DMSO supplementation during *in vitro* maturation of bovine oocytes improves blastocyst rate and quality

Amada Eugenia Ynsaurralde-Rivolta, Mariana Suvá, Carolina Griselda Luchetti, Romina Jimena Bevacqua, Sebastian Munilla, Lleretny Rodriguez-Alvarez, Alejandra Velasquez, Olinda Briski, Daniel Lombardo, Daniel Salamone

PII: S0093-691X(20)30157-6

DOI: https://doi.org/10.1016/j.theriogenology.2020.02.045

Reference: THE 15426

To appear in: *Theriogenology*

Received Date: 1 November 2019

Revised Date: 28 February 2020

Accepted Date: 29 February 2020

Please cite this article as: Ynsaurralde-Rivolta AE, Suvá M, Luchetti CG, Bevacqua RJ, Munilla S, Rodriguez-Alvarez L, Velasquez A, Briski O, Lombardo D, Salamone D, DMSO supplementation during *in vitro* maturation of bovine oocytes improves blastocyst rate and quality, *Theriogenology* (2020), doi: https://doi.org/10.1016/j.theriogenology.2020.02.045.

Dra Amada Eugenia Ynsaurralde-Rivolta: Conceptualization. investigation, formal analysis, funding acquisition, writing - original draft

Lic. Mariana Suvá: Investigation

Dra Carolina Griselda Luchetti: Investigation

Dra Romina Jimena Bevacqua: Conceptualization and investigation.

Dr Sebastian Munilla: Formal analysis.

Dra Lleretny Rodriguez-Alvarez: Investigation and funding acquisition.

Dra Alejandra Velasquez: Investigation.

MV Olinda Briski: Investigation.

Dr Daniel Lombardo: Investigation and funding acquisition.

Dr Daniel Salamone: Conceptualization, formal analysis, writing - original draft, supervision, project administration, funding acquisition, resources.

DMSO supplementation during *in vitro* maturation of bovine oocytes improves blastocyst rate and quality

Dr. Amada Eugenia Ynsaurralde-Rivolta[ab] eugeniaynsaurralde@gmail.com

Lic. Mariana Suvá[a] marianasuva@gmail.com

Dr. Carolina Griselda Luchetti[cd] caroluchetti@gmail.com

Dr. Romina Jimena Bevacqua[ae] bevacqua@agro.uba.ar

Dr Sebastian Munilla[de] munilla@agro.uba.ar

Dr. Lleretny Rodriguez-Alvarez[f] llrodriguez@udec.cl

Dr. Alejandra Velasquez[f] alevelasquez@udec.cl

MV Olinda Briski[ade] briskiolinda@agro.uba.ar

Dr Daniel Lombardo[c] dlombard@fvet.uba.ar

Dr Daniel Salamone*[ade] salamone@agro.uba.ar

[a]Animal Biotechnology Laboratory, Facultad de Agronomía, INPA-CONICET, Buenos Aires University, Buenos Aires, Argentina.

[b]Instituto Nacional de Tecnología Agropecuaria (INTA), Estación Experimental Agropecuaria Mercedes, Corrientes, Argentina.

[c]Universidad de Buenos Aires, Facultad de Ciencias Veterinarias, Instituto de Investigación y Tecnología en Reproducción Animal (INITRA), Cátedra de Histología y Embriología

[d]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

[e]Departamento de Producción Animal, Facultad de Agronomía, Universidad de Buenos Aires.

[f]Universidad de Concepción, Facultad de Ciencias Veterinarias, Chillán, Chile.

ABSTRACT

The molecule Dimethyl sulfoxide is widely used as drug solvent. However, its antioxidant property was poorly explored. In this study, we evaluated the effect of DMSO supplementation during oocyte *in vitro* maturation (IVM) on embryo development and quality. Bovine oocytes were matured with different DMSO concentrations (0, 0.1, 0.25, 0.5, 0.75, 1 and 10% v:v) followed by *in vitro* fertilization. Subsequently, quality indicators such as gene expression of SOX2, OCT4, CDX2, SOD1, oocyte and embryo redox status and DNA damage were evaluated. Polar body extrusion and blastocyst rates increased with 0.5 % v:v DMSO. Moreover, first polar body extrusion and blastocyst rates did not increase with 1%, and 10% of DMSO reduced polar body extrusion and did not produce blastocyst. Optimal concentration of DMSO for the use on the maturation was estimated at around 0.45% v:v. Supplementation with 0.5% v:v DMSO has not affected mRNA abundance of genes key in blastocyst, however 0.75% increased gene expression of OCT4 and SOX2. Oocytes matured with 0.5% v:v DMSO and blastocyst from DMSO group showed reduced lipid peroxidation respect control. Total Glutathione concentrations increased in blastocyst stage in DMSO group. DMSO increased the total cell number of blastocysts but not TUNEL positive cells. In conclusion, our results suggest that low DMSO concentrations used during bovine oocytes *in vitro* maturation increases the maturation, as well as the blastocyst rate and its quality, without demonstrating deleterious effect on embryo cells.

Key words: cattle, embryo, oxidative stress

1. Introduction

Dimethyl sulfoxide (DMSO) is a small organic polar aprotic molecule used as cryoprotectant and solvent for some drugs due to its amphipathic nature [1, 4]. Besides, the pharmacological actions of DMSO have been evaluated by properties like membrane penetration, carrier effect, collagen deposition and anti-inflammatory activity, among others [1, 2, 3, 4].

In vitro production systems use DMSO to solute drugs like roscovitine [5]. However, due to possible cellular toxicity, this compound must be used in low concentrations [6, 7]. Nevertheless, it has been shown that DMSO, used in low doses, has antioxidant effects since inhibits endoperoxide biosynthesis [8] and neutralizes the cytotoxic effects of reactive oxygen species (ROS) [9, 10].

ROS (superoxide anion radical O2-; hydrogen peroxide H2O2, and the hydroxyl radical HO-) are originated from normal cell metabolism, but its production is increased by external factors like oxygen tension [11]. The embryo has antioxidative mechanisms that counteract ROS action. However, protein, membrane and DNA damage have been observed when this balance between ROS and antioxidant mechanisms is broken. Consequently, the embryo development decreases [12, 13, 14, 15, 16, 17, 18, 19].

Nonetheless, the efficiency of *in vitro* embryo production increases when the oocytes are treated during *in vitro* maturation with antioxidants such as quercetin, vitamin C, resveratrol [20], melatonin [21] and glutathione (GSH) promoters as β-mercaptoethanol, cysteine and cystine [22]. The maturation process involves a number of events that gives oocyte the capacity for normal embryo development [23]. However, an antioxidant effect of low doses of DMSO on oocytes and embryonic development has not been explored yet.

Considering the wide use of DMSO in *in vitro* embryo production and its unexplored antioxidant property, this study evaluates the effect of DMSO supplementation at low concentrations on the maturation medium of oocyte over embryo development and quality.

2. Materials and methods

2.1. Experimental design

2.1.1. First polar body extrusion and embryo development

First, we determined the effect of DMSO on the extrusion of the first polar body (1PB) after IVM was determined. For this, five concentrations of DMSO were tested: 0, 0.1, 0.5, 1 and 10% v:v DMSO (N: 241, 195, 142, 192, 172 oocytes respectively). In all the repetitions, the IVM was performed in 35 mm dish with 3.5 ml of mineral oil (MO).

Second, the effect of DMSO on cleavage and blastocyst rates was evaluated (Figure 1). In this case the IVM medium was supplemented with 0, 0.1, 0.5, 1 or 10% v:v DMSO (N:

446, 322, 194, 250, 39 oocytes respectively). Concentration 0.25 and 0.75 % of DMSO were added to evaluation (N: 65 and 77) to calculate optimal dose.

Blastocyst produced in this experiment were destined to gene expression analysis and DNA fragmentation and total cell number evaluation. According results obtained and for practical purposes, 0.5% v:v was used as the optimum dose in analyses below.

