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Original Research Article

Influence of DMSO on gene expression in bovine embryos: Exploring solvent-specific effect on substance dissolution in fertilization medium

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Abstract

Purpose: *To investigate the role of dimethyl sulfoxide (DMSO) as a cryoprotectant and solvent in bovine embryogenesis, with a particular focus on its impact on gene expression during early and late developmental stages.*
Method: *Bovine embryos were used to evaluate effects of DMSO on expression of genes critical to apoptosis and developmental processes. Gene expression analysis was performed to assess changes in both pro-apoptotic and anti-apoptotic markers, as well as genes essential for growth and survival.*
Results: *Dimethyl sulfoxide influenced gene expression in a stage-specific manner. During early development, DMSO induced overexpression of the pro-apoptotic gene, BAX, and downregulated the anti-apoptotic gene, BCL2, indicating increased apoptotic activity. Furthermore, the expression of GDF9 and IGF1, which are crucial for growth and survival, was altered, suggesting interference with key developmental pathways. In contrast, late-stage embryos exhibited elevated levels of BCL2 and HSPB1, markers of anti-apoptotic activity, indicating a more complex regulatory role of DMSO at advanced stages of embryogenesis.*
Conclusion: *While DMSO is effective as a cryoprotectant, its impact on gene expression raises concerns about potential developmental consequences. These findings highlight the need for further investigation to better understand the specific effects of DMSO in the context of assisted reproductive technologies (ART).*

Keywords: *Gene expression, Bovine embryo, Embryo development, DMSO, Apoptosis*

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INTRODUCTION

Over the past few decades, significant advancements in *in vitro* fertilization (IVF) technologies have provided valuable insights into embryonic development, genetic engineering and reproductive medicine [1]. Despite these advancements, achieving optimal conditions for embryonic growth remains a considerable challenge, particularly concerning cryopreservation and the solvents used in assisted reproductive technologies (ART) [2]. One widely utilized solvent in cryopreservation is dimethyl sulfoxide (DMSO), which efficiently crosses cell membranes and solubilizes hydrophobic molecules [3]. While DMSO reduces ice crystal formation during freezing-thawing processes, it is also used in preserving cells and tissues such as oocytes and embryos [4].

Beyond its cryoprotective effect, DMSO is commonly employed to solubilize compounds such as growth factors, small molecules and hormones for *in vitro* use. However, its validity as a solvent in embryological study is increasingly questioned due to its potential to interfere with embryo viability and physiology, including effects on gene expression [5]. Reports indicate that DMSO modifies the expression of genes critical for embryonic development, affecting pathways related to stress response and epigenetic regulation. Such molecular changes impair embryo viability, cell differentiation and organogenesis [6]. Given the dynamic role of free amino acids in cultured cells and embryos, this study focuses on metabolomic pathways enriched during critical phases of bovine embryogenesis. The effects of DMSO on gene expression during early embryogenesis could lead to developmental failure and reduced reproductive efficiency. Understanding whether DMSO impacts the transcriptional profile of preimplantation embryos is essential [7].

A previous study has associated solvents like DMSO with oxidative stress, DNA damage and mitochondrial dysfunction in embryonic cells [8]. Maintaining intracellular stability during early development is crucial and DMSO's impact on the solubility and bioavailability of compounds in fertilization media is critical to this balance. The effects of DMSO on gene expression may depend on its dose and exposure duration, indicating a dual mode of action [9]. Recognizing that DMSO's impact is specific to its use as a solvent, this study examines the molecular pathways affected by DMSO and its role in regulating genes critical for embryonic growth and differentiation.

The study was designed to evaluate DMSO's effects on gene expression profiles in bovine embryos during early and late developmental stages and quantify the solvent-specific effects of DMSO in fertilization media in order to identify gene expression alterations that compromise embryo quality. This knowledge is vital for optimizing embryo culture conditions, which are integral to the success of ART.

EXPERIMENTAL

Ovaries, oocytes and maturation

Cow ovaries were collected from a local Slaughterhouse in Riyadh and transported to the Embryonic Studies and Reproductive Physiology Laboratory at King Saud University in 0.9 % NaCl solution. The transportation duration ranged from one to two hours. Oocytes were retrieved from ovarian follicles using a 10 mL syringe, a 10 mL vial and 0.5 mL of handling medium which consisted of TCM-199 (Hanks' solution) supplemented with 10 % fetal bovine serum, gentamycin and sodium pyruvate (100 mM stock solution; Smithfield, UT, USA). Cumulus-oocyte complexes were matured for 24 h at 37 °C in an atmosphere containing 5 % CO_2. Post-maturation, the complexes exhibited homogeneous ooplasm and more than three layers of cumulus cells, as described by Ammari *et al,* [10].

