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HOSSAM MAGHRABY

شبكة المعلومات الجامعية

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

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Improving Viability and Cryotolerance of *In Vitro* Produced Cattle Embryos

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Abstract

In vitro embryo production (IVEP) is considered as one of the most important technologies in cattle industry due to production of superior quality embryos with high developmental competence that use at low costs in embryo transfer programs. So, improvement of *in vitro* culture systems are the most critical and essential step to keep on this biotechnology. Hence, the current experiments were designed. Experiment 1, aimed to (I) study the effect of phenazine ethosulfate (PES) supplementation in culture media on the selected miRNAs (miR-205, miR-26a-5p, and *let-7b*) and their target genes (OCT4, DNMT, CASP3, ATF6, ATP5ME, and ELOVL5), during bovine embryo production. Therefore, a group of two-day bovine embryos was cultured in a medium with lipid-reducing agent, PES (0.3 mM). Another group of embryos without PES was left as a control. Embryos were vitrified and morphologically examined after warming and the viability was evaluated by culturing for 24 h. After evaluation, embryos were classified as good or poor. Afterwards, embryos (blastocyst and morula) were kept at -80°C for RNA extraction and qRT-PCR of miRNAs and their targets. Results revealed that the rate of morula was higher (P<0.01) in treated compared to control groups. After vitrifications, the percentage of good quality embryos increased in treated than control groups. Additionally, the rate of dead embryos was high in control groups. The *Let-7b* and miR-205 were significantly over-expressed in the treated good as well as poor embryos compared to control (untreated) good and poor embryos, respectively. However, miR-26 was suppressed in the treated good and poor embryos compared to control (untreated) embryos, respectively. Both of OCT4and DNMT1 transcripts up-regulated in the treated (good& poor) embryos compared to control groups. The ELOVL5 gene decreased in the treated (good&poor) embryos, compared to control untreated groups. In conclusion, PES supplementation reduced lipid droplets, and improved cryotolerance of morula and/or blastocysts through regulation the pattern of lipid metabolism and embryo quality genes.

Experiment 2, aimed to (I) study the effect of resveratrol supplementation in culture media on the quality of *in vitro* produced bovine embryos (II) monitoring changes in the expression of genes associated with oxidative stress and quality of embryos. So, three groups of bovine embryos were cultured with resveratrol at different concentrations (0.01, 0.001 and 0.0001µM). Another group without resveratrol was left as a control. Embryos were morphologically examined after vitrification and the viability was evaluated by culturing for 24 h. After evaluation, embryos were classified as good or poor. Afterwards, embryos were kept at -80°C for RNA extraction and qRT-PCR of target genes. Results revealed that the low concentrations of 0.001 µM (P<0.05) and 0.0001 µM (P<0.01) significantly improved the blastocyst rate than the control group. After vitrifications and culture for 24 hr, the percentage of good quality embryos increased (P<0.05) in treated groups with 0.001 and 0.0001 µM resveratrol compared to the control group. The OCT4 and DNMT1 genes were significantly over-expressed in the treated good as well as poor embryos compared to untreated good and poor embryos, respectively. However, the CASP3 was suppressed in the treated good embryos compared to control (untreated) embryos. Both of ELOVL5 and ATF6 transcripts down-regulated in the treated good embryos compared to control groups. The genes related to oxidative stress response (GPX4, SOD and CPT2) increased in the treated (good& poor) embryos, compared to the control group. While NFE2L2 mRNA decreased in the treated good embryos compared to the control untreated group. In conclusion, resveratrol supplementation with low concentration reduced oxidative stress, and improved cryotolerance of the blastocysts through regulation of some genes that related to oxidative stress response and embryo quality.

Keywords: Bovine; Gene expression; Phenazine ethosulfate; Resveratrol; Embryo quality; Viability; Cryotolerance.

DEDICATION

I would like to dedicate this humble dissertation with lots of love and respect to

My parents,

My wife,

My daughters

My sister and

My brothers

Without their support, love and care, I would not have realized my dreams in life.

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µM	Micro Mole
ACTB	β-actin
ATF6	Activating transcription factor 6
ATP	Adenosine triphosphate
ATP5ME	ATP synthase membrane subunit E
BP	Base pair
BSA	Bovine serum albumin
CASP3	Cysteine aspartic acid protease
cDNA	Complimentary DNA
COC	Cumulus oocyte complexes
CPT2	Carnitine palmitoyltransferase 2
Ct	Cycle threshold
DMSO	Dimethyl sulfoxide
DNA	Deoxy ribonucleic acid
DNMT	DNA methyltransferase
D-PBS	Dulbecco's phosphate buffer saline
EGA	Embryonic genome activation
ELOVL5	ELOVL fatty acid elongase 5
ER	Endoplasmic reticulum
GAPDH	Glyceraldehyde-3- phosphate dehydrogenase
GPX4	Glutathione peroxidase 4
GSH	Glutathione
H_2O_2	Hydrogen peroxide
ICM	Inner cell mass
IETS	International embryo technology society
IVC	In vitro culture
IVEP	In vitro embryo production
IVF	In vitro fertilization
IVM	In vitro maturation