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In vitro production of bovine embryos in medium supplemented with a serum replacer: Effects on blastocyst development, cryotolerance and survival to term

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Abstract

In this study, we evaluated a serum replacer (SR; Knockout SR®, Invitrogen) in our in vitro culture systems. We hypothesized that SR would benefit bovine embryo development, since SR supported survival of embryonic stem cells (which originate from embryos). Experiment 1 compared oocyte maturation with SR versus fetal bovine serum (FBS). Following fertilization, blastocyst development was lower for oocytes matured with SR (21.5 versus 34.1, $P < 0.05$). Experiment 2 evaluated SR for culturing embryos. Following fertilization, embryos were cultured for 3 days in KSOM, and then assigned to treatments: (1) KSOM static culture (KNM); (2) fresh KSOM (KD3); (3) KSOM + SR or (4) KSOM + FBS and cultured to Day 7 (fertilization = Day 0). Blastocyst development in FBS or SR was higher than either KNM or KD3 (48.2, 47.2, 32.7, and 35.5, respectively, $P < 0.05$). Experiment 3 evaluated cryosurvival of embryos cultured in the same manner as Experiment 2. On Day 7, embryos were vitrified and upon warming, embryos cultured in SR had greater 24 h survival rates (70.6%) than all other treatments ($P < 0.05$). Finally, Experiment 4 evaluated effects of SR on pregnancy rate and development to term. Culture in SR was not detrimental to pregnancy or calving rates (50 and 50%, respectively), and SR calves had normal birth weights (mean = 38.8 kg ± 1.5). In conclusion, the use of SR for maturation of oocytes was not beneficial; however, SR enhanced embryo culture by improving development in vitro, cryotolerance and survival, effectively replacing serum in culture.

Keywords: Maturation; In vitro embryo culture; Cryosurvival; Vitrification; Pregnancy rate

1. Introduction

Assisted reproductive technologies offer great opportunities to the livestock industries for increasing numbers of genetically superior animals and advancing reproductive efficiencies. However, the quality and survival of in vitro produced (IVP) bovine embryos has lagged behind that of in vivo derived embryos [1]. Although 70–80% of bovine oocytes are successfully fertilized in vitro, only 20–40% will survive the first week of life [2]. Culture conditions play a key role in cleavage, embryonic genome activation, compaction, differentiation, as well as later fetal viability and development [3,4]. The challenge is to develop a culture system or systems that meet the changing needs of the developing bovine embryo, minimize cellular stress and

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prevent loss of viability. This in turn will help make the assisted reproductive technologies, such as ovum pick up (OPU), intracytoplasmic sperm injection (ICSI), somatic cell nuclear transfer (SCNT), embryo cryopreservation and others, more feasible for use by the livestock industry.

Blood serum is a commonly used supplement for both oocyte maturation and embryo culture (see review [5]). Serum is known to contain growth factors, chelate heavy metal cations and serves as an osmolyte, as well as a surfactant [6]. Use of serum in embryo culture is known to benefit later stages of preimplantation embryo development by improving blastocyst yield [7,8]. However, it also gives variable results due to its source and the undefined components it contains, and has the added risk of possible contamination with viruses. Use of serum in culture can also alter the metabolism of the early embryo, and can lead to increased fatty acid content in blastocysts [9]. Serum supplementation has been associated with accumulation of cytoplasmic lipid droplets in IVP embryos [10] and can lead to poor cryosurvival [11]. Furthermore, serum in culture can increase the frequency of mixoploid embryos [12] and can induce alterations in gene expression profiles compared to in vivo derived embryos, giving further cause for concern [13–15]. Additionally, serum in culture reduces survival following embryo transfer and can cause problems later in development, including large offspring syndrome [13,16–18]. To circumvent problems related to serum, more defined culture systems have been developed.

In vivo derived embryos never come into contact with serum. It is therefore not surprising that some components may be detrimental. To overcome these issues with serum, commercially available serum replacers have been developed and tested extensively in human embryo culture. Some have demonstrated benefits over serum for production of blastocysts and increasing pregnancy rates [19–21]. However, use of serum replacers have been less common in bovine culture systems and have produced variable results [22,23].

One serum replacer, Knockout SR® (SR; Invitrogen Corp., Gaithersburg, MD, USA), was designed as a substitute for serum for culturing embryonic stem (ES) cells [24]. These cells are derived from the inner cell mass of the blastocyst stage embryo. Supplementation with SR allowed proliferation of ES cells while maintaining their pluripotency. Hence, SR may provide beneficial factors that maintain proper early embryonic development, without introducing the detrimental and highly variable constituents that are present in serum. Knockout SR® has not yet been evaluated for bovine embryo production.

The aim of this study was to test the commercially available serum replacer, Knockout SR® (Invitrogen) in our in vitro maturation (IVM), in vitro culture (IVC) and vitrification systems, as a possible means of removing the detrimental effects of serum, and improving long-term embryo survival.

2. Materials and methods

2.1. Collection and maturation of oocytes

Oocyte collection and maturation were performed as previously described [25]. Briefly, ovaries were collected from the abattoir and transported to the laboratory in saline supplemented with penicillin/streptomycin (1000 U penicillin and 1000 μg streptomycin, Invitrogen). Cumulus oocyte complexes (COCs) were recovered by slashing 2–8 mm follicles with a scalpel blade and washed in oocyte collection medium; COCs with at least one layer of intact compact cumulus and evenly granular ooplasm were selected for maturation (groups of 30–50) in 250 μL of pre-equilibrated oocyte maturation medium incubated in 5% CO_2 in humidified air at 39 °C for 22 h. The control oocyte maturation medium (OMM) consisted of Tissue Culture Medium-199 (TCM199, Invitrogen) supplemented with 10% (v:v) fetal bovine serum (FBS, Mediatech, Herndon, VA, USA), FSH (Folltropin, 5.0 μg/mL; AgTech, Manhattan, KS, USA), LH (0.3 μg/mL; National Hormone & Peptide Program, Torrance, CA, USA), epidermal growth factor (EGF, 50 ng/mL; Invitrogen), 0.2 mM sodium pyruvate (Sigma, St. Louis, MO, USA), 2.0 mM L-glutamine (Invitrogen) and penicillin/streptomycin (1000 U penicillin and 1000 μg streptomycin, Invitrogen).

2.2. In vitro fertilization

Fertilization followed a modified procedure of Parrish et al. [26]. The base culture media TL-Sperm, TL-IVF and TL-Hepes were purchased from Specialty Media (Phillipsburg, NJ, USA). Twenty-two hours after maturation, COCs were washed in Hepes-Tyrode's Albumin Lactate Pyruvate (TALP) and transferred in groups of 30–50 into 425 μL of pre-equilibrated IVF-TALP. Spermatozoa were prepared from frozen-thawed semen pooled from three Angus bulls (Southeastern Semen Services, Wellborn, FL, USA) by Percoll (Sigma) density gradient centrifugation and washed with Sperm-TALP. Cumulus–oocyte complexes and