2.1.2. *Gene expression*

Expression level of OCT4, SOX2, CDX2 and SOD1 transcripts were evaluated in blastocysts from oocytes supplemented with 0, 0.5 and 0.75% v:v DMSO in the IVM medium obtained from 2.1.1.

2.1.3. Redox balance

Superoxide dismutase (SOD) activity, total content of GSH and lipid peroxidation were evaluated. For this, separately in specific repetitions, IVM medium was supplemented with 0 and 0.5% v:v DMSO. Immature and mature cummulus oocytes complexes (COCs), 8/16 cell embryos and blastocysts were compared separately according to each developmental stage.

2.1.4. DNA fragmentation and total cell number

Damage in DNA and total cell number were evaluated by TUNEL assay in blastocysts from 0, 0.5 and 0.75% v:v DMSO group obtained from 2.1.1.

2.2.*Oocyte collection and in vitro maturation*

Ovaries were collected from slaughterhouse and transported to the laboratory. COCs were aspirated from small antral follicles (2-8 mm). COCs covered with at least 3 layers of granulosa cells were selected and maturated *in vitro*. Maturation medium was Tissue Culture Medium 199 (31100-035, Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum, 10 µg/mL follicle stimulating hormone (NIH-FSH-P1; Folltropin ®, Bioniche, Belleville, ON, Canada), 0.3 µM sodium pyruvate (P2256; Sigma-Aldich, St Louis, MO, USA), 100 µM cysteamine (M9768; Sigma-Aldich, St Louis, MO, USA), and 2% antibiotic-antimycotic (15240-096; Gibco, Grand Island, NY, USA). The COCs were incubated in 100 ul droplets (20-25 oocytes/droplet) covered with mineral oil (0121-1; Fisher Chemical, Pittsburgh, PA, USA) for 24 hours at 6.5% CO_2 in humidified air at

38.5°C. Different concentrations of DMSO (D2650; Sigma-Aldich, St Louis, MO, USA) were added to the maturation media, depending on the experiment. After IVM, COCs were vortexed for 2 min in hyaluronidase (H-4272; Sigma-Aldich, St Louis, MO, USA. 1 mg/mL) in Dulbecco´s phosphate saline (to remove cumulus cells). First polar body extrusion was evaluated by direct observation on stereoscopic microscope (SMZ 800; Nikon, Tokio, Japón).

2.3. *In vitro fertilization and culture*

Frozen semen was thawed for 30 seconds by bain-marie at 37 °C. Then, spermatozoa were centrifuged twice (490 x g, 5 minutes) in Brackett & Oliphant (BO) medium [24], with 16 x 10^6 spermatozoa/ml for 5 hours. Afterwards, presumptive zygotes were cultured in Synthetic Oviductal Fluid [25, 26] with 2.5% of fetal bovine serum for 7 days at 38.5°C and 5% O_2. Cleavage and blastocyst rates were observed on days 2 and 7, respectively.

2.4.*Gene expression analysis*

Three replicates of blastocysts (N5) treated with 0, 0.5 and 0.75% (v:v) DMSO were analyzed. The expression of OCT4, SOX2, CDX2 AND SOD1 genes was evaluated. Embryos were stored in RNAlater at -20°C until gene expression analysis. Embryos were treated with a Cells-to-cDNA TM II kit (Ambion Co., Austin, TX, USA) lyses buffer according to manufacturer's instruction. Briefly, embryos were washed twice in cold PBS to eliminate the RNAlater; 30 µl of lysis buffer was added and incubated 10 minutes at 75°C. All samples were treated with DNase I (0.04 U/µl) for genomic DNA digestion. For cDNA conversion, 10 µl of total RNA was used in a 20µl final reaction containing 5 µM random primers, 10 mM each dNTP, 2 µl first strand buffer (10×), 10 U of RNase inhibitor, and 200 U/ml M-MuLV (Ambion Co., Austin, TX, USA). Cycling parameters were: 70°C for 3 minutes, 42°C for 60 minutes, and 92°C for 10 minutes. The produced cDNAs were kept frozen at −20°C until use in PCR experiments.

Gene expression analysis was performed by real-time qPCR using the standard curve method. The standard curve was performed using 2 µl of PCR products for each gene, previously purified from agarose gel and eluted using the E.Z.N.A gel extraction kit (Omega, Biotek, Santiago, Chile) and quantified using a spectrophotometer (Epoch, BioTek Instruments, Inc., Winooski, VT, USA). Eight points of serial ten-fold dilutions of PCR

products were included in each standard curve, and 2 µl was used in duplicate for each point. For qPCR analysis, samples were loaded in duplicate (technical replicates). The qPCR reactions were made with 2 µl of cDNA from each sample, 1 µl of primers (10 pmol each, forward and reverse), 5 µl of 2x Sensimix^{TM} SYBR® Hi-ROX (Bioline, Berlin, B, Germany), in a final volume of 10 µl. The reaction was run on a MX3000P Real-Time PCR device (Agilent, Santa Clara, CA, USA). Melting curves (CP) and threshold values were calculated with built-in software for all the runs. The expression of each gene was normalized in relation to the expression of ACTB. Only PCR experiments with an efficiency within the range of 90% to 110% and a correlation coefficient of at least 0.9 were used for gene expression analysis. Similarly, only samples within the quantification range of the standard curve were considered for the analysis. The primers used and PCR conditions for each gene are presented in Table 1.

2.5.*Redox balance*

For each replicate, groups of COCs (10) and 8/16 cells embryos (10), and blastocyst (5), were homogenized in microtubes containing 100 µl Tris Base Buffer 20 mM, pH=7.6 with a Teflon-glass homogenizer in ice. The suspension was centrifuged at 4500 X g for 10 minutes at 4ºC. The supernatant was saved and stored at –80ºC until use. We have optimized the redox balance tests using a minimum amount of sample and reagents. This allowed us to carry out a greater number of replicates in each test and perform multiple evaluations from the same group of COCs/embryos. This was possible using a microplate reader and 96-well plates. The Biochrom EZ Read 400 Microplate Reader and Galapagos for EZ Read Software (Biochrom Ltd., Cambridge, UK) were used. In this way, we have made evaluations of: protein concentration, SOD activity, total glutathione concentration and lipid peroxidation [27].

Protein concentration

The concentration of proteins was determined in each group by the Bradford method adapted to microplate. A curve of known concentrations of albumin was used as a reference. Absorbance of 10 µl of sample and 100 µl of Bradford's reagent was read at 595 nm.

Lipid peroxidation

TBA reactive species (TBA-RS) method was used for the evaluation of the amount of malondialdehyde (MDA) formed from the breakdown of polyunsaturated fatty acids. Briefly, homogenate 30 µl was treated with trichloroacetic acid (TCA) 15% (w/v)- thiobarbituric acid (TBA) 0.375% (w/v)- HCI 0.25M in microtubes and heated for 15 minutes in water at 100ºC. After cooling, the absorbance of samples was registered at 492 nm. Lipid peroxidation was expressed as nmol MDA/unit (COCs/embryo).

Total Glutathione concentration

The measurement of acid-soluble thiol was used for estimating GSH content. Phosphate buffer (pH= 7) containing NADPH (N7505; Sigma-Aldich, St Louis, MO, USA) and GSH reductase (G3664; Sigma-Aldich, St Louis, MO, USA) incubated 10 µl of homogenates. The enzymatic reduction of GSSG to GSH was analyzed with Ellman's reagent (D8130; DTNB, Sigma and Co, St Louis, MO, USA) in a kinetic measurement for 5 minutes using microplate reader at 405 nm. Blanks consisting of homogenizing buffer were used instead of samples. Results were expressed as pmol GSH/ug protein.

Superoxide dismutase (SOD)

The RX MONZA - RANSOD - SD 125 kit was used, which employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals that react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction. One unit of SOD causes a 50% inhibition of the rate of reduction of INT at the conditions of the assay. The sample and reagent volumes were adapted to microplate, using one tenth of the volumes indicated in the kit. Absorbance was read at 492 nm, performing a kinetic measurement according to the manufacturer's instructions, reading initial absorbance after 30 seconds of mixing the reagents and final absorbance after 3 minutes. Results were expressed as USOD/mg protein.

