Theriogenology 115 (2018) 38–44

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Laminarin improves developmental competence of porcine early stage embryos by inhibiting oxidative stress

Hao Jiang [a, b], Shuang Liang [a], Xue-Rui Yao [b, c], Yong-Xun Jin [a], Xing-Hui Shen [d], Bao Yuan [a], Jia-Bao Zhang [a, **], Nam-Hyung Kim [a, b, *]

[a] Department of Laboratory Animals, College of Animal Sciences, Jilin University, Changchun, Jilin, 130062, China
[b] Department of Animal Science, Chungbuk National University, Cheongju, Chungbuk, 361-763, Republic of Korea
[c] Department of Animal Science, College of Agriculture, Yanbian University, Yanji, Jilin, 133000, China
[d] Department of Histology and Embryology, Harbin Medical University, Harbin, 150081, China

ARTICLE INFO

Article history:
Received 6 March 2018
Received in revised form
13 April 2018
Accepted 17 April 2018
Available online 23 April 2018

Keywords:
Laminarin
Porcine
Oxidative stress
Embryo development

ABSTRACT

Laminarin (LMA), a β-glucan mixture with good biocompatibility, improves the growth performance and immune response when used as food additives and nutraceuticals. The aim of the present research was to explore the effects of LMA on porcine early stage embryo development, as well as the underlying mechanisms. The results showed that the developmental competence of porcine early stage embryos was dramatically improved after LMA supplementation during the in vitro culture period. The presence of 20 μg/mL LMA during the in vitro culture period significantly improved cleavage rate, blastocyst formation rates, hatching rate, and total cell number in the blastocyst compared to that in the control group. Notably, LMA attenuated the intracellular reactive oxygen species generation induced by H_2O_2. Furthermore, LMA not only increased intracellular glutathione levels, but also ameliorated mitochondrial membrane potential. In addition, the expression of a zygotic genome activation related gene (*YAP1*), pluripotency-related genes (*OCT4*, *NANOG*, and *SOX2*), and hatching-related genes (*COX2*, *GATA4*, and *ITGA5*) were up-regulated following LMA supplementation during porcine early stage embryo development. These results demonstrate that LMA has beneficial effects on the development of porcine early stage embryos via regulation of oxidative stress. This evidence provides a novel method for embryo development improvement associated with exposure to LMA.

1. Introduction

In vitro embryo production (IVP) in livestock animals is a vital tool in embryo physiology research, animal production, and human assisted reproduction [1–3]. Although several animals have been successfully produced using IVP, its success rate remains extremely low, especially in pigs [4]. Compared with other species, such as cattle and mouse, porcine early stage embryos are more sensitive to alterations in the culture environment [5]. One of the underlying problems is porcine early stage embryos are highly sensitive to oxidative stress [6]. Oxidative stress occurs due to overproduction of reactive oxygen species (ROS) in cells, which could overwhelm the normal antioxidant capacity under normal physiological conditions. Previous studies suggest that excessive ROS generation causes abnormal embryo development by impairing the dynamic calcium balance as well as inducing mitochondrial dysfunction and cytoplasm abnormalities [7,8]. Therefore, development of effective protocols for the production of early stage porcine embryos will provide flexibility for the birth of healthy pig offspring using IVP technology.

Because in vitro culture conditions cannot fully simulate the in vivo physiological environment, embryos are more susceptible to oxidative stress [9,10]. Therefore, inhibition of ROS generation is an effective strategy for improving the developmental competence of embryos. Antioxidant supplementation of culture medium during embryo development could remove ROS, which is produced during cellular aerobic metabolism [11]. Various types of antioxidants such as vitamin C [12], L-carnitine [13], cysteine [14], melatonin [15] and lycopene [16] have been used in different combinations for

* Corresponding author. Department of Animal Science, Chungbuk National University, Cheongju, Chungbuk, 361-763, Republic of Korea.
** Corresponding author.
E-mail addresses: zjb515@163.com (J.-B. Zhang), nhkim@chungbuk.ac.kr (N.-H. Kim).

https://doi.org/10.1016/j.theriogenology.2018.04.019
0093-691X/

improving embryo developmental competence.

Laminarin or laminaran (LMA) contains β(1–3)-glucan with β(1–6)-branches and is a carbohydrate food reserve in brown algae. It also exists in many other plants, fungi, and bacteria. Previous studies show that LMA inhibits the growth, adhesion, and invasion of pathogenic bacteria [17–19]. Supplementation with LMA regulates the intestinal microbial community to prevent caecal putrefaction and improve the average daily weight gain, gut health, and growth performance by changing the lactate acid and indole content [20–23]. In vitro and in vivo experiments show that LMA and LMA sulfate have anti-cancer properties [24–27] and serve as functional food supplements or nutraceuticals [28]. Its special molecular structure also expands its application to cosmetic and therapeutic creams [29], low toxicity non-viral gene nanoparticle transfer vectors [30], and hydrogels [31]. According to current studies, LMA modulates or activates the immune response [24,32,33], reduces sepsis-induced oxidative stress/damage and lipid peroxidation [24,27,34,35], and has good biocompatibility [31].

