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Thermoprotective effect of insulin-like growth factor 1 on *in vitro* matured bovine oocyte exposed to heat shock

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ABSTRACT

The role of insulin-like growth factor 1 (IGF1) on cellular function and developmental capacity of heat-shocked oocytes has not been completely understood. Therefore, the objective of this study was to determine the effect of IGF1 on apoptosis, mitochondrial activity, cytoskeletal changes, nuclear maturation, and developmental competence of bovine oocytes exposed to heat shock. Cumulus–oocyte complexes were submitted to control (38.5 °C for 22 hours) and heat shock (41 °C for 14 hours followed by 38.5 °C for 8 hours) in the presence of 0 or 100 ng/mL IGF1 during IVM. Heat shock increased the percentage of TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling)–positive oocyte and reduced oocyte mitochondrial activity. However, addition of 100 ng/mL IGF1 minimized these deleterious effects of temperature. Caspase activity was affected neither by heat shock nor IGF1. Exposure of bovine oocytes to 41 °C during the first 14-hour IVM affected cortical actin localization and microtubule organization at the meiotic spindle and reduced the percentage oocytes that reached the metaphase II stage. However, in the presence of IGF1, cortical actin and percentage of metaphase II oocytes were not different between control and heat-shocked oocytes, suggesting a partial beneficial effect of IGF1. There was no effect of IGF1 on microtubule organization. Heat shock also reduced the percentage of oocytes that reached the blastocyst stage, blastocyst cell number, and increased the percentage of TUNEL-positive blastomeres. However, there was no effect of 100 ng/mL IGF1 on oocyte development to the blastocyst stage and blastocyst quality. Therefore, 100 ng/mL IGF1 prevented some heat shock–induced cellular damage in bovine oocytes but had no effect on oocyte developmental competence. In contrast, a low IGF1 concentration (25 ng/mL) had a thermoprotective effect on oocyte developmental competence to the blastocyst stage. In conclusion, IGF1 prevented part of the damage induced by heat shock on oocyte function. This effect was modulated by IGF1 concentration.

1. Introduction

Exposure of lactating Holstein cows to elevated temperature and humidity causes hyperthermia leading to maternal heat stress. It has been known that heat stress

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affects fertility of lactating dairy cows inducing deleterious effects on follicular growth [1,2], hormonal secretion [2,3], endometrial function [4], uterine blood flow [5], oocyte competence [6,7], and preimplantation embryonic development [8,9].

Despite the multiple deleterious effects of heat stress on fertility, there is increasing evidence that oocytes are major targets of maternal heat stress. For instance, heat stress during the periovulatory period increased the proportion of retarded embryos in heifers suggesting the negative effect of elevated temperature on oocyte maturation [10]. *In vitro* studies also reported the direct effects of heat shock on oocyte maturation. Exposure of bovine oocytes to severe (42 °C) or moderate (40 °C–41 °C) heat shock during the first 12-hour IVM (0–12 hours IVM) reduced oocyte ability to develop to the blastocyst stage [11,12]. Moreover, oocyte exposure to 43 °C for 45 and 60 minutes during IVM reduced blastocyst and expanded blastocyst rates [13]. The cellular mechanisms triggered by heat shock on that effect are not completely understood. There is evidence that exposure of bovine oocytes to heat shock during IVM affected cytoskeleton organization [14–16], altered cortical granule distribution [17], chromosome separation during fertilization and cleavage [16,18], decreased the proportion of oocytes that reached the MII stage after IVM [15], and increased oocyte apoptosis [12].

Although different oocyte cellular compartments have been shown to be affected by elevated temperature [19], nuclear transfer experiments found that exposure of donor cell nuclei to heat shock did not affect subsequent embryonic development in either *Bos indicus* or *Bos taurus* oocytes [20]. However, exposure of receptor ooplasm to heat shock decreased developmental competence of *Bos taurus* oocytes [20], indicating greater cytoplasmic susceptibility to elevated temperature.

It has been shown that exposure of dairy cows to heat stress reduced serum insulin-like growth factor 1 (IGF1) levels leading to impairment of oocyte quality [21]. Cumulus cells and oocytes express IGF1 receptor mRNA [22–24]. Indeed, several members of the IGF system have been reported in bovine oocytes and blastocysts. IGF1 mRNA was greater in heat-shocked embryos compared with control [25]. Bovine oocytes expressed mRNA for IGF1 and IGF2, IGF1 and IGF2 receptor (IGF1R and IGF2R), IGF-binding protein 2 and 4 (IGFBP2 and IGFBP4), and pregnancy-associated plasma protein A [25]. There is evidence that free IGF1 in follicular fluid can bind to oocyte IGFR activating phosphatidylinositol 3-kinase/protein kinase A (PI3K/AKT), and mitogen-activated protein kinases 3/1 (MAPK3/1) pathways [26]. In addition, activation of MAPK leads to phosphorylation of various subtracts like phospholipases, transcription factors, and cytoskeletal proteins [27]. PI3K/AKT activation is known to be involved in oocyte meiotic maturation [28] and activation of MAPK pathways by IGF1 promotes blastocyst development [29].

