexes were matured in Earl's-based Medium 199 (Invitrogen 11150-059, MA, USA) supplemented with 10% v/v heat-inactivated fetal bovine serum [FBS] (Gibco 10082-139, US-origin), 5 µl/ml pure FSH (Sigma F 2293, MO, USA), 10 µg/ml luteinizing hormone releasing hormone (Sigma H 8008), 1 µg/ml 17 beta-oestradiol (Sigma E 2758, MO, USA) and 1 µg/ml cysteamine (Sigma M 9768, MO, USA) in 5% CO2 in humidified air incubator at 38.5°C for 22 h. After 22 h of in-vitro maturation, oocytes were partially denuded of cumulus by gentle pipetting in 0.1% v/v hyaluronidase (350 IU, Sigma H 3506, MO, USA) solution. Oocytes were then washed three times in holding medium consisting of Hank's-base Medium 199

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