Effects of supplementation with heat shock cognate 17 KDA protein (HSC70) on in vitro bovine embryo viability

Aimé Jazmín Garza Arredondo (✉ aime_jazmin@hotmail.com)

Universidad Autónoma de Nuevo León <https://orcid.org/0000-0002-5923-2717>

Diana Elisa Zamora Ávila

Universidad Autonoma de Nuevo Leon Facultad de Medicina Veterinaria y Zootecnia

Uziel Castillo Velásquez

Universidad Autonoma de Nuevo Leon Facultad de Medicina Veterinaria y Zootecnia

Gustavo Moreno Degollado

Universidad Autonoma de Nuevo Leon Facultad de Medicina Veterinaria y Zootecnia

José Fernando De La Torre Sánchez

INIFAP: Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias

Sandra Pérez Reynozo

INIFAP: Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias

J. Rubén Cervantes Vega

Universidad Autonoma de Nuevo Leon Facultad de Medicina Veterinaria y Zootecnia

Research Article

Keywords: bovine, blastocyst, HSC70, apoptosis, gene expression

Posted Date: August 4th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-762557/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Endogenous heat shock cognate 71 kDa protein (HSC70) has a vital role in early embryonic development. This study assessed the effects of exogenous HSC70 on bovine embryo development and expression of genes associated with apoptosis. Expression analyses of HSPA1A, HSPA8, Bcl-2, and Bax genes were performed in bovine embryos in vivo on day 7 of development. Subsequently, expression of HSPA1A and HSPA8 were associated with apoptotic genes (Bcl-2 and Bax) in cultured bovine embryos in vitro that were supplemented with various concentrations (0 or control group, 50, and 100 ng) of HSC70. The results indicated that the control group (0 ng) in vitro embryos had higher expression of HSPA8, Bax, and Bcl-2 genes, compared with the vivo embryos ($P < 0.01$). In vitro-produced embryos supplemented with 50 ng or 100 ng HSC70 had higher expression of HSPA1A, HSC70, Bcl-2, and Bax genes, compared with the control group ($P < 0.01$). Embryos supplemented with 100 ng had greater expression of the HSPA8 gene compared with the control group and the group supplemented with 50 ng. However, embryos supplemented with 50 ng had better characteristics (i.e., stage of development and quality) than the control and 100-ng groups. In conclusion, supplementation of in vitro culture medium with HSC70 promoted development to the blastocyst stage and improved blastocyst quality.

1. Introduction

In vitro embryonic development is conditioned by factors such as origin of gametes, culture conditions, microenvironment, and environmental stress. The post-fertilization culture period is the time during which developmentally important events occur and is considered to be a key determinant of blastocyst quality [1]. During in vitro culture, embryos are exposed to various stresses that are not normally encountered within the cow's reproductive tract [2].

Exogenous stress factors such as exposure to light, higher concentrations of oxygen, and culture medium composition induce metabolic variations in oocytes and blastocysts. These variations result in metabolic imbalance and reactive oxygen species degradation [3]. Lipid peroxidation, enzyme inactivation, protein modification, and DNA fragmentation leads to apoptosis and developmental block of the oocyte to the blastocyst stages [4,5].

Apoptosis is a programmed cell death pathway and a natural and essential developmental process. It eliminates redundant or superfluous cells to allow for normal patterning to proceed [6]. Irreparably stressed and damaged cells also use this route to die. Pathological stimuli such as radiation, chemotherapy, and environmental toxicants can initiate apoptosis in oocytes [7–9]. Heat shock can induce apoptosis in pre-implanted embryos [10–12].

Molecular chaperones protect cells from damage caused by physical and chemical hazards like increasing temperatures (e.g., heat shock), anoxia, hypoxia, metabolic stress-associated cytokines, nitrogen oxide, exposure to ethanol, heavy metals, apoptosis-inducing agents, and other chemical

denaturants and drugs [13,14]. Chaperones such as heat shock proteins (HSPs) have important roles in facilitating protein folding and maintaining normal protein structures and functions [15].

HSPs are classified mainly based on molecular weight in kilodaltons (kDas) as HSP110, HSP100, HSP90, HSP70, HSP60, HSP40, and the small HSP family (20 to 25 kDa). Within the HSP70 family, the constitutively expressed HSC70 (73 kDa) is slightly expressed during stress conditions.

