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Effect of resveratrol supplementation during culture on the quality and cryotolerance of bovine in vitro produced embryos

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ABSTRACT

The aim of the study was to evaluate whether resveratrol supplementation of bovine culture medium improves in vitro blastocyst development, embryo cryotolerance and cell numbers. Abattoir-derived oocytes were matured and fertilized in vitro according to standard procedure. Twenty hours after IVF, zygotes were cultured in SOF medium, supplemented with 0 (control, $n = 439$), 0.25 μM ($n = 422$), 0.5 μM ($n = 447$) and 1 μM resveratrol ($n = 416$). On Day 7 (IVF = Day 0) blastocysts were vitrified by cryotop in 16.5% ethylene glycol, 16.5% dimethyl sulfoxide and 0.5 M sucrose. Development rate, i.e. the percentage of embryos resuming development to reach a more advanced stage, and hatching rate were evaluated after 24 and 48 h culture. Blastocysts cultured with (0.5 μM) and without resveratrol underwent differential staining to count inner cell mass (ICM) and trophectoderm (TE) cells. Resveratrol during culture did not increase blastocyst yields (57.1, 57.7, 59.2 and 46.6%, respectively in 0, 0.25, 0.5 and 1 μM resveratrol). However, 0.5 μM resveratrol improved embryo cryotolerance compared to the control, as indicated by higher development rates (67.3% vs 50.3%, respectively; $P < 0.01$) and hatching rates (58.9% vs 30.9%, respectively; $P < 0.01$) recorded after 48 h post-warming culture. Blastocysts produced in the control and in 0.5 μM resveratrol groups had similar numbers of ICM (34.1 and 36.4, respectively), TE (88.1 and 85.3, respectively) and total (122.2 and 121.7, respectively) cells. In conclusion, low levels of resveratrol during in vitro culture improve the quality of IVP bovine embryos, as indicated by their increased resistance to cryopreservation.

1. Introduction

A great improvement of in vitro embryo production (IVEP) efficiency in cattle has been recorded over the years; nevertheless, in vitro produced (IVP) embryos are still less viable and resistant to cryopreservation than their in vivo counterparts (Leibo and Loskutoff, 1993; Hasler, 2000). Differences between in vivo and in vitro embryos exist at the level of morphology, metabolism, gene expression and cryotolerance, and may have a knock-on effect further along the developmental axis (Rizos et al., 2008). The ability to cryopreserve embryos, without critical loss of viability, has a profound effect on the success of assisted reproductive techniques. However, the survival of cryopreserved IVP embryos, as measured either by post-warming survival in culture or by established pregnancies after

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embryo transfer, has lagged behind that of in vivo-derived embryos (Massip et al., 1995; Kaidi et al., 1998; Lonergan et al., 2003a,b; Gomez et al., 2009). Although these differences are not completely understood, culture medium has been shown to effect embryo developmental competence, and the ability to withstand cryopreservation (Hosseini et al., 2009). Deficiencies in culture conditions may lead to aberrant embryo development, indicated by lower frequency of blastocyst formation and lower cell numbers, that can affect fetal and postnatal life (Duranthon et al., 2008). Therefore, the optimization of in vitro culture system is critical to improve embryo viability.

A major factor impairing in vitro embryo development is the increased oxidative stress. Therefore, various antioxidants such as β-mercaptoethanol, cysteine and cysteamine have been added to the IVM (de Matos and Furnus, 2000) and IVC media (Hosseini et al., 2009; George et al., 2008) to improve the development of pre-implantation embryos.

Resveratrol (3,4′,5-trihydroxystilbene) is a phytoalexin identified in more than 70 plant species including grapes, plums, and peanuts (Baur and Sinclair, 2006). It is also an important antioxidant polyphenolic compound, which contributes to red wine's beneficial effects on the prevention of human cardiovascular disease. Recently, the interest in resveratrol has increased exponentially, following the major findings that this molecule has positive effects on cancer chemoprevention, cardioprotection, inflammatory processes, several aspects of metabolism, leading to increased lifespan of various organisms, from yeasts to vertebrates (Pirola and Fröjdö, 2008).