2.6. *DNA damage and total cell number*

DNA fragmentation was evaluated with a commercial kit (DeadEnd™ Fluorometric TUNEL System, Promega, Madison, WI, USA). Blastocysts derived from 0, 0.5 and 0.75% DMSO treatments (N=7, 7 and 14 respectively) were fixed in 4% paraformaldehyde solution. Labelling was performed according to the manufacturer's instruction. The

embryos were mounted on slides using commercial mounting medium (Vectashield, Vector Laboratories, Burlingame, CA, USA) and examined with confocal fluorescence microscope. The images of each embryo were analyzed using Fiji Software [28].

2.7.*Statistical analysis*

At least three replicates were performed in each experiment. First, polar body extrusion, cleavage, blastocyst rates, and TUNEL+ cells were statistically analyzed using Chi square test. A quadratic regression model was fitted to blastocyst rates data and a dose-response curve was estimated by means of weighted least squares. Parametric results were tested for normality and results of SOX2, SOD1 mRNA abundance and MDA concentration were transformed to Log10. Differences in gene expression, total cell number and oxidative stress were calculated through ANOVA, means differences were tested by Tukey`s post hoc test.

3. Results

3.1.*First polar extrusion*

The addition of 0.5 % DMSO increased first polar body extrusion rates. Nevertheless, 0.1 and 1% DMSO resulted in a similar polar body extrusion rate with control group, while 10% DMSO resulted in lower rates ($p<0.05$) (Table 2).

3.2.*Embryo development*

The addition of 0.1, 0.5 and 1% DMSO during *in vitro* maturation resulted in similar cleavage rates than with control group. In turn, the addition of 10% DMSO significantly decreased cleavage rates ($p<0.05$). On the other hand, blastocyst rates increased when IVM medium was supplemented with 0.5% DMSO, compared to control ($p<0.05$). Moreover, the addition of IVM with 10% DMSO failed in the development of blastocysts (Table 3). The R^2 for the quadratic dose-response curve for blastocyst rates was 0.43 and the regression coefficients were statistically significant ($p<0.0001$). Both 0.25 and 0.75% showed similar results to 0.5% v:v DMSO.

According to the estimated curve, the maximum blastocyst rate is achieved when the concentration of DMSO in the maturation medium is around 0.45% v:v (Figure 2).

Gene expression

Group 0.5 % DMSO showed similar mRNA abundance with control group. However, 0.75% DMSO resulted in increased OCT4 and SOX2 abundance ($p<0.05$). The expression levels of CDX2 and SOD1 did not differ among treatments (Figure 3).

3.3.*Redox balance*

Lipid peroxidation: As shown in Figure 4, control matured COCs showed higher MDA concentration than immature COCs ($p<0.05$). On the other hand, DMSO matured group showed a decrease MDA compared to control ($p<0.05$). MDA concentration did not differ between DMSO matured and immature COCs. In 8/16 cell embryos, no differences among treatments were observed. Blastocysts from oocytes matured with DMSO showed less lipid peroxidation compared to control ($p<0.05$).

Total GSH contents: Matured COCs showed lower GSH content than immature COCs ($p<0.05$). COCs treated with DMSO did not differ from control group. In 8/16 cell embryos, no differences among treatments were observed. Blastocyst from COCs treated with DMSO showed higher values of total GSH than control group ($p<0.05$).

SOD activity: The presence of DMSO in the maturation medium did not affect the activity of SOD enzyme.

3.4.*DNA damage and total cell number*

To evaluate the toxicity of DMSO, we analyzed the total number of cells and DNA fragmentation in blastocysts derived from 0, 0.5 and 0.75% DMSO matured oocytes. The number of blastocyst cells from DMSO treatments was higher than control. TUNEL positive cells did not differ among treatments (Figure 5 and Table 4).

4. Discussion

The present study showed that the use of low concentrations of DMSO in the maturation medium of cattle oocytes increases the rate of the blastocysts produced without apparent cell toxicity.

A dose dependent effect of DMSO was observed in maturation and blastocyst rates. Results suggest that 0.5% DMSO in *in vitro* maturation medium increases 1PB extrusion rate,

while 10% DMSO has a detrimental effect, where embryo development failed. In 1% DMSO group, 1PB extrusion and cleavage rates did not differ compared to control. However, previous reports showed that over 1% DMSO has cellular toxic effect through alterations in the structure of plasma membrane, cytoplasm [29, 30], extrusion of 1PB [29], dysregulated gene expression and apoptosis [7]. In turn, blastocyst rate in 1% DMSO was similar to control group and it showed a quadratic response with an optimum at 0.45% v:v. This response is probably due a long-term effect of maturation on oocyte developmental since part of oocyte competence is acquired during this complex and dynamic process [32, 33, 34, 35, 36, 37, 38, 39]. On the other hand, when similar doses of DMSO were used for a longer period, during the *in vitro* culture, effect on blastocyst rates was not observed [40, 6]. Nevertheless, DMSO used during IVC in concentrations up to 0.25% showed alterations in key genes expression. Relative expression of pro apoptotic gene BAX and fat metabolism CPT2 gene decreased in DMSO treatments compared to control while glucose metabolism G6PD gene was increased [41].

With a 0.5% v:v DMSO dose, the gene expression of SOX2, OCT4, CDX2 and SOD1 did not show differences compared to control. However, 0.75% DMSO increased SOX2 and OCT4 gene expression. These genes start to express in the 8-16 cell stage embryos, and they work closely together to maintain pluripotency in embryonic stem cells (ESC) [42, 43, 44] through downstream regulation of pluripotency specific genes [45]. Investigations carried out in induced pluripotent stem cells (iPSC) and ESC have demonstrated that the over-expression of SOX2 induces them to ectoderm and mesoderm differentiation, whereas the over-expression of OCT4 induces them to mesoderm and endoderm differentiation [46, 47]. On the other hand, related research has shown that DMSO associated with Activin A induces ESC to definitive endoderm differentiation through downregulation of OCT4 [48]. Then, an effect of DMSO on the modulation of the genetic expression is clearly evidenced. This increase in the gene expression of SOX2 and OCT4 can be attributed to an effect of DMSO on the epigenome [49]. It has been shown that DMSO upregulates Dnmt3as, which causes de novo hypermethylation of both genic and nongenic regions [50].

Although oxidative stress generates global damage in the cell, the main targets of ROS are proteins, plasma membrane and DNA [11]. In this research, 0.5% v:v DMSO showed

antioxidant effects during COCs maturation as well as in blastocyst stage. Oxidative stress induced by *in vitro* maturation of COCs was reflected in the increase of lipid peroxidation in matured control compared to immature COCs. This increase was prevented by the use of DMSO, resulting in similar levels of lipid peroxidation in matured DMSO compared to immature COCs. Similarly, lipid peroxidation decreased in DMSO blastocysts compared to control. Usually, during *in vitro* cell culture, ROS are accumulated. This generates lipid peroxidation of the membranes, the most important consequence and indicator of oxidative stress, which amplifies the initial damage by a chain reaction [51, 52, 53, 54]. The supplementation with antioxidant compounds such as Se or alpha linoleic acid reduces membrane damage [55, 57]. However, DMSO used at higher concentrations in this work, and below 15% v:v, has shown disorders in the membrane, such as lateral expansion, undulations and concurrent drops and membrane thickness [29, 30].