HSC70 is particularly abundant in mammalian embryos. It participates in the importation of proteins into the nucleus and other cellular organelles, such as endoplasmic reticula and mitochondria. HSC70 maintains protein homeostasis (protein folding, translocation, assembly, disassembly, differentiation, and degradation) during normal and stress conditions. HSC70 suppresses protein aggregation and reactivates heat-denatured proteins [15]. Due to the above and the low percentage of embryos produced in vitro, the aim of this study was to evaluate the effect of supplementation with HSC70 on in vitro bovine embryo viability.

2. Materials And Methods

Maturation (IVM), fertilization (FCDM), early culture (CDM1), and late culture (CDM2) media used in the experiment were supplemented with fatty acid-free bovine serum albumin prepared at the National Center for Genetic Resources of the National Institute for Agricultural and Livestock Forestry Research (Jalisco, Mexico).

Experiments were performed in accordance with Facultad de Medicina Veterinaria y Zootecnia de la Universidad Autónoma de Nuevo León guidelines for the care and use of animals (01/2021).

2.1. Experimental design

Simbrah cows maintained under standard managerial practices in Centro de Investigación y Producción Agropecuaria de la Universidad Autónoma de Nuevo León were used for in vivo embryo production.

2.2 In vivo embryo production

Estrus was synchronized using an intravaginal controlled internal drug release (CIDR) progesterone-releasing device (Pfizer®) for 7 days. Cows were treated with estradiol benzoate (Syntex®, 2.0 mg, intramuscular (i.m.) route) at the time insertion of the CIDR device (day 0). Follicle-stimulating hormone (FSH; Folltropin-V, Bioniche®, 360 mg i.m.) was administered on days 4, 5, and 6. On day 6, cows were treated with two doses of prostaglandin F2α (Lutalyse, Zoetis®, 5mg/ml Dinoprost Tromethamine; 25 mg i.m.) every 12 h. During day 7, the CIDR withdrawal time, one more dose of FSH was applied. Artificial insemination was performed twice on day 8, in the morning and afternoon.

Seven days after artificial insemination, embryos were recovered using two-way Foley catheters; complete flush medium (Syngro®, Bioniche) was introduced, and the medium containing the embryos was subsequently obtained. Embryos were washed using the same solution (Complete Flush, Syngro®,

Bioniche) and then placed in Petri dishes. A stereoscopic microscope-assisted search for embryos and identification through numerical codes (stage of development and quality of embryos) was performed according to Manual of the International Embryo Technology Society (IETS) guidelines. Subsequently, the blastocysts were stored in groups of three per Eppendorf tube in 100 µl Trizol® Reagent (Invitrogen) at 5°C until RNA extraction and real-time quantitative reverse transcription (qPCR) analysis.

2.2. Oocyte retrieval and in vitro maturation

Ovaries were obtained at the local abattoir and transported to the laboratory in physiological saline (0.9% NaCl) maintained at room temperature (20 to 24°C). Cumulus-oocyte complexes were recovered from follicles with a diameter of 3–6 mm using an 18-gauge needle attached to a 10-mL syringe. Follicular fluid and the cell package were placed in a conical tube (50 ml) at 38.5°C. Selected cumulus-oocyte complexes with a uniform and granular cytoplasm, surrounded by a compact mass of cumular cells, were subsequently cultivated in IVM medium for 23 h at 38.5°C in a humidified atmosphere of 5% CO₂ in air. The medium included hCG, FSH, β-estradiol, cysteamine, and epidermal growth factor.

2.3. In vitro fertilization and in vitro culture

Mature oocytes were transferred to FCDM medium for co-culture with spermatozoa. Semen was thawed and processed in Percoll® gradient (45:90%). Mature oocytes and sperm were incubated together at 5% CO₂ in humidified air at 38.5°C for 18 h before in vitro culture.

After co-incubation, cumulus cells were removed using a pipet. The presumptive zygotes were washed and transferred to CDM1 medium supplemented with various concentrations of HSC70 (Sigma-Aldrich) (0 or control group, 50, and 100 ng). The samples were incubated for 56 h at 38.5°C in a humidified atmosphere of 5% CO₂, 5% O₂, and 90% N₂ in air.