Frozen semen preparation

Thawed bull semen was first incubated in a 37 °C water bath and then subjected to separation using a discontinuous Percoll gradient, with 2 mL of 45 % Percoll on top of 2 mL of 90 % Percoll. After centrifugation, the supernatant was carefully discarded and the sperm pellet was resuspended in *in vitro* fertilization medium (IVF-B.O). The sperm cells were then incubated for three hours in a humidified atmosphere at 39 °C with 5 % CO_2 to enhance capacitation. After incubation, the sperm concentration was determined using a hemocytometer and adjusted to a final concentration of 2 x 10^6 cells/mL [11].

In vitro production

Cumulus cells surrounding the fertilized and unfertilized cumulus-oocyte complex (COCs) were removed via mechanical pipetting using a glass Pasteur pipette in a hyaluronidase enzyme solution. The COCs were then rinsed with an *in vitro* culture medium (IVC-SOF, Caisson IVL05). Afterward, batches of 20 to 25 zygotes were cultured in droplets of IVC-SOF medium enriched with 0.34 mM sodium pyruvate, 1 mM L-glutamine, 50X MEM-essential amino acids (Sigma B6766), 100X MEM-nonessential amino acids (Sigma M7145), 3 mg/mL BSA (Sigma A6003), 25 µg/mL gentamycin, 1.5 mM glucose and 1 µg/mL EDTA, placed in a 35 mm Petri dish. The culture drops were covered with mineral oil specifically tested for embryo safety. The embryos were incubated at 39 °C for a period of 7 to 9 days in a controlled environment of 5 % CO_2, 5 % O_2 and 90 % N_2, with high humidity inside the incubation chamber [12].

Assessment of DNA in embryos

Bovine embryos derived from *in vitro* fertilization (IVF) were monitored at various cleavage stages over a culture period of 7 to 9 days. Three replicates of 30 embryos each were prepared for analysis at specific developmental stages namely 2-cell, 4-cell, 8–16-cell, morula and blastocyst.

After collection, embryos were rinsed at least twice in 0.1 % PVA-PBS and subsequently stored at -80 °C in 5 µL aliquots of the same solution until RNA extraction was performed. The expression of selected genes, including B-cell lymphoma 2 (BCL2), Heat Shock Protein Family B, Small Member 1 (HSPB1), a gene associated with apoptosis (BAX), Growth Differentiation Factor (GDF9), and Insulin-like Growth Factor 1 (IGF1), was assessed at different stages of *in vitro* development [13].

RESULTS

Early stages of embryogenesis

In the early stages of embryogenesis, expression of BAX in control group, which did not receive DMSO treatment, was approximately 0.16, with minimal variability. In contrast, DMSO-treated group exhibited a significant increase in BAX expression to approximately 0.30, with higher variability, indicating a statistically significant difference ($p < 0.01$) between the two groups. This suggests that DMSO upregulates BAX, a key pro-apoptotic gene, in early embryogenesis. Expression of BCL2, an anti-apoptotic gene, was reduced in the DMSO-treated group (0.02) compared to control group (0.036). This reduction in BCL2, coupled with the increase in BAX, implies a potential shift towards pro-apoptotic pathways under DMSO treatment.

Also, expression of GDF9 exhibited significant changes. In control group, GDF9 expression was approximately 0.831, whereas in the DMSO-treated group, it significantly increased to 2.0 ($p < 0.01$), indicating that DMSO enhances GDF9 expression, which is associated with oocyte and follicular development. On the other hand, HSPB1 showed no significant differences in expression between control and DMSO-treated groups, with both maintaining a relative expression level of approximately 0.025, suggesting that DMSO does not alter HSPB1 expression during early embryogenesis. However, the expression of IGF1 was significantly reduced in DMSO-treated group (0.003) compared to control group (0.01; $p < 0.01$). This finding suggests that DMSO downregulates IGF1, a gene essential for growth and survival during early development (Figure 1).

Advanced stages of embryogenesis

During the late stages of embryogenesis, the expression of BAX in control group was approximately 0.215 increased slightly to 0.3 in the DMSO-treated group, although this change was not statistically significant, implying that the effect of DMSO on BAX expression diminishes as embryogenesis progresses. Conversely, the expression of BCL2 was significantly ($p < 0.01$) upregulated in the DMSO-treated group (0.060) compared to control group (0.04). This indicates that DMSO may enhance cellular survival mechanisms by upregulating BCL2 expression, thereby inhibiting apoptosis during late embryogenesis.