In oocytes, GSH synthesis decreases during maturation [57]. This tripeptide possesses a great antioxidant capacity since it oxidizes itself in a reaction mediated by glutathione peroxidase (GPX), and it reduces the environment [58]. Our results agree with this, and its effect could be related to the increase observed in lipid peroxidation. In oocytes, GSH could be raised to high levels through the supplementation in the maturation medium with β-mercaptoethanol, Cysteamine and Cystine, which has been associated to a greater development of bovine embryos [59, 14, 60, 22, 20]. It is remarkable that the homogenates used in this work to measure oxidant/antioxidant status were obtained from entire COCs. Given this, we cannot compare our results to those reported in denuded oocytes. The antioxidant effect of 0.5% DMSO on blastocysts that we observed in lipid peroxidation could also be related to total GSH concentrations, which showed an increase with DMSO treatment. Hence, we can affirm that this effect seen in blastocysts is induced by the maturation with DMSO. On other hand, SOD protein activity did not change between the development stages or treatments. However, the SOD1 mRNA expression was also unaffected by DMSO. Alike reported by others authors, a relation between protein activity and mRNA abundance was observed [61].

In this research, DMSO matured oocytes and their resulting blastocysts exhibited lower membrane damage and higher cell number. Despite this, no differences in DNA

fragmentation between treatments were observed. This disagrees with authors who report apoptosis triggered with high concentrations as those used in this work [6]. Apoptosis induced by DMSO occurs through the permeabilization of the mitochondrial membrane by Bax and, consequently, AIF translocates to the nucleus [7]. Once there, AIF condenses the chromatin and generates DNA fragments of high molecular weight (50 bp) [62]. Then, its fragments ends can be amplified by terminal desxynocleotidyl transferase using nitrogenous bases labeled with fluorochromes and, subsequently, be identified by fluorescence microscopy [60]. The total cells number observed in DMSO treatments were similar to the results shown in control IVF embryos [61]. Nevertheless, the amount of TUNEL positive cells was equal or greater than reported [65, 66, 67, 40].

5. Conclusions

In conclusion, our results suggest that low DMSO concentrations used during bovine oocytes in vitro maturation increases the maturation, as well as the blastocyst rate and its quality, without demonstrating deleterious effect on embryo cells.

Acknowledgements

We would sincerely like to thank IFFSA for offering the biological samples, CIALE Alta for donating semen, Maimónides University for allowing us to use the confocal microscope and Clarisa Pereira, English-Spanish Sworn Translator who collaborated on manuscript corrections.

References

[1] Szmant HH. Physical properties of dimethyl sulfoxide and its function in biological systems. *Ann N Y Acad Sci* 1975; 243:20–3. https://doi.org/10.1111/j.1749-6632.1975.tb25340.x

[2] Wood DC, Wood J. Pharmacologic and biochemical considerations of dimethyl sulfoxide. Ann N Y Acad Sci 1975; 243: 7-19. https://doi.org/10.1111/j.1749-6632.1975.tb25339.x

[3] Jacob SW, Herschler R. Pharmacology of DMSO. Cryobiology 1986; 23:14-27. https://doi.org/10.1016/0011-2240(86)90014-3

346 [4] Santos NC, Figueira-Coelho J, Martins-Silva J, Saldanha C. Multidisciplinary
347 utilization of dimethyl sulfoxide: pharmacological, cellular, and molecular aspects.
348 Biochem Pharmacol 2003; 65:1035-41. https://doi.org/10.1016/S0006-2952(03)00002-
349 9
350 [5] Mermillod P, Tomanek M, Marchal R, Meijer L. High developmental competence
351 of cattle oocytes maintained at the germinal vesicle stage for 24 hours in culture by
352 specific inhibition of MPF kinase activity. Mol Reprod Dev 2000; 55:89-95.
353 https://doi.org/10.1002/(SICI)1098-2795(200001)55:1<89::AID-MRD12>3.0.CO;2-M
354 [6] Kang MH, Das J, Gurunathan S, Park HW, Song H, Park C, Kim JH. The cytotoxic
355 effects of dimethyl sulfoxide in mouse preimplantation embryos: a mechanistic study.
356 Theranostics 2017; 7: 4735-52. https:// doi: 10.7150/thno.21662.
357 [7] Galvao J, Davis B, Tille M, Normando ER, Duchen MR, Cordeiro MF. Unexpected
358 low-dose toxicity of the universal solvent DMSO. The FASEB Journal 2014; 28:1317-
359 30 https:// doi: 10.1096/fj.13-235440.
360 [8] Panganamala RV, Sharma HM, Heikkila RE. Role of hydroxyl radical scavengers,
361 dimethyl sulfoxide alcohols and methional in the inhibition of prostaglandin synthesis.
362 Prostaglandins 1976; 11:599-607. https:// DOI: 10.1016/0090-6980(76)90063-0
363 [9] Shlafer M, Kane PF, Kirsh MM. Effects of Dimethyl Sulfoxide on the Globally
364 lschemic Heart: Possible General Relevance to Hypothermic Organ Preservation.
365 Cryobiology 1982; 19:61-69. https:// DOI: 10.1016/0011-2240(82)90125-0
366 [10] Cederbaum AI, Dicker E, Rubin E, Cohen G. The effect of dimethylsulfoxide and
367 other hydroxyl radical scavengers on the oxidation of ethanol by rat liver microsomes.
368 Biochem Biophys Res Commun. 1977; 78:1254-62. https:// DOI: 10.1016/0006-
369 291x(77)91428-0
370 [11] Guérin P, Mouatassim DE, And Ménézo Y. Oxidative stress and protection against
371 reactive oxygen species in the pre-implantation embryo and its surroundings. Human
372 Reproduction Update 2001; 7:175-89. https://doi.org/10.1093/humupd/7.2.175
373 [12] Al-Gubory KH, Fowler PA, Garrel C. The roles of cellular reactive oxygen
374 species, oxidative stress and antioxidants in pregnancy outcomes. Int J Biochem Cell
375 Biol. 2010; 42:1634-50. https://doi: 10.1016/j.biocel.2010.06.001.

[13] Agarwal A, Durairajanayagam D, du Plessis SS. Utility of antioxidants during assisted reproductive techniques: an evidence based review. Reprod Biol Endocrinol. 2014; 24:12-112. https://doi: 10.1186/1477-7827-12-112.

[14] Luvoni G, Keskintepe L, Brackett BG. Improvement in bovine production in vitro by glutathiones-containing culture media. Mol Rep Dev 1996; 43:437-43. https://doi.org/10.1002/(SICI)1098-2795(199604)43:4<437::AID-MRD5>3.0.CO;2-Q

[15] Balasubramaniana S, Son WJ, Mohana Kumar B, Ock SA, Yoo JG, Choe SY, Rho GJ. Expression pattern axygen and stress-responsive gene transcripts at various developmental stages of in vitro and in vivo preimplantation bovine embryos. Theriogenology 2007; 68:265-75 https://doi.org/10.1016/j.theriogenology.2007.05.044

[16] Thompson JG, Mitchell M, Kind KL. Embryo culture and long-term consequences. Reprod Fertil Dev 2007; 19:43-52. https:// doi.org/10.1071/RD06129

[17] Lott WV, Anchamparuthy VM, McGuilliard ML, Mullarky IK, Gwazdauskas FC, Influence of cysteine in conjunction with growth factors on the development of in vitro-produced bovine embryos. Reprod. Dom Anim 2011; 46:585-94. https:// doi: 10.1111/j.1439-0531.2010.01706.x.

[18] Sakatani M. Effects of heat stress on bovine preimplantation embryos produced in vitro. J Reprod Dev 2017; 63:347-352. https:// doi: 10.1262/jrd.2017-045.

[19] Cagnone G, Sirard MA. The embryonic stress response to in vitro culture: insight from genomic analysis. Reproduction 2016; 152:247-261. https://doi.org/10.1530/REP-16-0391

[20] Sovernigo TC, Adona PR, Monzani PS, Guemra S, Barros F, Lopes FG, Leal C. Effects of supplementation of medium with different antioxidants during in vitro maturation of bovine oocytes on subsequent embryo production. Reprod Domest Anim 2017; 52:561-69 https:// doi: 10.1111/rda.12946.

[21] Pang Y, Zhao S, Sun Y, Jiang X, Hao H, Du W, Zhu H. Protective effects of melatonin on the in vitro developmental competence of bovine oocytes. Anim Sci J 2018; 89: 648–660. https:// doi: 10.1111/asj.12970.