Embryos (≥ 6-cell stage) were transferred and cultured in CDM2 medium and again supplemented with HSC70 (Sigma-Aldrich) (0, 50, and 100 ng) according to the group to which they previously belonged. Embryos remained in culture for 120 h under the conditions described above. At the end of the culture period, blastocysts were transferred to a 1.5-mL Eppendorf tube with 100 µl Trizol® Reagent (Invitrogen) until RNA extraction for gene expression analysis.

2.4. RNA extraction using modified Trizol method and complementary DNA (cDNA) synthesis

Using a modified Trizol protocol [16], RNA was isolated from three biological replicates (with three blastocysts each) from each tube in 100 µl Trizol® Reagent (Invitrogen). The samples were then incubated at room temperature for 3 min. Next, 50 µl chloroform was added, each sample was inverted for 15 sec, and then centrifuged at 12,000 g for 30 min at 4°C. The aqueous phase was then transferred to a new tube. The RNA was precipitated by addition of 2.5 volumes of isopropanol to the aqueous phase collected. Each tube was then centrifuged at 12,000 g for 30 min at 4°C, the supernatant was discarded,

and the pellet was washed using 50 µl 70% ethanol and then centrifuged at 7,500 g for 5 min. Finally, the pellet was dried in an incubator for 30 min at 37°C and further dissolved in 20 µl of DEPC water.

Eluted RNA was reverse-transcribed into cDNA using a commercial kit (GoTaq Probe 2-Step RT-qPCR System kit, Promega®). Briefly, 5 µl RNA, 1 µl oligo deoxythymidine (oligo dT 0.5 mg/µl), and 1 µl random primer were added to each tube. Subsequently, the tubes were incubated in a heat block without shaking at 70°C for 5 min and were immediately placed in ice-water for at least 5 min. Then, 4.9 µl nuclease-free water, 4 µl GoScript™ 5X Reaction Buffer, 1.6 µl MgCl₂, 1 µl PCR Nucleotide Mix, 0.5 µl Recombinant RNasin Ribonuclease Inhibitor, and 1 µl GoScript™ Reverse Transcriptase were incubated in a heat block at 25°C for 5 min. Subsequently, the reaction was extended in a controlled-temperature heat block at 42°C for 45 min and was inactivated in a heat block at 70°C for 15 min. The complementary DNA obtained was quantified using a Quantus™ fluorometer and stored at -20°C until use.

2.5. Quantitative real-time PCR analysis

All primers and probes used (Table 1) were designed by Integrated DNA Technologies. Each reaction was performed in triplicate using the PROMEGA® GoTaq Probe qPCR Master Mix to improve reproducibility of the result. The protocol used consisted of activation using a real-time detection system (Applied Biosystems 7500/7500 Fast) and GoTaq polymerase for one cycle at 95°C for 2 min, followed by denaturation for 15 sec at 95°C, and alignment and extension using 40 cycles at 60°C for 1 min.

Table 1
Forward and reverse primer pairs and probes used for PCR

Gene	Accession number	Primer and probe sequences	Size (bp)
HSPA1A	NM_174550.1	F:CACCATTGAGGAGGTGGATTAG R:TAGCTGATGGCTGATGAAAGG S:FAM/ATGGAGACT/Zen/GTTGGGATCCAAGGC	128
HSPA8	NM-174345.4	F:CCAGGTTGCTGACTCTTTCA R:GGAAGACACCCACACAAGAATA S:FAM/TGCAGTTGG/Zen/CATTGATCTTGGCAC	96
Bax	NM_173894.1	F:CGAGTTGATCAGGACCATCAT R:ATGTGGGTGTCCCAAAGTAG S:HEX/TCGAAGGAA/Zen/GTCCAATGTCCAGCC	579
Bcl-2	NM_001166486.1	F:GATTTCTCCTGGCTGTCTCTG R:GCCTGTGGGCTTCACTTAT S:FAM/TTGCATCAC/Zen/CCTGGGTGCCTA T	792
MYO1B	NM_001102199.1	F:CTGGTTTGTGCTGGCTTTAC R:CCATCCTACAGGGTTCCTAATG S:FAM/CAGTTACTG/Zen/GCGATAATGGACAGC GT.	155
GAPDH	NM_001034034.2	F:TGAGATCAAGAAGGTGGTGAAG R:GCATCGAAGGTAGAAGAGTGAG S:FAM/CCAGGTTGT/Zen/CTCCTGCGACTTCAA	82
Bp, base pair; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2 Associated X-protein; F, forward; GAPDH, glyceradehyde-e-phosphate dehydrogenase; HSPA8, Heat shock protein family A member 8; HSPA1A, Heat shock 70kDa protein 1A; MYO1B, Myosin IB; R, reverse.			

