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Improved in vitro bovine embryo development and increased efficiency in producing viable calves using defined media

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Abstract

In this study, we developed a defined culture medium that supported improved in vitro bovine embryo development and calving rate after embryo transfer (ET). In vitro-matured bovine oocytes from abbatoir-derived ovaries from Korean native, HanWoo cattle were fertilized with frozen–thawed spermatozoa and embryos were cultured in two-step culture media. In Experiment 1, embryos were cultured in media supplemented with 8 mg/mL BSA, or 0.1 mg/mL PVA and 8 mg/mL BSA + 2.77 mM myo-inositol or 0.1 mg/mL PVA + 2.77 mM myo-inositol. Although defined culture media containing PVA supported lower developmental competence compared to undefined media (containing BSA; 8% versus 34%, respectively), defined culture media containing 2.77 mM myo-inositol increased rates of blastocyst formation up to 28%. In Experiment 2, the effect of energy substrate (1.5 mM glucose or 1.2 mM phosphate) in PVA–myo-inositol defined culture medium on in vitro embryo development was investigated. Defined culture media containing PVA, myo-inositol and phosphate supported better embryo development to blastocysts compared to medium supplemented with both glucose and phosphate (43% versus 31%). In Experiment 3, the effect of epidermal growth factor (EGF) in PVA + myo-inositol–phosphate two-step culture medium on in vitro embryo development was investigated. Among 0, 1, 10 and 100 ng/mL EGF concentrations, the maximal effect was observed with 10 ng/mL EGF (52% blastocyst formation). In Experiment 4, total cell number and calving rate were compared between defined PVA–myo-inositol–phosphate–EGF medium and undefined medium containing BSA, glucose and phosphate. No differences in total cell number of blastocysts obtained from the two groups were observed, however, the rate of viable offspring production was increased using the defined culture medium, compared to the undefined culture medium. In Experiment 5, the relative abundance of mRNA transcripts [interferon-tau (If-τ), glucose transporter-1 (glut-1) and insulin like growth factor 2 receptor (Igf2r)] were analyzed in blastocysts derived from undefined or defined culture media. Gene expression of If-τ, glut-1 was significantly increased in defined culture medium compared to undefined medium. In conclusion, chemically defined culture media without BSA or FBS improved developmental competence of in vitro cultured bovine embryos and delivery of viable calves after ET.

Keywords: Culture media; PVA; Embryo development; Embryo transfer; Calving rate

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1. Introduction

In order to improve in vitro culture systems for bovine blastocyst production, culture media have been supplemented with a variety of antioxidants, growth factors and/or macromolecules. Among the latter, bovine serum albumin (BSA) and fetal bovine serum (FBS), which are widely used as protein sources for embryo culture media, are complex and undefined mixtures of proteins, growth factors, peptides, etc. Serum and BSA can have a stimulatory effect on embryo growth [1–3]. Thompson [4] clearly demonstrated that BSA played a substantial nutritive role during blastocyst development, especially post-compaction. This is perhaps not surprising given that albumin is the most prevalent extracellular protein in the mammalian reproductive tract. Although BSA had a beneficial effect on embryo development, it is difficult to elucidate specific functions of growth factors or other stimulants because BSA is an undefined mixture of compounds. Chemically defined conditions without serum or serum-derived proteins allow more precise observation of the effects of growth or other embryotrophic factors in a given medium [5–7]. It was demonstrated that chemically defined medium supported bovine zygote development to blastocysts, with varying rates of success [8,9].

In a large field study with in vitro produced (IVP) bovine preimplantation embryos, pregnancy rates following transfer of IVP embryos ranged from about 12 to 50% [10]. Factors shown to influence the maintenance of pregnancy following transfer of IVP embryos include the embryo culture system, embryo quality, embryo evaluator, number of embryos transferred per recipient, synchrony of embryo development with the recipient's day of estrous cycle, transfer technician, fresh versus frozen embryos and heat stress effects on the embryo or recipient [11–15]. Among these factors, embryo quality produced in vitro culture system was considered a critical factor.

Accordingly, the present study was performed to: (1) optimize a BSA- and FBS-free chemically defined culture medium by supplementing it with myo-inositol, EGF, or both, and modifying energy substrates; (2) produce offspring by transferring preimplantation stage embryo obtained using the improved chemically defined culture medium; (3) compare the gene expression patterns of preimplantation embryos produced with the chemically defined and undefined media. The genes were interferon tau (If-τ), insulin-like growth factor 2 receptor (Igf2r), glucose transporter-1 (glut-1) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

2. Materials and methods

2.1. Oocyte collection and in vitro maturation (IVM)

Korean native, *HanWoo* cattle ovaries were collected from a local abbatoir into saline at 35 °C and transported to the laboratory within 2 h. Cumulus-oocyte complexes (COCs) from follicles 2–8 mm in diameter were aspirated using an 18 gauge needle attached to a 10 mL disposal syringe. The COCs with evenly-granulated cytoplasm and enclosed by more than three layers of compact cumulus cells were selected, washed three times in HEPES-buffered tissue culture medium-199 (TCM-199; Invitrogen, Carlsbed, CA, USA), supplemented with 10% FBS, 2 mM $NaHCO_3$ (Sigma–Aldrich Corp., St. Louis, MO, USA), and 1% penicillin–streptomycin (v/v). For IVM, COCs were cultured in four-well dishes (30–40 oocytes per well; Falcon, Becton-Dickinson Ltd., Plymouth, UK) for 22 h in 450 μL TCM-199 supplemented with 10% FBS, 0.005 AU/mL FSH (Antrin, Teikoku, Japan), and 1 μg/mL 17β-estradiol (Sigma–Aldrich Corp.) at 39 °C in a humidified atmosphere of 5% CO_2.

2.2. Sperm preparation and in vitro culture of embryos

Motile spermatozoa were selected by a swim-up technique [16]. At 22 h of IVM, oocytes were inseminated (day 0) with 1×10^6 spermatozoa/mL for 18 h in 50 μL/well of tyrode's albumin-lactate-pyruvate (TALP)-IVF medium on a four-well plate. Groups of six or seven zygotes were cultured in 25 μL microdrops of the two-step defined culture medium, as described in Table 1 [17], overlaid with mineral oil (Sigma–Aldrich Corp.). Presumptive zygotes were cultured in early stage medium for the first 5 days post-insemination, and then moved into later stage medium. All incubations were done at 39 °C in an atmosphere of 5% O_2, 5% CO_2 and 90% N_2. Cleavage and blastocyst formation were recorded on days 2 and 7, respectively. Hatching of blastocysts was recorded on day 9.

2.3. Evaluation of blastocyst quality

Randomly selected blastocysts at day 8 were used for differential staining. The cell numbers of blastomeres, inner cell mass (ICM) and trophectoderm (TE) cells in blastocysts were counted after staining, as described [18]. Blastocysts were incubated in 500 μL of BSA-free, HEPES-buffered TCM-199 supplemented with 1% (v/v) Triton X-100 and 100 μg/mL propidium