[22] de Matos DG. Fumus CC. The importance of having high glutathione (gsh) level after bovine In vitro maturation on embryo development: effect of P-mercaptoethanol,

406 cysteine and cysteine. Theriogenology 2000; 53:761-71 https://doi.org/10.1016/S0093-
407 691X(99)00278-2
408 [23] Watson AJ. Oocyte cytoplasmic maturation: a key mediator of oocyte and embryo
409 developmental competence. J Anim Sci. 2007; 85:1-3. https://
410 ttps://doi.org/10.2527/jas.2006-432
411 [24] Brackett BG, Oliphant G. Capacitation of rabbit spermatozoa in vitro. Biol Reprod.
412 1975; 12:260-74. https://DOI: 10.1095/biolreprod12.2.260
413 [25] Tervit HR, Whittingham DG, Rowson LE. Successful culture in vitro of sheep and
414 cattle ova. J Reprod Fertil. 1972; 30:493-497. https:// DOI: 10.1530/jrf.0.0300493
415 [26] Holm P, Booth PJ, Schmidt MH, Greve T, Callesen H. High bovine blastocyst
416 development in a static in vitro production system using SOFaa medium supplemented
417 with sodium citrate and myo-inositol with or without serum-proteins. Theriogenology
418 1999; 52:683-700. https:// DOI: 10.1016/S0093-691X(99)00162-4
419 [27] Luchetti CG, Cruzans PR, Lombardo DM. Antioxidant effects of metformin plus
420 ITS during in vitro maturation of porcine COC. Medicina 2017; 77.
421 [28] Schindelin J, Arganda-Carreras I, Frise E. "Fiji: an open-source platform for
422 biological-image analysis". Nature methods 2012; 9:676-82, PMID 22743772 https://
423 doi: 10.1038/nmeth.2019.
424 [29] Gurtovenko AA, Anwar J. Modulating the Structure and Properties of Cell
425 Membranes: The Molecular Mechanism of Action of Dimethyl Sulfoxide. J. Phys.
426 Chem 2007; 111(35):10453-60 https://doi.org/10.1021/jp073113e
427 [30] Ménorval MA, Mir LM, Fernández ML, Reigada R. Effects of Dimethyl Sulfoxide
428 in Cholesterol-Containing Lipid Membranes: A Comparative Study of Experiments In
429 Silico and with Cells. PLoS One 2012; 7(7):e41733. https:// doi:
430 10.1371/journal.pone.0041733.
431 [31] Zhou D, Shen X, Gu Y, Zhang N, Li T, Wu X, Lei L. Effects of dimethyl
432 sulfoxide on asymmetric division and cytokinesis in mouse oocytes. *BMC Dev Biol*
433 2014; 21: 14- https:// doi: 10.1186/1471-213X-14-28.
434 [32] Dekel N. Cellular, biochemical and molecular mechanisms regulating oocyte
435 maturation. Molecular and Cellular Endocrinology 2004; 234(1):19–25 https://
436 doi.org/10.1016/j.mce.2004.09.010

[33] Mao L, Lou H, Lou Y, Wang N, Jin F. Behaviour of cytoplasmic organelles and cytoskeleton during oocyte maturation. Reproductive BioMedicine Online 2014; 28: 284-99. https:// doi: 10.1016/j.rbmo.2013.10.016.

[34] You J, Lee E, Bonilla L, Francis J, Koh J, Block J, Chen S, Hansen PJ. Treatment with the proteasome inhibitor MG132 during the end of oocyte maturation improves oocyte competence for development after fertilization in cattle. *PLoS One* 2012; 7(11):e48613. https:// doi: 10.1371/journal.pone.0048613.

[35] Huang W, Nagano M, Kang SS, Yanagawa Y, Takahashi Y. Effects of in vitro growth culture duration and prematuration culture on maturational and developmental competences of bovine oocytes derived from early antral follicles. Theriogenology. 2013; 80:793-9. https:// doi: 10.1016/j.theriogenology.2013.07.004.

[36] Gilchrist RB, Thompson JG. Oocyte maturation: Emerging concepts and technologies to improve developmental potential in vitro. Theriogenology 2007; 67:6–15 https:// https://doi.org/10.1016/j.theriogenology.2006.09.027

[37] Ottosen LD, Hindkjaer J, Ingerslev J. Light exposure of the ovum and preimplantation embryo during ART procedures. J Assist Reprod Genet 2007; 24: 99-103. https:// doi: 10.1007/s10815-006-9081-x

[38] Wu Y, Zhang N, Li YH, Zhao L, Yang M, Jin Y, Xu YN, Guo H. Citrinin exposure affects oocyte maturation and embryo development by inducing oxidative stress-mediated apoptosis. Oncotarget 2017; 8:34525-33 https:// doi: 10.18632/oncotarget.15776.

[39] Nikseresht M, Toori MA, Rahimi HR, Fallahzadeh AR, Kahshani IR, Hashemi SF, Bahrami S, Mahmoudi R. Effect of Antioxidants (β-mercaptoethanol and Cysteamine) on Assisted Reproductive Technology In vitro. J Clin Diagn Res 2017; 11, BC10-BC14. https:// doi: 10.7860/JCDR/2017/21778.9298.

[40] Stöhr J, Grothmann H, Wrenzycki C. Effect of dimethyl sulfoxide supplementation on bovine In vitro embryo development. Reprod Fert Dev 2016; 157-158. https:// doi.org/10.1071/RDv27n1abs

[41] Stöhr J, Grothmann H, Wrenzycki C. Effect of dimethyl sulfoxide supplementation on bovine In vitro embryo development. Abstract book 18TH International Congress on Animal Reproduction. Tours; 2016

[42] Graf A, Krebs S, Heininen-Brown M, Zakhartchenko V, Blum H, Wolf E. Genome activation in bovine embryos: review of the literature and new insights from RNA sequencing experiments. Anim Reprod Sci 2014; 149:46-58. https:// doi: 10.1016/j.anireprosci.2014.05.016.

[43] Rizzino A, Wuebben EL. Sox2/Oct4: A delicately balanced partnership in pluripotent stem cells and embryogenesis. Biochim Biophys Acta 2016; 1859:780-91. https:// doi: 10.1016/j.bbagrm.2016.03.006.

[44] Goissis MD, Cibelli JB. Functional characterization of SOX2 in bovine preimplantation embryos. Biol Reprod 2014; 90:30 (2). https:// doi: 10.1095/biolreprod.113.111526.

[45] Masui S, Nakatake Y, Toyooka Y, Shimosato D, Yagi R, Takahashi K, Okochi H, Okuda A, Matoba R, Sharov AA, Ko MS, Niwa H. Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells. Nat Cell Biol. 2007; 9: 625-35. https://doi.org/10.1038/ncb1589

[46] Niwa H, Miyazaki J, Smith AG. Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nat Genet 2000; 24:372-6. https://doi.org/10.1038/74199

[47] Yamaguchi S, Hirano K, Nagata S, Tada T. Sox2 expression effects on direct reprogramming efficiency as determined by alternative somatic cell fate. Stem Cell Res 2011; 6:177-86. https:// doi: 10.1016/j.scr.2010.09.004

[48] Czysz K, Minger S, Thomas N. DMSO efficiently down regulates pluripotency genes in human embryonic stem cells during definitive endoderm derivation and increases the proficiency of hepatic differentiation. PLoS One 2015. 210(2), e0117689. https:// doi: 10.1371/journal.pone.0117689.

