Theriogenology 81 (2014) 683–688

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Effect of ATP-binding cassette subfamily B member 1 on bovine blastocyst implantation

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ARTICLE INFO

Article history:
Received 7 August 2013
Received in revised form 26 November 2013
Accepted 30 November 2013

Keywords:
ABCB1
Bovine blastocyst
IVF
Cell proliferation
Conception

ABSTRACT

The ATP-binding cassette subfamily B member 1 (ABCB1) is an efflux transporter that excretes xenobiotics and waste matter. High expression of ABCB1 induced by forskolin (FSK) and rifampicin (RIF) in the bovine blastocysts reportedly improves the cellular quality. In the present study, interferon-α, similar to FSK and RIF, was highly potent in inducing the expression of ABCB1 in the bovine blastocysts but did not exhibit an additive effect with FSK and RIF. Bovine blastocysts stimulated by the combined treatment with FSK, RIF, and interferon-α to express high levels of ABCB1 displayed better freezing resistance as indicated by higher cell numbers in post thawing cultures. On transfer to recipients, such embryos established pregnancies with significantly higher frequencies in repeat breeder cows rather than normal ones.

1. Introduction

Since the first report on beef calf production using nonsurgical embryo transfer [1], attempts have been made to improve the survival of transferred embryos by supplementation of nutrients and other substances in the culture medium [2–4], selection of high-quality embryos [5] and their manipulation [6], improvements in cryopreservation [7], and analysis of embryonic genes associated with development and pregnancy [8,9]. However, the rate of conception of cryopreserved embryos derived from IVF still remains low [3,7,10].

We reported previously that the treatment with forskolin (FSK) and rifampicin (RIF) induced the expression of the ATP-binding cassette subfamily B member 1 (ABCB1) in bovine oocytes, developing embryos, blastocysts, and uterine stromal cells [11]. Furthermore, embryos with high expression of ABCB1 showed a significant improvement in survival and cell proliferation after cryopreservation [11]. The ABCB1 protein is an efflux transporter in the plasma membrane of many cell types including the trophoblastic cells of the placenta, in which the fetus is protected by transporting toxic substances [12,13]. Although Janus kinase-signal transducer and activator of transcription–like motifs activated by cytokines (e.g., interferons [IFNs]), are present in the upstream regulatory promoter region of ABCB1, there are no reports on the cytokine-induced expression of the *ABCB1* gene in embryos.

Therefore, the present study investigated the effects of (1) interferon-α (IFNA) on ABCB1 expression; (2) IFNA, FSK, and RIF on ABCB1 expression and cell numbers after cryopreservation in bovine blastocysts; and (3) the conception ability of cryopreserved bovine blastocysts expressing high levels of ABCB1.

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http://dx.doi.org/10.1016/j.theriogenology.2013.11.015

2. Materials and methods

2.1. In vitro maturation, fertilization, and culture of bovine embryos

All experimental procedures were approved by the Animal Care and Use Committee of the Fukuoka Prefectural Agricultural Research Center. Ovarian follicles (2–8 mm in diameter) from bovine ovaries collected at a slaughterhouse were aspirated using a 20-ga needle to obtain cumulus-oocyte complexes. Approximately 50 cumulus-oocyte complexes were matured for 20 hours in 500 μL of IVMD101 medium (Research Institute for the Functional Peptides, Yamagata, Japan) covered with mineral oil (Sigma-Aldrich, St. Louis, MO, USA). All cultures were maintained at 38.5 °C in a humidified atmosphere of 3% CO_2, 10% O_2, and 87% N_2. Frozen semen from a Japanese black bull was used for IVF, as previously described [14]. After IVF for 5 hours, presumptive zygotes were cultured with cumulus cells in a glucose-free modified medium 199 [14] containing 10% fetal calf serum (FCS), 100 IU/mL penicillin, and 100 μg/mL streptomycin (1× PS). On Day 6 (IVF = Day 0) of culture, the culture medium was changed from the modified medium 199 to medium 199 (GIBCO, Invitrogen) containing 10% FCS and 1× PS. More than 10 replicates in control and treated cumulus-oocyte complexes were cultured, and blastocysts were stored in liquid nitrogen until analysis.

