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The relationship between oxygen consumption rate and viability of *in vivo*-derived pig embryos vitrified by the micro volume air cooling method

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ABSTRACT

The aim of this study was to assess the viability of vitrified-warmed *in vivo*-derived pig embryos after measuring the oxygen consumption rate. Six days after artificial insemination, blastocysts were collected from gilts and vitrified by the micro volume air cooling method. The oxygen consumption rate was measured in 60 vitrified-warmed embryos, which were then cultured for 48 h to assess the viability. The survival (re-expansion) rate of embryos after warming was 85.0%. The average oxygen consumption rate of embryos immediately after warming was greater in embryos which could re-expand during subsequent culture ($F = 0.75 \pm 0.04$) than that in those which failed to re-expand ($F = 0.33 \pm 0.05$). Moreover, the oxygen consumption rate of vitrified-warmed embryos was greater in the hatched ($F = 0.88 \pm 0.06$) than that in the not-hatched group ($F = 0.53 \pm 0.04$). When the oxygen consumption rate of the vitrified-warmed embryos and the numbers of viable and dead cells in embryos were determined, there was a positive correlation between the oxygen consumption rate and the number of live cells ($P < 0.01$, $r = 0.538$). A total of 29 vitrified embryos after warming and measuring the oxygen consumption rate were surgically transferred into uterine horns of two recipients. Both of the recipients become pregnant and farrowed 12 healthy piglets. These results demonstrate that the oxygen consumption rate of vitrified-warmed pig embryos can be related to the number of live cells and that the measurement of oxygen consumption of embryos after cryopreservation may be useful for estimating embryo survivability.

1. Introduction

In general, pig embryos are collected surgically by uterine flushing. It is difficult to perform embryo collection at farms. Therefore, embryos are collected at facilities with surgical and laboratory equipment, and subsequently cryopreserved except for when embryo transfers occur immediately after collection. Pig embryos are more sensitive to chilling than those of other mammals (Polge et al., 1974; Wilmut, 1974). The sensitivity of pig embryos to low temperature is mainly due to abundant intracellular lipids (Nagashima et al., 1994). Nagashima et al. (1995a) removed cytoplasmic lipids from pig embryos and successfully

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produced offspring from frozen–thawed embryos. Moreover, piglets were also obtained from use of frozen–thawed embryos at the hatched blastocyst stage, which tolerate slow freezing (Fujino et al., 1993; Nagashima et al., 1995b).

Various vitrification methods have been developed for cryopreservation of pig blastocysts (Cuello et al., 2005; Fujino et al., 2008; Gomis et al., 2012; Maehara et al., 2012) because these embryos have greater survivability than when a slower freezing method is used.

Misumi et al. (2013) also reported a greater survival rate of vitrified-warmed pig embryos after using a micro volume air cooling (MVAC) method. With this method, embryos were vitrified using a chemically defined vitrification medium and cooled in a straw placed vertically in liquid nitrogen (LN_2). Therefore, embryos could be effectively preserved and transferred into recipients without direct contact to the undefined factors of serum and LN_2.

Selection of live embryos immediately after vitrification and warming by the evaluation of the morphology is rather difficult, because during vitrification and warming blastocysts shrink. Thus, an objective method is required for evaluating of embryo quality after cryopreservation.

In a previous report (Sakagami et al., 2015), freshly collected pig embryos that hatched after the subsequent culture had greater oxygen consumption rates than those that did not hatch. Moreover, a greater conception rate was observed and normal piglets were obtained after transferring embryos with greater oxygen consumption rates into sows by non-surgical transfer. Oxygen consumption rates were correlated with cell numbers and viability in freshly collected embryos (Sakagami et al., 2015). However, there has been no report investigating the relationship between oxygen consumption rate and viability of vitrified-warmed pig embryos using the MVAC method.

The objectives of the present study were to assess the correlation among the oxygen consumption rate, cell numbers and viability of pig vitrified-warmed embryos using the MVAC method.