[49] Thaler R, Spitzer S, Karlic H, Klaushofer K, Varga F. DMSO is a strong inducer of DNA hydroxymethylation in pre-osteoblastic MC3T3-E1 cells. Epigenetics 2012; 7: 635-51. https:// doi: 10.4161/epi.20163

[50] Iwatani M, Ikegami K, Kremenska Y, Hattori N, Tanaka S, Yagi S, Shiota K. Dimethyl sulfoxide has an impact on epigenetic profile in mouse embryoid body. Stem Cells 2006; 24: 2549-56. https://doi.org/10.1634/stemcells.2005-0427

[51] Fridovich I. Oxygen toxicity: a radical explanation. J Exp Biol 1998; 201:1203-9 https://jeb.biologists.org/content/jexbio/201/8/1203.full.pdf

[52] Imlay JA. Pathways of oxidative damage. Annu Rev Microbiol 2003; 57: 395-418. https://doi.org/10.1146/annurev.micro.57.030502.090938

[53] Dalvit GC, Cetica PD, Pintos LN, Beconi MT. Reactive oxygen species in bovine embryo in vitro production. Biocell 2005; 29:209-12. https://doi.php?id=685

[54] Fridovich I. Oxygen: how do we stand it?. Med Princ Pract. 2013; 22:131-7 https:// doi: 10.1159/000339212.

[55] Ghafarizadeh AA, Vaezi G, Shariatzadeh MA, Malekirad AA. Effect of in vitro selenium supplementation on sperm quality in asthenoteratozoospermic men. Andrologia 2018; 50(2). https:// doi: 10.1111/and.12869.

[56] Chirino-Galindo G, Barrera-Argüelles JI, Trejo-González NL, Mejía-Zepeda R, Palomar-Morales M. Biphasic effect of alpha-linolenic acid on glucose-induced dysmorphogenesis and lipoperoxidation in whole rat embryo in culture. Biochem Biophys Res Commun 2017;484:878-83. https:// doi: 10.1016/j.bbrc.2017.02.011.

[57] Yoshida M, Ishigaki K, Nagai T, Chikyu M, Pursel VG. Glutathione concentration during maturation and after fertilization in pig oocytes: relevance to the ability of oocytes to form male pronucleus. Biol Reprod 1993; 49:89-94. https://doi.org/10.1095/biolreprod49.1.89

[58] Meister A, Tate SS. Glutathione and related gamma-glutamyl compounds: biosynthesis and utilization. Annu Rev Biochem 1976; 45:559-604. https://doi.org/10.1146/annurev.bi.45.070176.003015

[59] de Matos DG, Furnus CC, Moses DF, Martinez AG. Matkovic M. Stimulation of glutathione synthesis of in vitro matured bovine oocytes and its effect on embryo development and freezability. Mol Reprod Dev 1996; 45:451-57 https:// https://doi.org/10.1002/(SICI)1098-2795(199612)45:4<451::AID-MRD7>3.0.CO;2-Q

[60] Takahashi M, Nagai T, Hamano S, Kuwayama M, Okamura N, Okano A. Effect of thiol compounds on in vitro development and intracellular glutathione content of bovine embryos. Biol Reprod. 1993; 49:228-32. https://doi.org/10.1095/biolreprod49.2.228

[61] Forsberg H, Borg LA, Cagliero E, Eriksson UJ. Altered levels of scavenging enzymes in embryos subjected to a diabetic environment. Free Radic Res 1996; 24:451-59. https://doi.org/10.3109/10715769609088044

[62] Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM, Mangion J, Jacotot E, Costantini P, Loeffler M, Larochette N, Goodlett DR, Aebersold R, Siderovski DP, Penninger JM, Kroemer G. Molecular characterization of mitochondrial apoptosis-inducing factor. *Nature* 1999; 397: 441–46. https:// doi.org/10.1038/17135

[63] Gavrieli Y, Sherman Y, Ben-Sasson SA. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 1992; 119:493-501. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2289665/

[64] Bevacqua RJ, Pereyra-Bonnet F, Fernandez-Martin R, Salamone DF. High rates of bovine blastocyst development after ICSI-mediated gene transfer assisted by chemical activation. Theriogenology 2010; 74:922-31. https:// doi: 10.1016/j.theriogenology.2010.04.017.

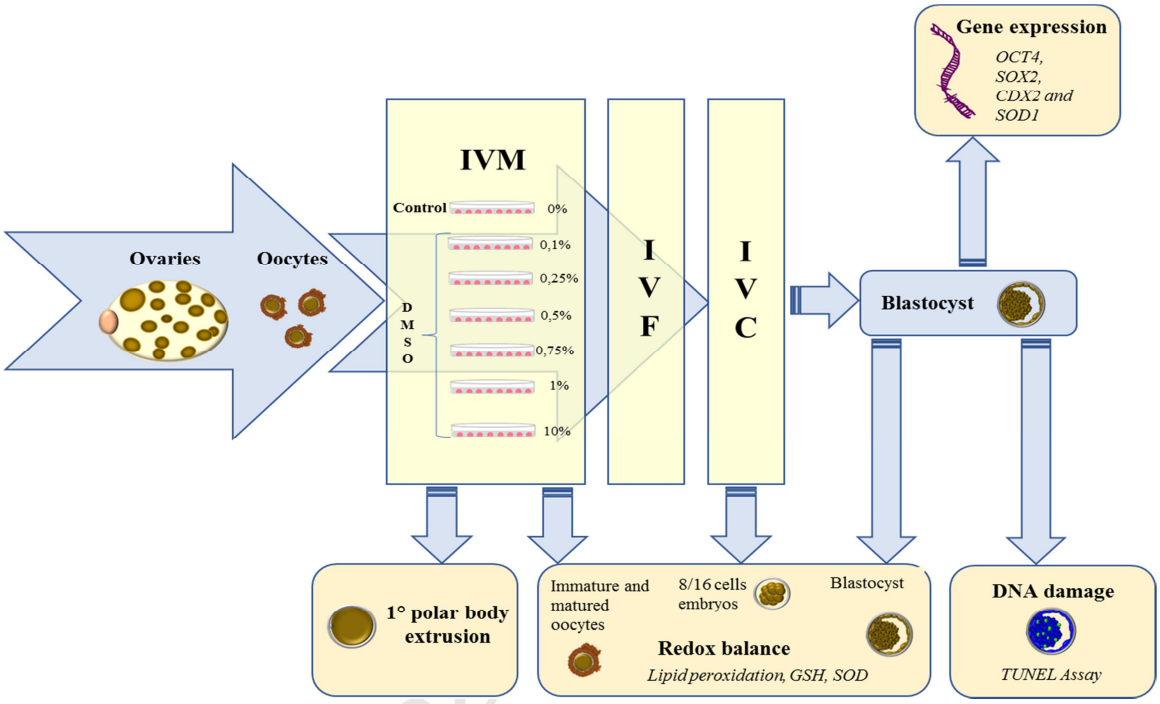
[65] Canel NG, Bevacqua RJ, Hiriart MI, Rabelo NC, de Almeida Camargo LS, Romanato M, de Calvo LP, and Salamone DF. Sperm pretreatment with heparin and l-glutathione, sex-sorting, and double cryopreservation to improve intracytoplasmic sperm injection in bovine. Theriogenology 2017; 93:62-70. https:// doi: 10.1016/j.theriogenology.2016.12.018.

[66] Chowdhury MMR, Choi BH, Khan I, Lee KL, Mesalam A, Song SH, Xu L, Joo MD, Afrin F, Kong IK. Supplementation of lycopene in maturation media improves bovine embryo quality in vitro. Theriogenology 2017; 103:173-184. https:// doi: 10.1016/j.theriogenology.2017.08.003.

[67] Mesalam A, Khan I, Lee KL, Song SH, Chowdhury MMR, Uddin Z, Park KH, Kong IK. 2-Methoxystypandrone improves in vitro-produced bovine embryo quality through inhibition of IKBKB. Theriogenology 2017; 99:10-20. https:// doi: 10.1016/j.theriogenology.2017.05.012.