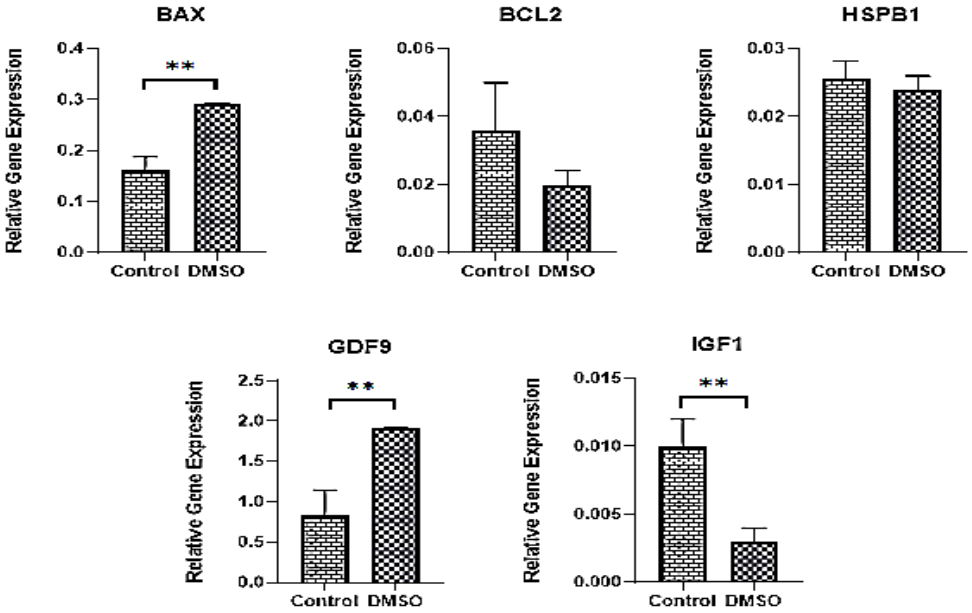

Figure 1: Impact of DMSO on gene expression in early-stage bovine embryos

HSPB1 demonstrated a significant upregulation following DMSO treatment, with expression levels increasing to 0.4 compared to 0.2 in control group ($p < 0.01$). This finding suggests that DMSO enhances the cellular stress response during late embryogenesis.

In contrast, the expression of GDF9 was significantly downregulated in the DMSO-treated group (0.02) compared to control group (0.030), with a statistically significant difference ($p < 0.05$), indicating that DMSO may disrupt pathways associated with oocyte maturation and follicular development during the later stages of embryogenesis. Finally, expression of IGF1 remained consistent between control and DMSO-treated groups, with both exhibiting similar expression levels of approximately 0.003, thus indicating that DMSO has no measurable effect on IGF1 expression in late embryogenesis (Figure 2).

DISCUSSION

In comparison to the study by Saber *et al,* which examined the effects of resveratrol on viability and stress-response genes in vitrified bovine embryos [14], this study demonstrates that DMSO induces pro-apoptotic activity during early embryogenesis by upregulating BAX and downregulating BCL2. Conversely, Saber *et al,* reported that resveratrol exhibited antioxidative properties, enhancing embryo viability by upregulating OCT4 and DNMT1 while downregulating CASP3, a critical gene involved in apoptosis. The studies differ in their focus, with DMSO linked to apoptotic processes and resveratrol demonstrating protective effects. Resveratrol mitigated oxidative stress, while DMSO appeared to promote apoptotic pathways early on, implying different impacts on embryo quality. In addition, both studies highlight the importance of understanding the molecular pathways activated by different treatments with the findings of this study indicating opposing biological effects between DMSO and resveratrol. While DMSO appears to induce cellular stress and apoptosis by upregulating BAX and downregulating BCL2, resveratrol promotes stress resilience and viability by upregulating OCT4 and DNMT1 and downregulating CASP3, a gene critical to apoptosis.

A similar study by Alsolami and co-workers investigated the role of DMSO in inducing trophectoderm differentiation and clonal blastoid formation from single human pluripotent stem cells [15].