Results of the differences in expression of the genes studied were analyzed by relative quantification using $\Delta\Delta\text{ct}$. The results were reported as relative expression or n-fold differences compared with the calibrator after normalization of the level of transcript to the mean level of the endogenous control genes, glycerol e-phosphate dehydrogenase (GAPDH), and Bos taurus myosin (MYO1B).

2.6. Statistical analysis

Gene expression was analyzed using analysis of variance (ANOVA) followed by multiple pairwise comparison tests. Dunn's test was used to compare embryos in vitro versus in vivo, Bonferroni tests were used to compare for transferable with degenerate embryos, and Kruskal-Wallis tests were used to compare HSC70 protein-supplemented versus unsupplemented embryos. GraphPad Prism version 4.0 software was used to graph the obtained results.

3. Results

3.1. Expression of HSPA1A and HSPA8 genes and their relationships with apoptotic genes in bovine embryos in vivo and in vitro

The basal expression levels of three of the four genes of the study (HSPA8, Bcl-2, and Bax) were significantly higher in in vitro embryos than in those obtained in vivo ($***p < 0.001$ / $**p < 0.01$) (Fig. 1). Only the HSPA1A gene did not show a significant difference between in vitro and in vivo embryos.

The expression of the HSPA1A was significantly differed ($***p < 0.001$) between degenerate embryos compared with blastocysts, and expression of the anti-apoptotic Bcl-2 gene was higher in transferable embryos compared with degenerate embryos. Conversely, expression of the pro-apoptotic Bax gene was higher in degenerate embryos compared with the other treatment groups (Fig. 2).

At the end of the culture period, embryos were identified and classified based on IETS manual numerical codes corresponding to stage of development and quality.

Embryos that were supplemented with 50 ng of HSC70 had better developmental and quality characteristics; 63% of the resulting embryos in this group were categorized as blastocysts and 100% as code 1 (excellent or good) quality. This group was followed by the control group (without supplementation), with 63% blastocysts and 60% with quality code 1. Only degenerate embryos were found in the treatment group supplemented with 100 ng of HSC70.

Embryos supplemented with recombinant protein HSC70 also expressed greater amounts of the HSPA1A, HSPA8, Bcl-2, and Bax genes ($***p < 0.001$ / $*p < 0.05$).

The results also indicated that there was higher expression of the HSPA1A gene, which codes for the stress-inducible protein (HSP70), when the embryos were supplemented with low doses (50 ng) ($***p < 0.001$). Supplementation with high doses (100 ng HSC70) resulted in higher expression of the HSPA8 gene compared with the other treatments (unsupplemented and 50 ng; $***p < 0.001$).

The results for the genes associated with apoptosis indicated that the pro apoptotic Bax gene had higher expression in both treatment groups compared with the control group ($***p < 0.001$); and the anti-apoptotic Bcl-2 gene had higher and significant expression at the low supplementation doses (50 ng, $*p < 0.05$) (Fig. 3).

4. Discussion

HSPs are molecular chaperones that protect cells from physical or chemical damage [13,14]. They keep cells alive via regulation of homeostasis [17]. This study investigated effects of supplementation with the HSC70 protein on viability and expression of genes related to apoptosis in bovine embryo cultures.

HSPs are among the first proteins produced during embryonic development and are important for cell functions. Although they have been extensively studied in humans and mice, there is little information on the functions of genes that encode HSPs in bovine embryos [18].

Identification of HSP genes that specifically contribute to embryo survival may be an opportunity to improve protection of embryos in vitro against different toxic conditions and improve gestation rates in

cattle [19]. Embryos produced in vitro have changes in gene expression that reflect a stress response to sub-optimal conditions. During embryonic development, conditions such as apoptosis and oxidative stress are related to differences in expression of specific genes [1,20–22]. The results of this study were consistent with this characteristic; the HSPA1A gene was expressed differently between blastocysts and degenerated embryos. Embryos with poor development had greater expression of the gene that codes for the stress-induced protein, HSP70. Relatively small changes (decreases or increases) in levels of HSP expression can result in growth abnormalities and cell death [23].