2.2. Cryopreservation

Blastocysts were transferred to a cryoprotective solution (5% ethylene glycol, 6% propylene glycol, 0.1 M sucrose, and 4 mg/mL BSA in Dulbecco's PBS [D-PBS]), and then one or two blastocysts were introduced into a 0.25-mL straw (IMV Technologies, L'Aigle, France) at 23 °C to 25 °C. After the blastocysts were equilibrated at 23 °C to 25 °C for 13 minutes, the straws were directly set in a programmable freezer (Fujihira) at −7 °C where seeding was manually performed. Subsequently, the straws were cooled at a rate of −0.3 °C/min to −30 °C and then directly transferred to liquid nitrogen for storage until use. The straws were thawed in air for 10 seconds and then immersed in a water bath at 30 °C for 10 seconds.

2.3. Stimulation of ABCB1 expression in bovine embryos

The ABCB1 level enhancement in bovine blastocysts was investigated by screening several regulatory sites upstream of the transcription start site of the bovine *ABCB1* gene. A Janus kinase-signal transducer and activator of transcription–like motif (canonical: AGTTTCNNTTTCNC/T, TCAAAGNNAAAGNG/A, A/GNGAAANNGAAACT, T/CNCTTTNNCTTTGA) was located at −1370 (Fig. 1A).

After IVF, zygotes were cultured with cumulus cells in a culture medium (Days 0–6, modified medium 199; Days 6–7, medium 199) and IFNA (100 U) and/or FSK + RIF (each 10 μM) containing 10% FCS and 1× PS. Supplementation with a cocktail of FSK + RIF + IFNA in the culture medium was conducted over a period of 192 hours (Days 0–7) and 24 hours (Days 6–7). Some embryos were cultured without the supplements as controls.

2.4. RNA isolation and reverse transcription reaction

Total RNA was isolated from five blastocysts using an RNeasy Mini kit according to the manufacturer's protocol. Samples of RNA were treated with RNase-free DNase (Qiagen). The cDNAs were generated using reverse transcription (RT) with oligo $(dT)_{15}$ and random primers using a GoTaq 2-Step RT-qPCR System (Promega, Madison, WI, USA). The RNA concentration was determined using a 260/280 UV spectrophotometer, and RNA integrity was checked using agarose gel electrophoresis.

2.5. Reverse transcriptase-polymerase chain reaction

Expression of the *interferon receptor* (*IFNAR1*) was determined using polymerase chain reaction with the primer sets listed in Table 1. Reactions were carried out in a total volume of 20 μL containing 1 μL cDNA (10 ng/μL), deoxynucleotide triphosphates (at a final concentration of 0.2 mM), 200 nM of each primer, and 0.4 U KOD-Plus-enzyme (Toyobo, Osaka, Japan). The samples were first denatured at 94 °C for 2 minutes, followed by 35 cycles of 94 °C for 15 seconds, 60 °C for 30 seconds, and 68 °C for 15 seconds. They were then held at 68 °C for 5 minutes. The polymerase chain reaction products were analyzed using electrophoresis on 2% agarose gels. The bands were stained with ethidium bromide and visualized under UV fluorescence. The *H2AFZ* gene was used as the internal control.

2.6. Western blotting

The level of ABCB1 in bovine embryos was analyzed using immunoblotting. The following aspects were investigated: (1) the effect of IFNA and/or FSK and RIF during development and (2) the duration required for the maximal expression of ABCB1 induced by the cocktail of the three reagents. On Day 7, four embryos lysed in Laemmli sample buffer were run on 7.5% SDS-PAGE for the immunodetection of ABCB1 and electrotransferred onto Immobilon-polyvinylidene fluoride membranes (Millipore, Bedford, MA, USA). The membranes were treated with blocking solution (1% dry milk) in 10 mM TRIS–HCl (pH 7.5, containing 0.15 M NaCl, 1 mM EDTA, and 0.05% Tween 20) at room temperature for 1 hour. The membranes were then incubated with an anti-ABCB1 antibody (1:400; H-241; Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4 °C. They were then incubated in a

Table 1
Primer sequences for the targeted genes in real-time polymerase chain reaction.

Gene	Accession no.	Sequence 5′–3′	Amplicon (bp)
IFNAR1	NM_174552	F: TGGAACAGCAGCAGTGAGTC R: CACCCAGACAATTTCTTCCAG	92
H2AFZ	NM_174809	F: AGGACGACTAGCCATGGACGTGTG R: CCACCACCAGCAATTGTAGCCTTG	208