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Effect of estrous cow serum during bovine embryo culture on blastocyst development and cryotolerance after slow freezing or vitrification

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Abstract

The present study investigated the effect of estrous cow serum (ECS) during culture of bovine embryos on blastocyst development and survival after cryopreservation by slow freezing or vitrification. Embryos were derived from in vitro maturation (IVM) and in vitro fertilization (IVF) of abbatoir-derived oocytes. At Day 3, embryos were cultured in three different media: Charles Ronsenkrans medium + amino acids (CR1aa; without bovine serum albumin (BSA)) + 5% estrous cow serum (CR1-ECS), CR1aa + 3 mg/mL BSA (CR1-BSA) or CR1aa + 5% ECS + 3 mg/mL BSA (CR1-ECS-BSA). At 7.5 d post-insemination (PI), blastocyst yield and quality were evaluated; blastocysts and expanded blastocysts from each media were cryopreserved by Open Pulled Straw (OPS) vitrification method or slow freezing (1.5 M ethylene glycol, EM). Total blastocyst yield did not differ among CR1-ECS, CR1-BSA and CR1-ECS-BSA (30.9, 33.1 and 32.9%, respectively, $P < 0.05$). Embryo survival (hatching rate) was higher in vitrified versus slow-frozen embryos (43% versus 12%, respectively, $P < 0.01$), and in embryos cultured in CR1-BSA (40.3%) compared with those cultured in serum-containing media (CR1-ECS, 21.5% and CR1-ECS-BSA, 19.8%; $P < 0.01$). In conclusion: (a) it was possible to produce in vitro bovine embryos in serum-free culture medium without affecting blastocyst yield and quality; (b) serum-free medium produced the best quality

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embryos (in terms of post-cryopreservation survival); and (c) vitrification yielded the highest post-cryopreservation survival rates, regardless of the presence of serum in the culture medium.

Keywords: Cryopreservation; Bovine embryo; In vitro culture; Serum

1. Introduction

With current in vitro techniques, large numbers of bovine preimplantation embryos can be produced at relatively low cost. These embryos can be used for basic scientific research (e.g. embryo development, transgenesis, cloning) or for commercial purposes. However, in vitro produced (IVP) embryos are characterised by increased chilling sensitivity and decreased ability to tolerate cryopreservation, compared to their in vivo counterparts [1,2]. Until now, the major obstacle associated with the extensive use of this technology is the lack of suitable methods to preserve in vitro produced embryos. There are at least two approaches to overcome this problem; adjust cryopreservation methods or improve embryo quality by optimising the in vitro environment for embryo production.

At present, slow freezing and vitrification are commonly used to cryopreserve bovine embryos. Slow freezing, which is most widely used, has the advantage of using low concentrations of cryoprotectants, and allows post-thaw direct transfer [3]. Calving rates are slightly lower with in vivo produced embryos after freezing than with fresh ones. However, slow freezing of IVP bovine embryos reduced post-thaw survival rates compared with their in vivo counterparts, mostly due to their susceptibility to ice crystal formation [4]. Vitrification is the solidification of a solution brought about not by crystallisation but by extreme elevation in viscosity during rapid cooling. Although vitrification eliminates ice crystal injury, the high cryoprotectant concentration required to achieve the glass state increases the risk of osmotic and toxic damage [5]. Direct comparisons between the two cryopreservation methods has led to different results, but it seems that vitrification procedures are most suitable for in vitro fertilization (IVF)-produced embryos [6], especially when high cooling rates are applied (in order to reduce adverse effects) [7].

Whereas innate oocyte quality profoundly affects the percentage of oocytes developing to the blastocyst stage, recent data suggested that the in vitro culture environment following fertilisation is a key determinant of blastocyst quality [8]. Almost all media used for embryo development contain serum or bovine serum albumin (BSA) as source of protein; several authors have demonstrated the favourable effects of these products on embryo development [9]. The presence of serum accelerated the development of morula and blastocysts compared with those produced in its absence [10]. However, the role of serum in in vitro embryo development is still unknown. It is expected that serum provides energy substrates, amino acids, vitamins, growth factors, and heavy-metal chelators; however, the presence or concentrations of these components may vary among batches [11]. Several studies have demonstrated that culture medium supplemented with serum can cause morphological [12,13] and physiological [14,15] differences in embryos compared to those produced in vivo or in serum-free media; these differences include increased number and size of lipid droplets [16,17] and differences in embryo quality [18,19]. Interestingly,