The results of this study were also consistent with Wrenzycki et al. [24] results. There were significant differences in levels of gene expression (HSPA8, Bcl-2, and Bax) between the embryos obtained in vivo and those produced in vitro. The increase in the expression of genes in vitro supports the hypothesis that they were subjected to greater stress conditions during production. The results suggested that this change was associated with oxygen tension and composition of the medium used during culture.

Generally, the increase in oxygen tension during in vitro embryo production increases the generation of reactive oxygen species, which causes DNA damage, lipoperoxidation, and oxidative modification of proteins that increases susceptibility to proteolysis [4,25]. Therefore, antioxidants are used in oocyte maturation media and in vitro culture to improve the developmental competence of pre-implanted blasts [25–29].

Culture media supplemented with HSPs can be used as a treatment to reduce the toxicity and aggregation of polyglutamine in cells with Huntington's disease. Novoselova et al. [30] purified HSP70 and HSC70 from bovine muscle and added them to the culture media of cells transfected with Huntington's disease. Compared with the control group (without supplementation), the number of apoptotic cells decreased up to 50%. They also found that apoptosis was due to aggregation of insoluble proteins. Similarly, in this study we found that supplementation of embryo culture media with recombinant protein HSC70 promoted cell viability. Supplementation with the HSC70 protein stimulated expression of the HSPA1A, HSPA8, Bcl-2, and Bax genes at both supplementation concentrations (50 and 100 ng), compared with the control group. But, addition of the low dose (50 ng) also resulted in higher expression of the HSPA1A gene and in embryos with better characteristics at the time of stage and quality identification. These results could be attributed to regulation of expression of anti- and pro-apoptotic (Bcl-2 and Bax, respectively) genes.

HSPs are molecular chaperones whose expression increases after cells are subjected to different stressors; they have a protective function that helps the cell to cope with lethal conditions [15]. HSP70 has an anti-apoptotic function [31,32], which we also found in this study. It interacts with the intrinsic and extrinsic pathways of apoptosis at several steps. These steps include inhibition of Bax translocation in mitochondria, release of cytochrome c from mitochondria, apoptosome formation, and inhibition of activation of caspase activity. It also modulates the c-Jun N-terminal kinase, nuclear factor kappa B, and Akt signaling pathways in the apoptotic cascade.

The results of this study were consistent with Arya et al. [32] results; they found that HSC70 also has a pro-apoptotic role. In this study we found that supplementation with high doses (100 ng HSC70) resulted in a null number of blastocysts, which was related to the increase in the expression of the pro-apoptotic Bax gene.

In conclusion, supplementation of bovine embryo culture media with 50 ng recombinant protein HSC70 increased expression of the HSPA1A gene and the numbers of blastocysts produced in vitro.

Declarations

ACKNOWLEDGEMENTS

We thank the Aquatic-Livestock Laboratory of the National Center for Genetic Resources of the National Institute for Agricultural and Livestock Forestry Research (Tepatitlán de Morelos, Jalisco, México) for use of the facilities provided to perform the experiments of this study.

Funding:

No funding was received for conducting this study.

Conflicts of interest/Competing

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Availability of data and material

The rest of the datasets generated during the current study are available from the corresponding author on reasonable request.

Code availability

GraphPad Prism version 4.0 software was used to graph the obtained results.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Aimé Jazmín Garza Arredondo, Uziel Castillo Velásquez, José Fernando De La Torre Sánchez and Sandra Pérez Reynozo. The first draft of the manuscript was written by Aimé Jazmín Garza Arredondo, J. Ruben Cervantes Vega,

Gustavo Moreno Degollado and Diana Elisa Zamora Ávila, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval:

The studies have been approved by the Bioethics and animal welfare committee at the Facultad de Medicina Veterinaria y Zootecnia of the Universidad Autónoma de Nuevo León at which the studies were conducted, by the 01/2021 permit number.

Consent to participate:

Not applicable.

Consent for publication:

Not applicable.