Legends

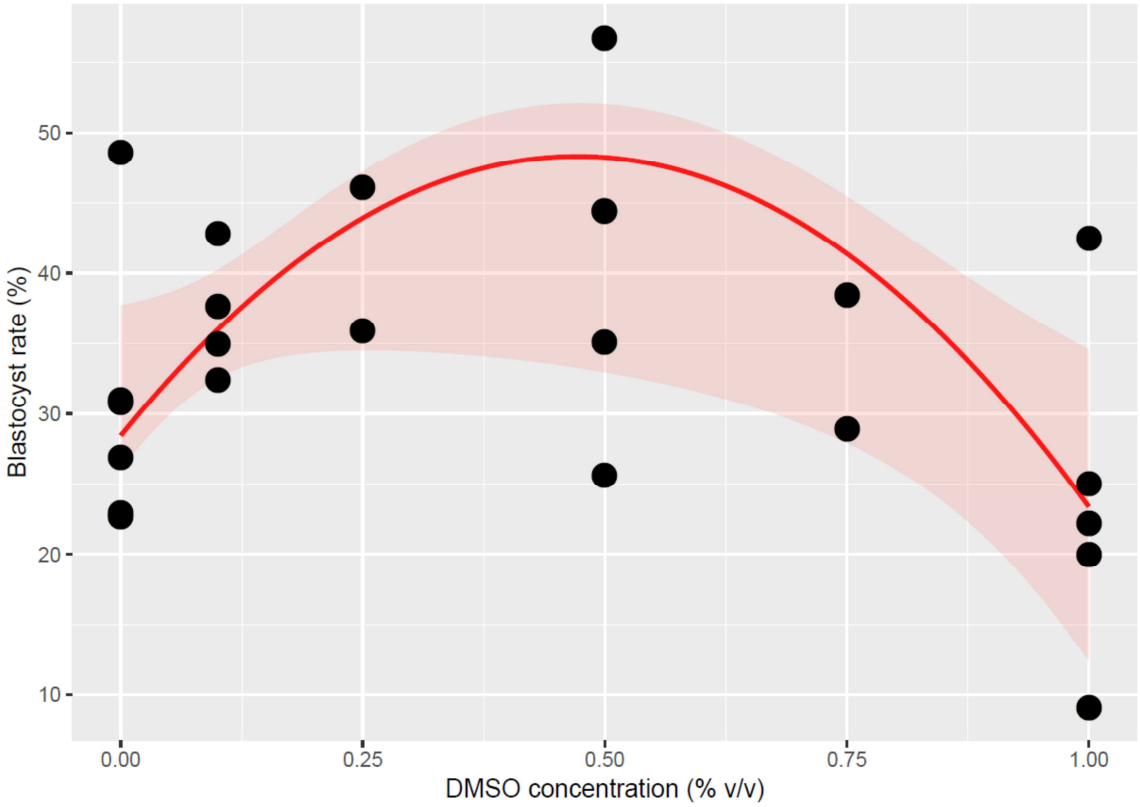
556 Figure 1. Schematic description of the methodology used to study the effect of the use of
557 DMSO during maturation on embryo development and quality. IVM, In vitro maturation;
558 IVF, In vitro fertilization; IVC, In vitro culture.



559

560

561 Figure 2. Adjustment curve and confidence interval for the average response on values of
562 blastocyst rates obtained from different % v:v DMSO ($p<0.0001$).



563

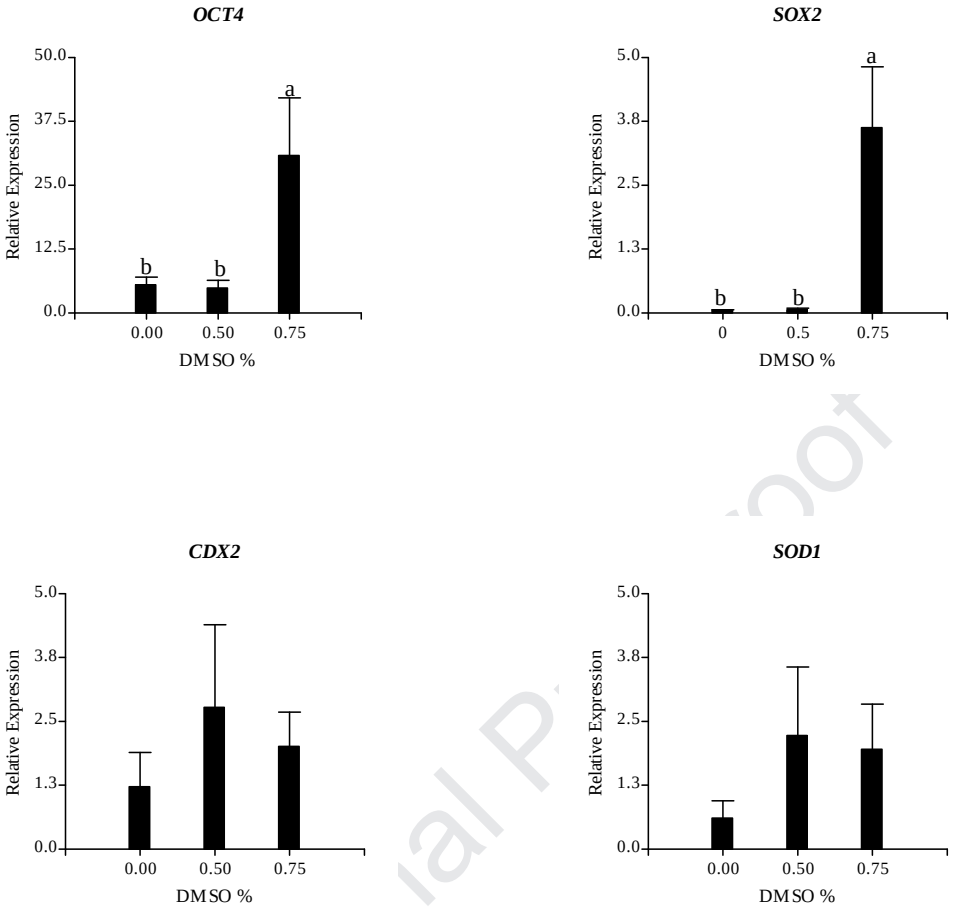
564

565 Figure 3. Expression level of OCT4, SOX2, CDX2 and SOD1 genes in bovine blastocyst
566 produced from oocytes supplemented with 0, 0.5 and 0.75% v:v DMSO during IVM. All
567 genes were normalized with the ACTB gene. [a, b] Significant differences was observed for
568 OCT4 and SOX2 expression in blastocyst from 0.75% v:v DMSO group (ANOVA, Tukey
569 test $p<0.05$)

570

571

572

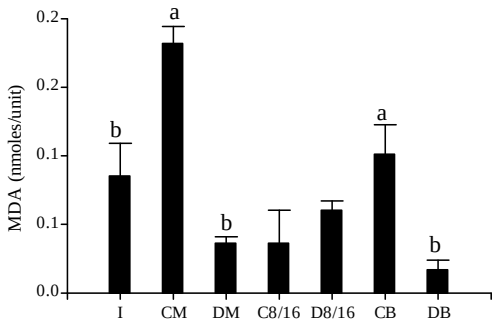


573

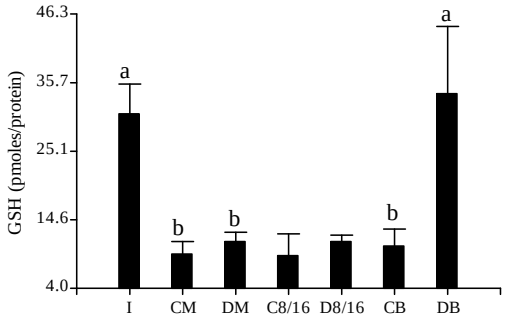
574 Figure 4. SOD activity, total GSH and lipid peroxidation in immature and mature oocytes,
575 8/16 embryos and blastocyst from oocytes supplemented with 0 and 0.5% v:v DMSO
576 during IVM. I: immature oocytes; CM: mature control oocytes; DM: DMSO mature
577 oocytes; C8/16: control 8/16 cells embryos; D8/16: DMSO 8/16 cells embryos; CB; control
578 blastocyst and DB: DMSO blastocyst. [a, b] Significant differences were observed in Lipid
579 peroxidation and total GSH (ANOVA, Tukey test $p<0.05$).