2. Materials and methods

2.1. Embryo collection from gilts or sows

All animal-related procedures followed in this study were conducted after the approval of the Institutional Animal Experiment Committee of the Kanagawa Prefectural Agriculture Facilities. A total of 10 pre-pubertal (Landrace and Large White; 6–8 months of age) and two estrous cycling (Duroc; 9 and 11 months of age) gilts were used for collection of embryos, as previously described with some modifications (Hirayama et al., 2011; Nakazawa et al., 2008). Briefly, superovulation was induced by intramuscular administration of 1500 IU eCG (Peamex, Sankyo, Tokyo, Japan), followed 72 h later by 500 IU hCG (Puberogen, Sankyo). The donors were artificially inseminated twice in the afternoon 1 day and in the morning 2 days after the hCG administration.

On the morning of Day 6 (Day 0 = the day of the first artificial insemination), the embryos were recovered from the uterine horns by laparotomy under general anesthesia (4–5% [v/v] isoflurane) by flushing with a porcine oocyte/embryo collection medium (POE-CM; Research Institute for the Functional Peptides [IFP], Yamagata, Japan; Yoshioka et al., 2008). The recovered embryos were morphologically evaluated under an inverted microscope (100×), and Code 1 blastocyst stage embryos classified based on the IETS manual (Stringfellow and Seidel, 1998) were used for the experiments. The blastocysts were preserved in porcine blastocyst medium (PBM, IFP; Mito et al., 2012) at 38.5 °C in atmospheres of 5% CO_2, 5% O_2 and 90% N_2 until vitrification.

2.2. Measurement of the oxygen consumption rate

To objectively assess embryo quality, the oxygen consumption rate of embryos was measured using an embryo respirometer (HV-405, IFP) by the method of Abe et al. (2004). Briefly, an embryo was placed into the solution for the measurement (ERAM-2, IFP) on the flat bottom of a cone-shaped microwell plate (RAP-1, IFP). A platinum microelectrode was moved close to the embryo, and the potential of −0.6 V *vs.* Ag/AgCl was applied to reduce the oxygen concentration in the solution surrounding the embryo. The respiration rate gradients in the solution surrounding the embryo were measured by scanning the *z*-axis (*i.e.*, vertical direction) at a speed of 30.0 μm/s over a scanning distance of 160 μm. The oxygen consumption rate (respiration rate) of the embryos was estimated according to spherical diffusion theories (Shiku et al., 2004).

2.3. Vitrification and warming of embryos

The embryos were vitrified using MVAC method (Misumi et al., 2013). The MVAC method was conducted using the HI-Stick (2.0 mm wide, 60 mm long and 0.1 mm thick stainless steel board bended 90°; Misawa Medical Industry, Ibaraki, Japan; Fig. 1). Embryo handling was performed at 22–25 °C and all media were maintained at 38 °C. The embryos were kept in PBM for 2 min, equilibrated for 5 min in 20 mM Hepes-buffered PBM (Hepes-PBM) supplemented with 1.8 M ethylene glycol (EG; equilibrate medium 1), and then kept in equilibrate medium 2

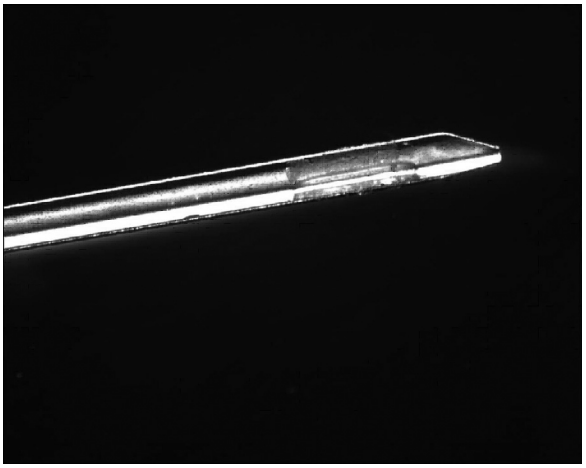

Fig. 1. A stainless steel vitrification instrument (HI Stick).