References

- [1] Rizos D, Lonergan P, Boland MP, Arroyo-Garcia R, Pintado B, de la Fuente J, Gutiérrez-Adán A. Analysis of differential mRNA expression between bovine blastocyst produced in different culture systems: implications for blastocyst quality. *Biol Reprod* 2002; 66: 589-95.
- [2] Gordon I. Laboratory production of cattle embryos. Wallingford, Oxon, UK: CAB International 2003; 2nd ed: 273.
- [3] Thiyagarajan B, Valivittan K. Ameliorating effect of vitamin E on in vitro development of preimplantation buffalo embryos. *J Assist Reprod Genet* 2009; 26: 217-225.
- [4] Johnson M, Nasr-Esfahani M. Radical solutions and cultural problems: ¿could free oxygen radicals be responsible for the impaired development of preimplantation mammalian embryos in vitro? *Bioessays* 1994; 16: 31-38.
- [5] Kowaltowski AJ, Vercesi AE. Mitochondrial damage induced by conditions of oxidative stress. *Free Radic Biol Med* 1999; 26: 463-471.
- [6] Jacobson MD, Weil M, Raff MC. Programmed cell death in animal development. *Cell* 1997; 88: 347-354.
- [7] Perez GI, Knudson CM, Leykin L, Korsmeyer SJ, Tilly JL. Apoptosis associated signaling pathways are required for chemotherapy-mediated female germ cell destruction. *Nat Med* 1997; 3: 1228-1232.

- [8] Hu X, Christian PJ, Thompson KE, Sipes IG, Hoyer PB. Apoptosis induced in rats by 4-inylcyclohexenene diepoxide is associated with activation of the caspase cascade. *Biol Reprod* 2001; 65: 87-93.
- [9] Matikainen TM, Perez GI, Jurisicova A, Schlezinger JJ, Ryu H-Y, Pru JK, Saki T, Korsmeyer SJ, Casper RF, Sherr DH, Tilly JL. Aromatic hydrocarbon receptor-driven Bax gene expression is required for premature ovarian failure caused by biohazardous environmental chemicals. *Nat Genet* 2001; 28: 335-360.
- [10] Paula-Lopes FF, Hansen PJ. Heat-shock induced apoptosis in bovine preimplantation embryos is a developmentally-regulated phenomenon. *Biol Reprod* 2002; 66: 1169-1177.
- [11] Krininger III CE, Stephens SH, Hansen PJ. Developmental changes in inhibitory effects of arsenic and heat shock on growth of preimplantation bovine embryos. *Mol Reprod Dev* 2002; 63: 335-340.
- [12] Paula-Lopes FF, Hansen PJ. Apoptosis is an adaptive response in bovine preimplantation embryos that facilitates survival after heat shock. *Biochem Biophys Res Commun* 2002; 295: 37-42.
- [13] Smith DF, Whitesell L, Katsanis E. Molecular chaperones: biology and prospects for pharmacological intervention. *Pharmacol Rev* 1998; 50: 493-513.
- [14] Tsukahara F, Yoshioka T, Muraki T. Molecular and functional characterization of HSC54, a novel variant of human heat-shock cognate protein 70. *Mol Pharmacol* 2000; 58: 1257-1263.
- [15] Liu T, Daniels CK, Cao S. Comprehensive review on the HSC70 functions, interactions with related molecules and involvement in clinical diseases and therapeutic potential. *Pharmacol Ther* 2012; 136: 354-374.
- [16] Pavani KC., Baron EE, Faheem M, Chaveiro A, Moreira Da Silva F. Optimisation of total RNA extraction from bovine oocytes and embryos for gene expression studies and effects of cryoprotectants on total RNA extraction. *Cytol Genet* 2015; 49: 232-239.
- [17] Rodriguez-Ariza A, López-Sánchez LM, González R, Corrales FJ, López P, Bernardos A, Muntané J. Altered protein expression and protein nitration pattern during D-galactosamine-induced cell death in human hepatocytes: a proteomic analysis. *Liver Int* 2005; 25: 1259-1269.
- [18] Zhang B, Peñagaricano F, Driver A, Chen H, Khatib H. Differential expression of heat shock protein genes and their splice variants in bovine preimplantation embryos. *J Dairy Sci* 2011; 94: 4174-4182.
- [19] Hansen PJ. To be or not to be. Determinants of embryonic survival following heat shock. *Theriogenology* 2007; 68: 40-48.
- [20] Lonergan P, Rizos D, Gutiérrez-Adán A, Moreira PM, Pintado B, de la Fuente J. Temporal divergence in the pattern of Messenger RNA expression in bovine embryos cultured from the zygote to blastocyst stage

in vitro or in vivo. Biol Reprod 2003a; 69: 1424-31.

