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Effect of addition of hyaluronan to embryo culture medium on survival of bovine embryos in vitro following vitrification and establishment of pregnancy after transfer to recipients

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Abstract

Two experiments were conducted to determine whether addition of hyaluronan to culture medium could improve survival of bovine embryos after vitrification or following embryo transfer. In Experiment 1, embryos were produced in vitro and cultured for 7 days in modified synthetic oviductal fluid (SOF) containing one of four concentrations of hyaluronan (0, 0.1, 0.5, or 1 mg/mL), with or without 4 mg/mL of bovine serum albumin (BSA). On Day 7 after insemination, blastocysts and expanded blastocysts were vitrified using open-pulled straws. At a concentration of 1 mg/mL, hyaluronan increased ($P < 0.05$) the percentage of oocytes that were blastocysts and re-expansion rate at 24 h after warming. At 0.5 mg/mL, hyaluronan tended ($P < 0.10$) to increase re-expansion rate at 48 h after warming and increased ($P < 0.05$) embryo hatching rate at 24 and 72 h. Treatment with BSA caused a slight reduction in cleavage rate ($P < 0.05$), but only for cultures containing hyaluronan (BSA × hyaluronan, $P = 0.10$), an increase in the percentage of oocytes that became blastocysts ($P < 0.001$), and a reduction in re-expansion rates ($P < 0.001$) and hatching rates ($P < 0.05$ or $P < 0.01$) at all times examined. In Experiment 2, embryos were produced in vitro and cultured in modified SOF containing 4 mg/mL BSA, with or without 1 mg/mL hyaluronan. At 159–162 h after insemination, grade 1 morula, blastocysts and expanded blastocysts were harvested for embryo transfer. Harvested embryos were transferred individually to lactating Holstein recipients with a palpable corpus luteum on Day 7 after presumptive ovulation. There was an interaction ($P < 0.05$) between hyaluronan and embryo stage on pregnancy rate. Recipients that received morula and blastocyst stage embryos treated with hyaluronan had a higher pregnancy rate than recipients that received control embryos of the same stage. There was no effect of hyaluronan on pregnancy rates of recipients that received expanded blastocysts. In conclusion, addition of hyaluronan to embryo culture enhanced blastocyst yield, improved survival following vitrification, and enhanced the post-transfer survival of fresh morula and blastocyst stage embryos.

Keywords: Hyaluronan; Bovine; Vitrification; Embryo transfer

1. Introduction

In vitro embryo production has great potential for enhancing genetic selection, improving fertility, and optimizing crossbreeding schemes in beef and dairy cattle production systems [1,2]. Despite this potential, embryos produced in vitro represent only 30% of the total

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number of embryos transferred worldwide [3]. One reason for the limited commercial application of in vitro embryo transfer is that embryos produced in vitro are associated with altered ultrastructural and physiological features compared to embryos produced by super-ovulation [4–7]. Moreover, such alterations can affect post-culture viability. In particular, bovine embryos produced in vitro are more sensitive to cryopreservation [8–10], have reduced embryo and fetal survival following transfer [11,12], and result in an increased number of fetuses and calves with abnormalities [12–14].

Recent research using the sheep oviduct as a model for in vivo embryo development has highlighted the sub-optimal nature of embryo culture and the consequences of such an environment for post-culture viability [7–9,15]. Thus, a potential strategy for improving in vitro produced embryos is to modify culture conditions to more closely mimic the microenvironment found in vivo, through addition of growth factors, hormones and other regulatory molecules. Such molecules include the growth factor, insulin-like growth factor-1 (IGF-1), and the cytokine, granulocyte macrophage-colony stimulating factor, which have been reported to improve the post-transfer survival of embryos produced in vitro [16–18].

The glycoaminoglycan hyaluronan is another molecule that has the potential to improve post-culture viability of embryos produced in vitro. Hyaluronan plays an important role in several aspects of cell function, including cell proliferation, cell migration, cell adhesion and intracellular signaling [19,20]. Hyaluronan is present in the oviductal and uterine fluids of cattle [21] and receptors for hyaluronan are expressed on the preimplantation bovine embryo [22,23]. Addition of hyaluronan to bovine embryo culture has been reported to improve development to the blastocyst stage [24,25] and increase re-expansion and hatching rates following cryopreservation [26–28]. Hyaluronan can alter the abundance of several developmentally-important gene transcripts in bovine embryos [28,29] and promote the differentiation of extra-embryonic structures in mouse embryos [30].

Culture of mouse embryos in the presence of hyaluronan increased implantation rates and fetal development following embryo transfer [31]. In addition, treatment of ovine embryos with 6 mg/mL hyaluronan during culture increased pregnancy and lambing rates following the transfer of vitrified/warmed in vitro produced blastocysts [32]. The aim of the present study was to determine whether addition of hyaluronan to bovine embryo culture could improve survival following vitrification and increase pregnancy rates following embryo transfer.

2. Materials and methods

2.1. Materials

All materials were purchased from Sigma (St. Louis, MO, USA) or Fisher Scientific (Fairlawn, NJ, USA) unless specified otherwise. Hepes-Tyrode's Lactate and IVF-Tyrode's Lactate were purchased from Caisson Laboratories Inc. (Logan, UT, USA). These media were used to prepare Hepes-Tyrode's albumin lactate pyruvate (TALP) and IVF-TALP, as described previously [33]. Oocyte collection medium (OCM) consisted of Tissue Culture Medium-199 (TCM-199) with Hank's salts without phenol red (Atlanta Biologicals, Norcross, GA, USA) and supplemented with 2% (v/v) bovine steer serum (Pel-Freez, Rogers, AR, USA), 2 U/mL heparin, 100 U/mL penicillin-G, 0.1 mg/mL streptomycin, and 1 mM glutamine. Oocyte maturation medium (OMM) was prepared as TCM-199 (Invitrogen, Carlsbad, CA, USA) with Earle's salts supplemented with 10% (v/v) bovine steer serum, 2 μg/mL estradiol 17-β, 20 μg/mL bovine FSH (Folltropin-V; Bioniche, Belleville, ON, Canada), 22 μg/mL sodium pyruvate, 50 μg/mL gentamicin sulfate, and 1 mM glutamine. Percoll® was from Amersham Pharmacia Biotech (Uppsala, Sweden). Recombinant human insulin-like growth factor-1 was purchased from Upstate Biotech (Lake Placid, NY, USA). A modified synthetic oviductal fluid (mSOF) was purchased from Millipore (Billerica, MA, USA). The formula was as described by Takahashi and First [34], except that phenol red was omitted and bovine serum albumin (BSA) was modified for the dictates of each specific experiment. The mSOF was further modified prior to use to contain 1.0 mM alanyl-glutamine, 5.3 mM sodium lactate, 0.5 mM tri-sodium citrate, 2.77 mM myo-inositol, 0.5 mM fructose, essential amino acids (basal medium Eagle), non-essential amino acids (minimum essential medium) and culture treatments as described below. Hyaluronan (1100 kDa from *Streptococcus pyrogenes*) was purchased from R&D Systems (Minneapolis, MN, USA), Syngro® holding medium was from Bioniche, and lidocaine was from Pro Labs (St. Joseph, MO, USA).

2.2. Experiment 1: Effect of bovine serum albumin and hyaluronan during culture on embryo development and survival following vitrification

2.2.1. Embryo production

All procedures related to in vitro embryo production were as described previously [35] unless noted otherwise. Immature cumulus oocyte complexes (COC) were