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ARTICLE

Hydroxypropyl cellulose as an option for supplementation of cryoprotectant solutions for embryo vitrification in human assisted reproductive technologies

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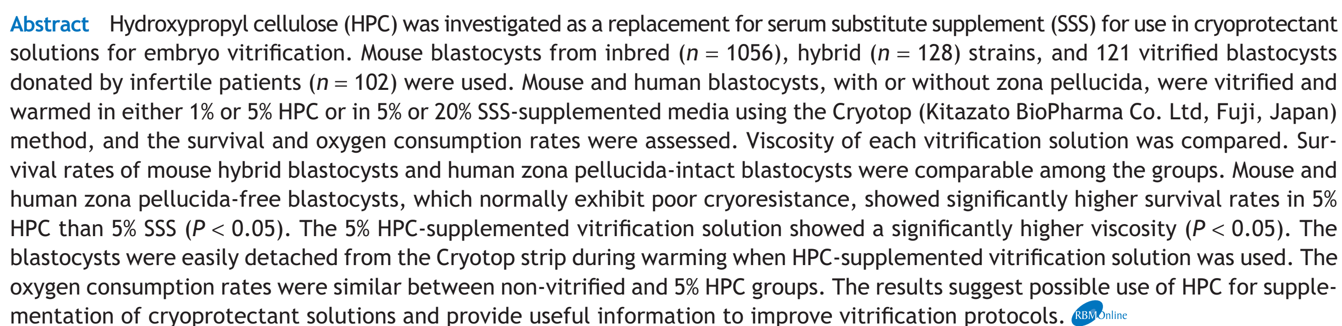

Abstract Hydroxypropyl cellulose (HPC) was investigated as a replacement for serum substitute supplement (SSS) for use in cryoprotectant solutions for embryo vitrification. Mouse blastocysts from inbred ($n = 1056$), hybrid ($n = 128$) strains, and 121 vitrified blastocysts donated by infertile patients ($n = 102$) were used. Mouse and human blastocysts, with or without zona pellucida, were vitrified and warmed in either 1% or 5% HPC or in 5% or 20% SSS-supplemented media using the Cryotop (Kitazato BioPharma Co. Ltd, Fuji, Japan) method, and the survival and oxygen consumption rates were assessed. Viscosity of each vitrification solution was compared. Survival rates of mouse hybrid blastocysts and human zona pellucida-intact blastocysts were comparable among the groups. Mouse and human zona pellucida-free blastocysts, which normally exhibit poor cryoresistance, showed significantly higher survival rates in 5% HPC than 5% SSS ($P < 0.05$). The 5% HPC-supplemented vitrification solution showed a significantly higher viscosity ($P < 0.05$). The blastocysts were easily detached from the Cryotop strip during warming when HPC-supplemented vitrification solution was used. The oxygen consumption rates were similar between non-vitrified and 5% HPC groups. The results suggest possible use of HPC for supplementation of cryoprotectant solutions and provide useful information to improve vitrification protocols.

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Introduction

Over the past decade, vitrification has been refined as an alternative to slow freeze for human oocyte and embryo cryopreservation (Boyer et al., 2013; Roque et al., 2013), and is becoming increasingly accepted as a preferred method. It is widely applied in IVF centres worldwide (Gosden, 2011).

Vitrification involves the solidification of the cell into a glass-like amorphous state without ice crystal formation, and can be achieved by rapid cooling with highly concentrated cryoprotectant (CPA) (Vajta, 2013). In oocyte and embryo vitrification, the addition of CPAs in the vitrification media, such as ethylene glycol, dimethylsulphoxide (DMSO), propane-1–2-diol (PrOH) or various polymers are important to yield a high survival rate after vitrification and warming (Checura and Seidel, 2007; Horvath and Seidel, 2006; Kuleshova et al., 2001).

The Cryotop device (Kitazato BioPharma Co. Ltd, Fuji, Japan) is becoming widely used for human oocyte and embryo cryopreservation (Kato et al., 2012; Maheshwari et al., 2012). A literature search identified that the human oocyte survival rate after vitrification and warming by the Cryotop® method was between 90 and 97%, and no differences were found in fertilization rates or pregnancy rates compared with non-vitrified fresh oocytes (Cobo et al., 2010; Forman et al., 2012). The composition of CPA in Cryotop (Kitazato BioPharma Co. Ltd, Fuji, Japan) vitrification media includes ethylene glycol, DMSO, and serum substitute supplement (SSS) as supplements for CPA solutions. It has been shown that SSS supplementation of CPA solutions results in protection against cell injury and, indeed, survival rates declined when SSS was removed from vitrification media (Mori and Kuwayama, 2009).