[21] Lonergan P, Rizos D, Kanka J, Nemcova L, Mbaye AM, Kingston M, Wade M, Duffy P, Boland MP. Temporal sensitivity of bovine embryos to culture environment after fertilization and the implications for blastocyst quality. Reproduction 2003b; 126: 337-346.

[22] Mortensen CJ, Choi Y-H, Ing NH, Kraemer DC, Vogelsang MM., Katrin-Hinrichs. Heat shock protein 70 gene expression in equine blastocysts after exposure of oocytes to high temperatures in vitro or in vivo after exercise of donor mares. Theriogenology 2010; 74: 374-383.

[23] Nollen EA, Morimoto RI. Chaperoning signaling pathways: molecular chaperones as stress-sensing heat shock proteins. J Cell Sci 2002; 115: 2809-2816.

[24] Wrenzycki C, Herrmann D, Keskinetepe L, Martins Jr A, Sirisathien S, Brackett B, Niemann H. Effects of culture system and protein supplementation on mRNA expression in preimplantation bovine embryos. Hum Reprod 2001; 16: 893-901.

[25] Wang ZG, Yu SD, Xu ZR. Improvement in bovine embryo production in vitro by treatment with green tea polyphenols during in vitro maturation of oocyte. Anim Reprod Sci 2007; 100: 22-31.

[26] Salzano A, Alberio G, Zullo G, Neglia G, Abdel-Wahab A, Bifulco G, Zicarelli L, Gasparrini B. Effect of resveratrol supplementation during culture on the quality and cryotolerance of bovine in vitro produced embryos. Anim Reprod Sci 2014; 151: 91-96.

[27] Wang F, Tian X, Zhang L, He C, Ji P, Li Y. Beneficial effect of resveratrol on bovine oocyte maturation and subsequent embryonic development after in vitro fertilization. Fertil Steril 2014; 101: 577-586.

[28] Fakruzzaman M, Ghanem N, Bang JI, Ha AN, Lee KL, Sohn SH, Wang Z, Lee DS, Kong IK. Effect of peroxiredoxin II on the quality and mitochondrial activity of pre-implantation bovine embryos. Anim Reprod Sci 2015; 159: 172-183.

[29] Rodrigues-Cunha MC, Mesquita LG, Bressan F, del Collado M, Balieiro JCC, Schwarz KRL, de Castro FC, Watanabe OY, Watanabe YF, de Alencar Coelho L, Leal CLV. Effects of melatonin during IVM in defined médium on oocyte meiosis, oxidative stress, and subsequent embryo development. Theriogenology 2016; 86: 1685-1694.

[30] Novoselova TV, Margulis BA, Novoselov SS, Sapozhnikov AM, van der Spuy J, Cheetham ME., Guzhova IV. Treatment with extracellular HSP70/HSC70 protein can reduce polyglutamine toxicity and aggregation. J Neurochem 2005; 94: 597-606.

[31] Lanneau D, de Thonel A, Maurel S, Didelot C, Garrido C. Apoptosis versus cell differentiation: role of heat shock proteins HSP90, HSP70 and HSP27. Prion 2007; 1: 53-60.

Figures

Figure 1

Baseline expression of study genes (HSPA1A, HSPA8, Bcl-2, BAX) in in vitro versus in vivo embryos. Asterisks indicate a significant difference (** $p < 0.001$ / ** $p < 0.01$)

Figure 2

Baseline expression of study genes, based on embryonic quality (blastocysts and degenerate embryos). Asterisks indicate a significant difference (**p < 0.01, ***p < 0.001)

Figure 3

Comparison of gene expression of in vitro embryos supplemented with 50 ng or 100 ng HSC70, compared with the control group (no supplementation). Asterisks indicate a significant difference (**p < 0.01/ * p < 0.05).