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Defined media optimization for *in vitro* culture of bovine somatic cell nuclear transfer (SCNT) embryos

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Abstract

The objective was to establish an efficient defined culture medium for bovine somatic cell nuclear transfer (SCNT) embryos. In this study, modified synthetic oviductal fluid (mSOF) without bovine serum albumin (BSA) was used as the basic culture medium (BCM), whereas the control medium was BCM with BSA. In Experiment 1, adding polyvinyl alcohol (PVA) to BCM supported development of SCNT embryos to blastocyst stage, but blastocyst formation rate and blastocyst cell number were both lower ($P < 0.05$) compared to the undefined group (6.1 vs. 32.6% and 67.3 ± 3.4 vs. 109.3 ± 4.5, respectively). In Experiment 2, myo-inositol, a combination of insulin, transferrin and selenium (ITS), and epidermal growth factor (EGF) were added separately to PVA-supplemented BCM. The blastocyst formation rate and blastocyst cell number of those three groups were dramatically improved compared with that of PVA-supplemented group in Experiment 1 (18.5, 23.0, 24.1 vs. 6.1% and 82.7 ± 2.0, 84.3 ± 4.2, 95.3 ± 3.8 vs. 67.3 ± 3.4, respectively, $P < 0.05$), but were still lower compared with that of undefined group (33.7% and 113.8 ± 3.4, $P < 0.05$). In Experiment 3, when a combination of myo-inositol, ITS and EGF were added to PVA-supplemented BCM, blastocyst formation rate and blastocyst cell number were similar to that of undefined group (30.4 vs. 31.1% and 109.3 ± 4.4 vs. 112.0 ± 3.6, $P > 0.05$). In Experiment 4, when blastocysts were cryopreserved and subsequently thawed, there were no significant differences between the optimized defined group (Experiment 3) and undefined group in survival rate and 24 and 48 h hatching blastocyst rates. Furthermore, there were no significant differences in expression levels of *H19*, *HSP70* and *BAX* in blastocysts derived from optimized defined medium and undefined medium, although the relative expression abundance of *IGF-2* was significantly decreased in the former. In conclusion, a defined culture medium containing PVA, myo-inositol, ITS, and EGF supported *in vitro* development of bovine SCNT embryos.

Keywords: Defined culture medium; Embryo culture; SCNT; Bovine; Embryo development

1. Introduction

Since the birth of Dolly [1], many other animal species have been successfully cloned from somatic cells. Bovine somatic cell nuclear transfer (SCNT) is widely studied to explore mechanisms of somatic cell nuclear reprogramming, improve efficiency of bovine cloning, and produce cloned transgenic cattle.

Because culture media has major effects on *in vitro* production of embryos, various media have been developed for culture of bovine embryos [2]. In general, most culture media for *in vitro* culture of bovine SCNT

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embryos contain serum or bovine serum albumin (BSA), as they usually accelerate *in vitro* development of embryos [3–7]. Furthermore, BSA can bind heavy metal ions, free radicals and toxins, and regulate redox potential, pH, and osmolarity [8], thereby promoting embryo development. However, blastocysts derived from serum-supplemented media have several morphologic differences and more cytoplasmic fragmentations, as well as more cytoplasmic lipid droplets compared to those derived *in vivo* or from serum-free media [9,10]. In addition, embryos cultured in media supplemented with serum or BSA often have "Large offspring syndrome" (LOS) with a greater incidence of stillbirths and premature deaths [11,12], thereby lowering cloning efficiency and raising biosecurity risks in production of cloned cattle. Furthermore, the highly variable composition of serum and BSA [12] makes it difficult to clarify functions of factors involved in early development of SCNT embryos. Therefore, an efficient, serum/BSA free, chemically defined culture medium is required for cloned cattle production. At present, the most widely used culture medium for bovine embryos is modified synthetic oviductal fluid (mSOF) [13], a medium with high competence to support *in vitro* and *in vivo* development of bovine embryos [2,14,15]. Accordingly, mSOF without BSA was used as the basic culture medium (BCM) in the present study.

Polyvinyl alcohol (PVA), a synthetic polymer, was a suitable serum/BSA substitute in chemically defined embryo culture media, especially for bovine embryos [16,17]. However, PVA supplement only acted as a surfactant to assist embryo handling [18]. Although BSA has impurities, it also provides energy substrates, amino acids, vitamins, growth factors and heavy-metal chelators which support development of early embryos [19]. Compared to BSA, PVA provided less support for embryo development. Therefore, to compensate for the shortcomings of PVA and enhance the development capacity of embryos in a defined medium, myo-inositol, insulin, transferrin and selenium (ITS) and epidermal growth factor (EGF) were also added to the defined culture medium. In that regard, myo-inositol belongs to the vitamin B complex group [20] and is important in metabolic interconversions during embryo development [6,21]; ITS can promote energy uptake and remove toxic factors [22,23]; and EGF is a well-known growth factor important for *in vitro* embryo development [24].

The objective of the present study was to develop an efficient defined culture medium for bovine SCNT embryos.

2. Materials and methods

2.1. Materials

Unless otherwise noted, all chemicals were purchased from the Sigma Chemical Company (St. Louis, MO, USA). Disposable, sterile plasticware was purchased from Nunclon (Roskilde, Denmark). All procedures were approved by the Animal Care and Use Committee of Northwest A and F University and performed in accordance with animal welfare and ethics.

2.2. Oocyte collection and in vitro maturation (IVM)

Bovine ovaries were obtained from local abattoirs and transported to the laboratory (within 4 h after death) in sterile 0.9% NaCl saline at 15 to 20 °C [25] in a vacuum bottle. Cumulus oocyte complexes (COCs) were aspirated from antral follicles (2–8 mm in diameter) using a 12-gauge disposable syringe. The COCs with evenly granulated cytoplasm and more than three layers of compact cumulus cells were selected and washed three times in maturation medium, comprised of TCM-199 supplemented with 10% (v/v) FBS, 1 μg/mL 17β-estradiol, and 0.075 IU/mL Human menopausal gonadotropin (TCM-199 and FBS, GIBCO, Grand Island, NY, USA; HMG, Livzon, Zhuhai, Guangdong, China). Then, the COCs were transferred into maturation medium and incubated at 38.5 °C in a humidified incubator of 5% CO_2 in air for approximately 20 h.

2.3. Preparation of donor cells

Donor somatic cells were derived from a 1-wk-old Holstein heifer. Briefly, ear skin was collected and minced with sterile scissors in a 35 mm Petri dish. Explants (approximately 1 mm in diameter) were cultured in Dulbecco's Modified Eagle's medium (DMEM, GIBCO, Grand Island, NY, USA) containing 10% FBS, 1 mM sodium pyruvate, 100 IU/mL penicillin, and 100 mg/mL streptomycin under 5% CO_2 in air at 37.5 °C. Once cells reached 90% confluence, they were trypsinized and reconstituted at a concentration of 1×10^6 cells/mL. The second to fifth passage of cell line were used as nuclei donors.

2.4. Production of SCNT embryos

Nucleus transfer was performed essentially as described [26]. Briefly, matured oocytes were denuded of cumulus cells by treatment with 0.1% bovine testicular hyaluronidase in PBS. Only oocytes with a first polar body were selected and used for SCNT; they were transferred into droplets of PBS supplemented with 7.5