580

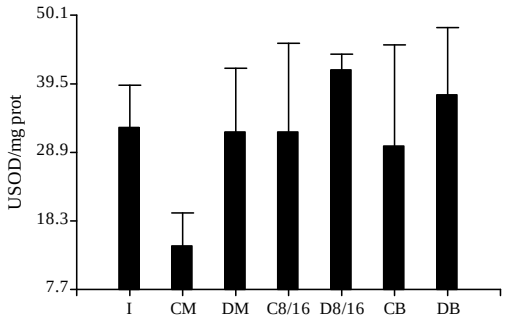
Lipid Peroxidation



Total GSH



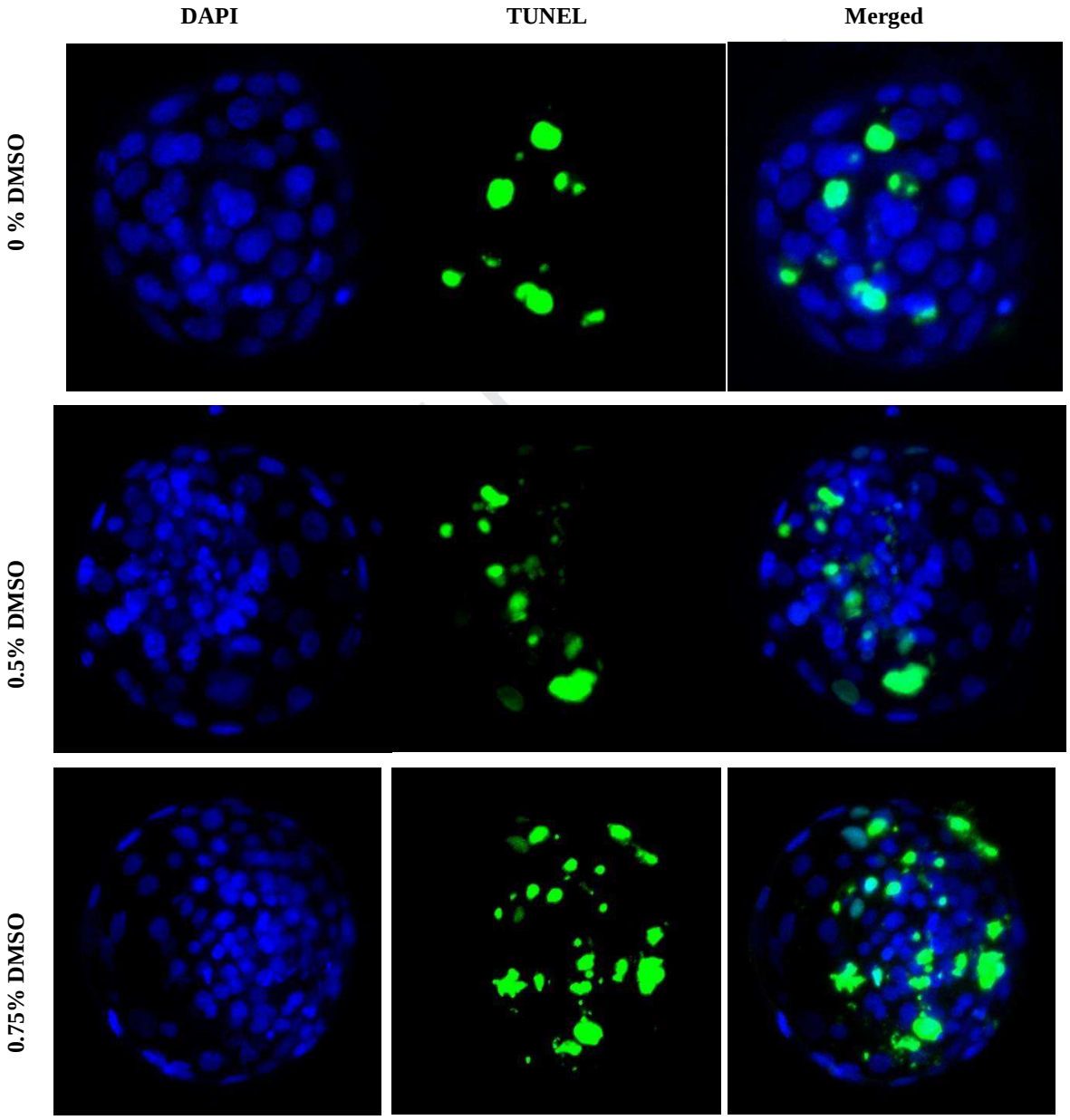
SOD activity



581

582 Figure 5. TUNEL assay in blastocyst from oocytes matured with 0, 0.5 and 0.75% v:v
583 DMSO. TUNEL positive cells were labeled with fluorescein-12-dUTP (green), and nuclei
584 are counterstained with DAPI (blue).

585



586

Table 1

List of primers used for RT-qPCR

Gene	**Primer sequences** (5'-3')	**Annealing temperature** (°C)	**Product lenght (pb)**	**Accession number** (NCBI)
OCT4	F: 5´-GGAGAGCATGTTCCTGCAGTGC 3´ R: 5´-ACACTCGGACCACGTCCTTCTC 3´	58	95	NM_174580
SOX2	F: 5´-CGAGTGGAAACTTTTGTCCG 3´ R: 5´-GGTATTTATAATCCGGGTGTT 3´	55	101	NM_001105463
CDX2	F: 5´-CCTGTGCGAGTGGATGCGGAAG 3´ R: 5´-CCTTTGCTCTGCGGTTCT 3´	58	230	XM_871005
SOD1	F: 5´-CCATCCACTTCGAGGCAAAG 3´ R: 5´-TCTCCAAACTGATGGACGTGG 3´	58	100	NM_174615.2
ACTB	F: 5´-GGCCAACCGTGAGAAGATGACC 3´ R: 5´-GAGGCATACAGGGACAGCACAG 3´	58	96	BT030480.1

Table 2

First polar body extrusion in oocytes matured with different DMSO concentrations

% DMSO	N	Polar Body extrusion (%)
0	241	178 (73.9)[b]
0.1	195	143 (73.3)[b]
0.5	142	135 (95.1)[a]
1	192	127 (66.1)[b]
10	172	13 (7.6)[c]

Within a column, values with a different superscript (a or b) differed (Chi square test, $p < 0.05$).

Table 3

Blastocyst rates obtained from oocytes matured with different DMSO concentrations

DMSO %	N	Cleavege (%)	Blastocyst (%)
0	446	334 (74.9)[a]	117 (26.2)[b]
0.1	322	248 (77.2)[a]	117 (36.6)[ab]
0.5	194	153 (78.9)[a]	80 (41.2)[a]
1	250	193 (77.0)[a]	58 (23.2)[b]
10	39	1 (2.6)[b]	0 (0)[c]

Within a column, values with a different superscript (a or b) differed (Chi square test, $p< 0.05$).

593

594

Table 4

TUNEL positive cells in blastocysts obtained from oocytes matured with 0, 0.5 or 0.75 % v:v DMSO

DMSO %	N	Nº Cells±SD	TUNEL±SD (%)
0	8	71±35[b]	10±6 (14.1)
0.5	7	110±25[a]	19±5 (18.2)
0.75	14	98±23[a]	18±6 (19.7)

Within a column, values with a different superscript (a or b) differed (ANOVA and Chi square test, $p< 0.05$).

595

596 Conflict of interest: The authors declare no conflicts of interest.

Highlights:

- Bovine oocytes were matured with different DMSO concentrations (0, 0.1, 0.5, 1 and 10% v:v) followed by *in vitro* fertilization. The optimal concentration of DMSO to use in the maturation was estimated in around 0.45% v:v.
- DMSO at 0.5% v:v concentration has not effect on mRNA abundance of genes explored. However 0.75% v:v DMSO increased gene expression of OCT4 and SOX2.
- Oocytes matured with 0.5% v:v DMSO showed reduced lipid peroxidation and increased glutathione.
- Low DMSO concentrations used during *in vitro* maturation of bovine oocytes increases the maturation, as well as the blastocyst rate and its quality without any deleterious effect on embryo cells.