Although the beneficial biological and physiological functions of LMA for immune activation are well documented, the information regarding the influence of LMA and its β-glucan analogues (important component of cell wall in bacteria and fungus) on the reproductive system, especially on early stage embryo development, is lacking. Whether LMA affect the physiological status of reproductive tract is not clear. Here, we hypothesized that LMA will improve the developmental potential of porcine early stage embryos due to its protective effect on reducing oxidative stress from excess ROS generation during the in vitro culture period. In the present research, we first investigated the effect of LMA on early stage porcine embryos cultured in vitro. Subsequently, the underlining mechanism of LMA on porcine early stage embryo development was detected after oxidative stress induced with H_2O_2.

2. Materials and methods

All chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless expressly stated otherwise.

2.1. Oocyte collection and in vitro maturation

Porcine ovaries were obtained from a local slaughterhouse and transferred to the lab at 30–35 °C within 1 h. Cumulus-oocyte complexes (COCs) were aspirated from the 3–6 mm antral follicles using a 10 mL syringe with an 18-gauge needle. Oocytes with a minimum of three layers of cumulus cells were selected for further experiments. After washing three times with Tyrode's Lactate HEPES (TL-HEPES), about 50 oocytes were transferred into 500 μL in vitro maturation medium (M199 with 20 ng/mL epidermal growth factor, 1 μg/mL insulin, 75 μg/mL kanamycin, 0.91 mM Na pyruvate, 0.57 mM L-cysteine, 10% porcine follicular fluid, 0.5 μg/mL follicle stimulating hormone, and 0.5 μg/mL luteinizing hormone) covered with mineral oil and cultured at 38.5 °C in an atmosphere of 5% CO_2 and 100% humidity for 44 h.

2.2. Parthenogenetic activation and embryo in vitro culture (IVC)

Matured oocytes were parthenogenetically activated according to our previous study [36]. The activated oocytes were cultured in bicarbonate-buffered PZM-5 containing 4 mg/mL BSA (IVC medium) and 7.5 mg/mL cytochalasin B for 3 h to suppress extrusion of the pseudo-second polar body. After thoroughly washing, about 40 parthenogenetically activated oocytes were transferred and cultured in four-well plates containing 500 μL mineral oil-covered IVC medium with/without 20 μg/mL LMA at 38.5 °C and in 5% CO_2 without changing the medium. Blastocyst rate and hatching rate were detected on days 5, 6, and 7.

2.3. TUNEL assay and cell counting

Cell apoptosis was detected using the TUNEL (Terminal deoxynucleotidyl transferase mediated dUTP Nick End Labeling) assay kit (Roche Diagnostics, Indianapolis, IN, USA). Blastocysts were gently washed three times with PBS–PVA (phosphate-buffered saline with 0.1% polyvinyl alcohol) and fixed with 3.7% paraformaldehyde in PBS for 30 min at room temperature. Then, the embryos were washed with PBS–PVA and permeabilized by incubation in 0.1% Triton X-100 for 30 min at room temperature. Next, the embryos were washed twice with PBS–PVA and incubated with fluorescein-conjugated dUTP and the terminal deoxynucleotidyl transferase enzyme (Roche) in the dark for 1 h at 37 °C. After incubation with 10 μg/mL Hoechst 33342 for 5 min at 37 °C to label the nuclei, a fluorescence microscope and ImageJ software (NIH, MD, USA) were used to analyze fluorescence intensities and the number of apoptotic nuclei and total number of nuclei. Apoptosis was evaluated based on the percentage of apoptotic nuclei in blastocysts.

2.4. Intracellular ROS and glutathione (GSH) levels assay

Intracellular ROS and GSH levels were determined by incubating the 4-cells stage embryos in PBS-PVA medium containing 10 μM 2′,7′-dichlorodihydrofluorescein diacetate (DCFH) (Invitrogen, NY, USA) and 10 μM 4-chloromethyl-6,8-difluoro-7-hydroxycoumarin (Invitrogen) for 15 min and 30 min, respectively. After washing five times in PBS-PVA, Image J software was used to analyze fluorescence intensities.

2.5. Mitochondrial membrane potential assay

Mitochondria membrane potential (MMP, ΔΨm) is commonly used as an indicator of mitochondrial function and cellular viability in embryos. To measure ΔΨm, 4-cell stage embryos were washed three times with PBS-PVA and incubated in the PBS-PVA containing 2 μM 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide (JC-1; Invitrogen) dye for 30 min. After washing three times with PBS-PVA, red/green fluorescence signals were captured using a fluorescence microscope. The ΔΨm of average entire 4-cell stage embryos was then calculated as a ratio of red fluorescence intensity (J-aggregates; corresponding to activated mitochondria) to green fluorescence intensity (J-monomers; corresponding to inactive mitochondria) using ImageJ software.

2.6. Quantitative RT-PCR (qRT-PCR) analysis

Total RNA was extracted from 200 embryos using the Dynabeads™ mRNA DIRECT™ Purification Kit (Invitrogen) according to the manufacturer's instructions. Gene expression was quantified using the CFX96 Real-Time System (Bio-Rad, Hercules, CA, USA) and $2^{-\Delta\Delta Ct}$ method with 18S rRNA as the standard. PCR primers used to amplify each gene are listed in Supplementary Table S1.

2.7. Statistical analysis

Results are presented as mean ± SD. Total numbers of embryos (n) used in each group and independent repeat times (*Re*) of experiments are shown at the data column and figure notes, respectively. Data obtained from two groups were compared using the Student's t-test. Tests with three or more means were analyzed using a one-way ANOVA (Tukey-Krame). All statistical analyses were performed using SPSS version 22.0 (IBM, IL, USA) software.