Several studies have been conducted to identify the biologic functions and activities of resveratrol for mammalian reproduction (Kwak et al., 2012; Huang et al., 2013). A positive effect of resveratrol on in vitro embryonic development was demonstrated in swine, as indicated by enhanced blastocyst formation and improved embryo quality (Lee et al., 2010). Recently, Wang et al. (2014) demonstrated that the addition of resveratrol to the in vitro maturation medium increases progesterone secretion and decreases estradiol secretion by cumulus cells, promoting cumulus expansion and polar body formation in bovine oocytes and improves blastocyst yield. Preliminary results obtained in our laboratory in a small-scale trial suggested a beneficial effect of 0.5 μM resveratrol during culture on the resistance to cryopreservation of IVP bovine embryos (Abdel-Wahab et al., 2012). However, the limited numbers of embryos cryopreserved recommended further investigations to increase case history and test lower concentrations of the compound. Therefore, the aim of this work was to evaluate whether supplementation of culture medium with resveratrol improves in vitro blastocyst development and embryo quality in cattle, the latter assessed both in terms of cryotolerance and allocation of blastocyst cells into the inner cell mass (ICM) and trophectoderm (TE) lineages.

2. Materials and methods

Unless stated otherwise, all chemicals were obtained from Sigma–Aldrich Chemical (Milano, Italy)

2.1. Experimental design

A preliminary dose response trial (0–10 μM) demonstrated deleterious effects of resveratrol on both cleavage and blastocyst development at concentration ≥5 μM. Therefore, the range of concentrations to test was reduced in the following experiment, in which presumptive zygotes were cultured in vitro with 0 (control, $n = 439$), 0.25 μM ($n = 422$), 0.5 μM ($n = 447$) and 1 μM resveratrol ($n = 416$), over six replicates. To assess the embryo quality, the vitrified-warmed day 7 (end of culture) blastocysts (on average 153 per culture group) were cultured for 2 days to evaluate their resistance to cryopreservation. Finally, zygotes were cultured in the absence (control) and presence of 0.5 μM resveratrol to produce blastocysts for subsequent differential staining.

2.2. Reagents and media

The oocyte aspiration medium was TCM 199 supplemented with 25 mM Hepes, 2 mM sodium bicarbonate, 2 mM sodium pyruvate, 1 mM L-glutamine, 10 μL/mL amphotericin B (H199) supplemented with 2% bovine serum (BS) and 95.6 SI/mL heparin. The IVM medium was TCM 199 supplemented with 15% bovine serum (BS), 0.5 μg/mL FSH, 5 μg/mL LH, 0.8 mM L-glutamine and 50 μg/mL gentamycin. The IVF medium was Tyrode's modified medium (Parrish et al., 1986) without glucose and bovine serum albumin (BSA), supplemented with 5.3 SI/mL heparin, 30 μM penicillamine, 15 μM hypotaurine, 1 μM epinephrine and 1% of BS. The IVC medium consisted of synthetic oviduct fluid (SOF) medium (Tervit et al., 1972), with 30 μL/mL essential amino acids, 10 μL/mL non-essential amino acids and 5% BS. Resveratrol was dissolved in dimethyl sulfoxide (DMSO) to make a 1000× stock (1 mM) that was kept frozen until day of use, when the lower stock concentrations were obtained by dilutions. Then the relative stock solutions were diluted 1:1000 in SOF, to reduce to the minimum the amount of DMSO during culture. Likewise, DMSO (1:1000) was added to the control group.

The base medium for the vitrification and warming solutions was H199 with 20% fetal calf serum (FCS). The equilibration medium (VS1) consisted of 7.5% DMSO and 7.5% ethylene glycol (EG) in base medium and the vitrification solution (VS2) consisted of 16.5% DMSO and 16.5% EG with 0.5 M sucrose in the base medium. The warming solutions consisted of 0.25 M and 0.15 M sucrose in the base medium.

2.3. In vitro embryo production

Abattoir-derived oocytes were matured and fertilized in vitro according to our standard procedure (Rubessa et al., 2011). Briefly, bovine ovaries were recovered from a local abattoir and transported to the laboratory, in physiological saline at 30–35 °C. Cumulus–oocyte complexes (COCs) were aspirated from follicles of 2–8 mm in diameter and only those with uniform cytoplasm and multilayered cumulus cells were selected, washed twice in the aspiration medium and once in the IVM medium. Groups of 25 COCs were matured in 400 μL of IVM medium, covered