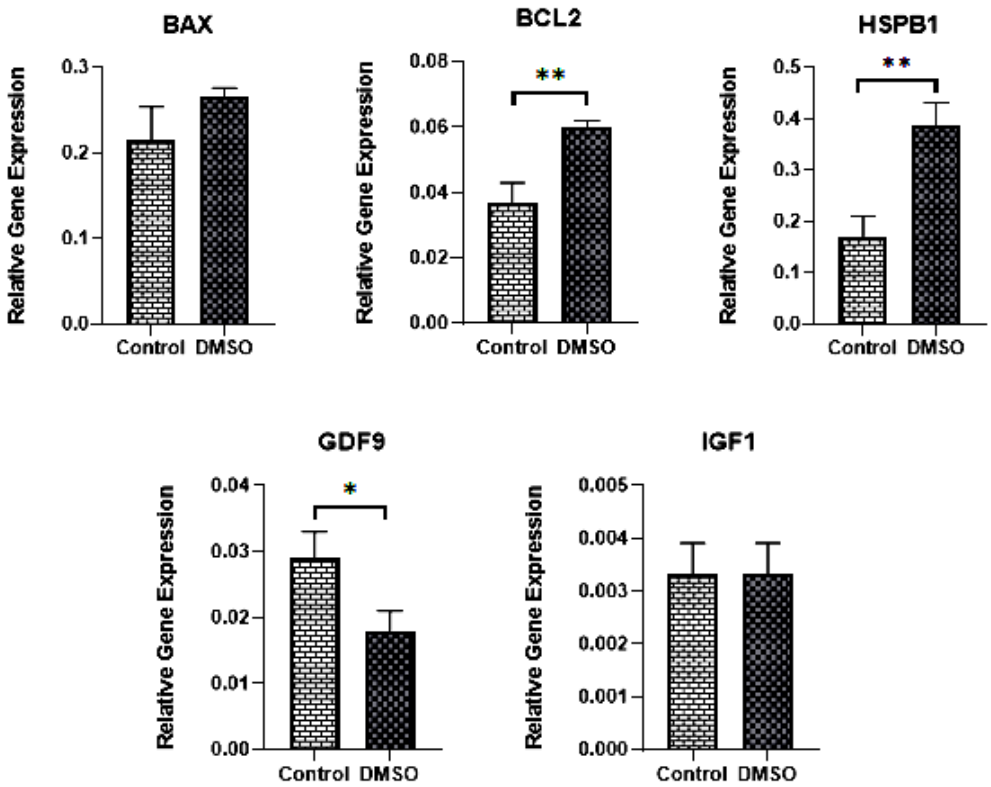

Figure 2: Impact of DMSO on gene expression in late-stage bovine embryos

While this study demonstrates that DMSO promotes apoptosis in bovine embryos, Alsolami's findings emphasize its ability to enhance trophectoderm formation through PKC signaling in human stem cells. These studies highlight the context-dependent effect of DMSO, which activates either apoptotic or developmental pathways depending on the cellular context and species. Furthermore, the findings of this study contrast with those of Zhao *et al,* who investigated the effect of dendrobine on bovine oocyte maturation and embryonic development [16]. Findings reveal that dendrobine, a plant alkaloid, significantly improved oocyte maturation, subsequent embryonic development and overall embryo quality by enhancing the maturation environment, improving cytoplasmic conditions and reducing oxidative stress. This resulted in better blastocyst rates and embryo quality during *in vitro* maturation (IVM).

Though DMSO in this study was shown to promote pro-apoptotic activity, particularly by increasing BAX expression and reducing BCL2, leading to potential cellular stress, dendrobine appears to support cell viability and development. Dendrobine's positive impact on oocyte maturation contrasts with the potential stress-inducing effects of DMSO, especially during early embryogenesis. Both studies underscore the critical importance of regulating cellular environment to achieve optimal embryonic development, albeit through distinct mechanisms. Thus, DMSO exerts its effects by modulating apoptosis-related genes, while dendrobine enhances maturation conditions and mitigates oxidative stress, highlighting complementary pathways for improving embryonic outcomes.

Furthermore, the results of this study indicate that DMSO impacts cell fate by triggering apoptotic pathways in embryonic cells. Similarly, studies by Dubois-Pot-Schneider and his group demonstrated that DMSO influences transcriptional and epigenetic regulation in HepaRG cells, a human liver cell line, by inducing changes in cell cycle regulation and metabolic pathways. Specifically, they observed that DMSO modulated the expression of genes associated with drug metabolism and induced epigenetic modifications of histone markers, affecting cellular differentiation and proliferation. Both studies highlight DMSO's capacity to elicit significant alterations in gene expression and cellular behavior, albeit within different contexts. While our study focuses on DMSO's role in apoptosis during early embryogenesis, Dubois-Pot-Schneider *et al,* highlight its regulatory effects on metabolic and differentiation pathways in liver cells [17]. These findings are crucial for understanding how DMSO influences cellular processes beyond simple solvation, with potential implications in drug testing and liver cell biology.

CONCLUSION

Results show that DMSO modulates the expression of apoptosis-related genes, including BAX and BCL25, during early embryogenesis. Additionally, upregulation of BCL2 and HSPB1 during later embryonic stages indicates that DMSO activates alternative mechanisms to support cell survival during this phase of development. Exploring the wider biological consequences of these changes could inform about how DMSO affects aspects of embryonic development and may be beneficial or detrimental in reproductive biology.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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