Serum substitute supplement (SSS) is generated from a purified fraction of human serum and consists of 16% alpha- and beta- globulins and 84% human serum albumin. Meintjes et al. (2009) reported that SSS supplementation in IVF embryo culture media resulted in better clinical outcomes compared with human serum albumin supplementation alone. On the other hand, the use of human serum also may bear a number of disadvantages such as cost, qualitative variations and the risk of contamination by unidentified viruses (Shaw et al., 1997). Despite the fact that no incidences of infection have been reported by the use of SSS in vitrification media, recent trends towards the use of non-human products have been observed.

As a candidate for a non-human supplement to CPA solutions, hydroxypropyl cellulose (HPC) was selected as a substitute for SSS. It is formulated by reacting propylene oxide with cellulose. It is a large molecule and its molecular weight is comparable to SSS (HPC: ranges from 45,000–100,000 Da, SSS: approximately 66,000 Da). It becomes soluble by introducing hydroxypropoxy groups into cellulose to prevent the formation of hydrogen bonds between the hydroxyl groups in the cellulose. Compared with SSS, HPC is more cost effective, and is also listed as pharmacopeia in many countries including the USA, the UK and Japan. It is widely used in drug manufacturing and as a food additive. Therefore, the safety of using HPC has already been addressed.

In the present study, HPC is proposed as an option for supplementation of CPA solutions by examining the survival rate of human and mouse expanded blastocysts after vitrification and warming. Additionally, human hatched blastocysts are generally less tolerant to cryopreservation in assisted reproduction techniques (Cobo et al., 2012; Escriba et al., 2010; Valbuena et al., 2012). Therefore, zona pellucida-free blastocysts, which mimic the hatched blastocysts, were also used to examine whether HPC-supplemented media may induce an increase in the survival of hatched blastocysts during vitrification and warming.

Materials and methods

Animal maintenance

All mice were housed in an isolator for experimental animals (Innorack® IVC; Oriental Giken Inc., Tokyo, Japan) under a 12-h light–12-h dark cycle at 24°C. Free access to a standard mouse diet and water was provided. All experimental procedures were carried out in accordance with the 'Guidelines for Proper Conduct of Animal Experiments' established by the Science Council of Japan.

Institutional review board approval

All studies with human blastocysts were approved by the Institutional Review Board of Kato Ladies Clinic on 12 September 2013 (Approval number: 13-06). Informed consent was obtained from all couples.

Mouse embryo collection

Female mice (C57BL/6J) aged 4–5 weeks (Sankyo laboratory, Tokyo, Japan, $n = 56$) were superovulated by intraperitoneal injection of 5 IU pregnant mare's serum gonadotropin (PMSG, ASKA Pharmaceutical Co., Ltd., Tokyo, Japan) followed by 5 IU human chronic gonadotropin (HCG, ASKA Pharmaceutical Co., Ltd., Tokyo, Japan) 48 h later. On the night of HCG injection, females were mated with C57BL/6J or CBA males to obtain fertilized embryos of inbred C57BL/6J or B6CBA (C57BL/6J x CBA) hybrid strains. At 44–48 h after HCG administration, mice were killed and two-cell embryos were collected from oviducts. The two-cell embryos were then cultured in KSOM+AA media (EmbryoMax Medium (1X) w1/2 Amino Acids & Phenol Red, Merck Millipore, MA, USA) at 37°C in 5% CO_2 and 95% air for 72 h, until the blastocyst stage.

To mimic human hatched blastocysts, zona pellucida-free mouse blastocysts were prepared. Blastocysts were treated with acid Tyrode's solution for 30 s to dissolve the zona pellucida, and zona pellucida-free blastocysts were transferred into KSOM+AA media until vitrification.

Warming of human blastocysts donated by IVF patients

Human vitrified blastocysts included in this study were donated from 102 patients (under 39 years of age) who had stored

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