IGF1 can modulate effects of elevated temperature on cellular function. Addition of 100 ng/mL IGF1 to *in vitro* culture medium improved preimplantation embryo resistance to heat shock preventing the deleterious effect of temperature on development to the blastocyst stage and blastocyst apoptosis [30,31]. Similarly, heat-stressed lactating dairy cows had higher pregnancy rates after transfer of IGF1-treated embryos compared with control embryos [32]. However, the effects of IGF1 during IVM of heat-shocked bovine oocytes are still controversial. In a study conducted by Zhandi et al. [33], addition of 100 ng/mL IGF1 during IVM enhanced heat shock–induced apoptosis and compromised developmental competence of heat-shocked oocytes. In contrast, Meiyu et al. [34] found that addition of 100 ng/mL IGF1 during IVM prevented heat shock–induced apoptosis in bovine oocytes. Therefore, the objective of this study was to determine the role of IGF1 on nuclear maturation, apoptosis, mitochondrial activity, cytoskeleton organization, and developmental competence of bovine oocytes exposed to heat shock during IVM.

2. Materials and methods

2.1. Materials

Unless otherwise stated, all chemicals were purchased from Sigma–Aldrich (St Louis, MO, USA). Antibodies were purchased from Molecular Probes (Eugene, OR, USA). Recombinant human IGF1 expressed in *E. coli* was purchased from Upstate Biotechnology (Lake Placid, NY, USA), *In Situ* Cell Detection Kit Fluorescein was from Boehringer Mannheim/Roche Diagnostics (Penzberg in Upper Bavaria, Germany), and PhiPhiLux-G_1D_2 was from OncoImmunin, Inc. (Gaithersburg, MD, USA). MitoTracker Red CMX-Ros was purchased from Invitrogen (Carlsbad, CA, USA), VECTASHIELD Mounting Medium was from Vector Laboratories, Inc. (Burlingame, CA, USA), and goat serum from Cripion (Andradina, São Paulo, Brazil).

Oocyte collection medium was tissue culture medium-199 (TCM-199) containing L-glutamine and phenol red (GIBCO, Grand Island, NY, USA) supplemented with 2.2 mg/mL sodium bicarbonate, 1% (vol/vol) fetal bovine serum (FBS) (GIBCO) (containing 2 U/mL heparin), 0.01 μg/mL streptomycin, and 0.01 U/mL penicillin-G. Pre-IVM medium was TCM-199 HEPES with Hanks salts (GIBCO), supplemented with 10% (vol/vol) FBS, 50 μg/mL gentamicin, and 0.2 mM sodium pyruvate. Oocyte maturation medium was TCM-199 sodium bicarbonate with earle's salts (GIBCO) supplemented with 10% (vol/vol) FBS, 50 μg/mL gentamicin, 0.2 mM sodium pyruvate, 1 μg/mL estradiol 17-β, 10 μg/mL FSH (FOLLTROPIN–V from Bioniche Animal Health Canada Inc., Belleville, Ontario, Canada), and 10 μg/mL LH (Chorulon from Intervet Schering Plow, Roseland, NJ, USA). Pre-IVF medium was TCM-199 HEPES (GIBCO) supplemented with 3 mg/mL bovine serum albumin fraction V (BSA-V), 50 μg/mL gentamicin, and 0.2 mM sodium pyruvate. IVF medium was a modified Tyrode's albumin-lactate pyruvate (TALP) medium [35] containing 6 mg/mL essentially fatty acid–free BSA, 50 μg/mL gentamicin, 0.2 mM sodium pyruvate, 100 μg/mL heparin, and 41.66 μL/mL PHE (penicillamine 2.7 μg/mL, hipotaurine 1 μg/mL, epinephrine 0.33 μg/mL in 0.9% [wt/vol] NaCl). SP-TALP medium used during sperm purification was as described by Parrish et al. [35]. Frozen semen from Nelore was acquired from CRV Lagoa (Sertãozinho, São Paulo, Brazil). *In vitro* culture medium was potassium simplex optimized medium (KSOM, MR-107-D; Millipore, Darmstadt,