Effect of oxygen tension and antioxidants on the developmental competence of buffalo oocytes cultured in vitro

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Received: 05-10-2020, Accepted: 24-11-2020, Published online: 11-01-2021

doi: www.doi.org/10.14202/vetworld.2021.78-84 How to cite this article: El-Sanea AM, Abdooon ASS, Kandil OM, El-Toukhy NE, Abo El-Maaty AM, Ahmed HH (2021) Effect of oxygen tension and antioxidants on the developmental competence of buffalo oocytes cultured in vitro, Veterinary World, 14(1): 78-84.

Abstract

Aim: Oxidative stress (OS) is one of the major disruptors of oocyte developmental competence, which appears due to the imbalance between the production and neutralization of reactive oxygen species (ROS).

Materials and Methods: In Experiment 1, buffalo oocytes were in vitro matured, fertilized, and cultured at 38.5°C under 5% CO2 + 20% O2 in standard CO2 incubator (OS) or under 5% O2 + 5% CO2 + 90% N2 (Multi-gas incubator, low O2). In Experiment 2, buffalo cumulus oocytes complexes (COCs) were matured in Basic maturation medium (BMM) composed of TCM199+ 10% FCS+ 10 µg/ml gentamicin (control group) or in BMM supplemented with 50 µM ascorbic acid (ascorbic acid group) or 3.0 mM glutathione (glutathione group) or 10-5 M melatonin (melatonin group) and cultured at 38.5°C under 20% O2 for 24 h. Matured buffalo oocytes in control, ascorbic acid, or melatonin groups were fertilized and zygotes were cultured for 8 days under the same conditions.

Results: In both experiments, maturation, cleavage, and blastocyst rates were recorded. Results showed that culture of buffalo oocytes under low O2 (5% O2) significantly increased maturation, cleavage, and blastocyst rates (p<0.05). Meanwhile, under 20% O2, addition of 10-4 M melatonin or 50 µM ascorbic acid to in vitro maturation (IVM) medium significantly improved cumulus cell expansion, nuclear maturation rates of buffalo oocytes (p<0.05), and increased cleavage and blastocyst rates (p<0.05).

Conclusion: About 5% O2 is the optimum condition for in vitro production of buffalo embryos, and addition of 10-5 M melatonin to IVM medium for oocytes cultured under 20% O2 could alleviate the adverse effect of high oxygen tension and increased embryo yield.

Keywords: antioxidants, buffalo oocytes, developmental competence, in vitro embryo production, O2 tension.

Introduction

The application of assisted reproductive technologies such as in vitro embryo production (IVEP) and embryo transfer is slower in buffalo than in cattle. Oocytes matured in vitro showed lower quality and reprogramming competence compared to oocytes matured in vivo [1]. Oxidative stress (OS) during IVEP is one of the major disruptors of oocyte developmental competence; it appears due to the imbalance between the production and neutralization of reactive oxygen species (ROS) [2]. OS induces granulosa cell apoptosis and reduces the transfer of nutrients and growth factors to oocytes leading to apoptosis [3]. Moreover, buffalo oocytes possess higher lipid content as compared with many other species and they are very sensitive to OS, which leads to deterioration in oocyte quality and affect their developmental potentials [4].

OS of 20% O2 during IVEP was associated with higher H2O2 formation within the bovine embryos [5] and a reduction in the number of inner cell mass within the bovine blastocyst compared with 5% O2 tension [6]. Furthermore, 5% O2 tension during in vitro culture of embryos produced a higher blastocyst rate in buffalo [7] and cattle [8-10]. There is a lack of information on the effect of OS on maturation, fertilization, and blastocyst rates of in vitro produced buffalo embryos. Furthermore, a variety of antioxidants has been used to decrease ROS during the IVEP [11,12]; however, which antioxidant is the most suitable to support the development of buffalo embryos is still undefined. Melatonin ameliorates oocyte OS and improves subsequent in vitro development and decreased apoptosis levels, recovered the integrity of mitochondria, amend the spindle assembly and chromosome alignment in oocytes, and enhances embryo development in bovine [1,13], pigs [14,15], mice [16], and human [17]. In addition, antioxidant
glutathione (GSH) plays an important role in the antioxidant system of cells [18]. The concentrations of GSH in matured oocytes were significantly higher than that in the immature oocytes and play an important role in successful fertilization [19]. Furthermore, in vitro maturation (IVM) of bovine oocytes in the presence of Vitamin C significantly increases the blastocyst rate [20]. Better understanding of the direct effect of manipulation of oxygen tension and addition of antioxidants to maturation medium could improve the developmental competence of buffalo oocytes.

Therefore, the present study was undertaken to investigate: (1) The effect of in vitro culture of buffalo oocytes and embryos under OS condition of 20% O₂ or that of 5% O₂ tension on the developmental competence of buffalo oocytes and (2) the effect of addition of antioxidants (50 µM ascorbic acid, 3.0 mM GSH, or 10⁻⁵ M melatonin) to IVM medium of buffalo oocytes cultured under 20% oxygen tension on cumulus cell expansion, nuclear maturation rates, and their ability to develop to blastocyst stage.

Materials and Methods

Ethical approval
All animal studies in the present work were conducted in accordance with the requirement of the Institutional Animal Care Committee and were reviewed and approved by the Animal Ethics Committee of the National Research Centre of Egypt (NRC, ID: 12/1/7).

Experimental procedures
All chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA), unless otherwise mentioned.

Experiment 1: Effect of OS (20% O₂) on IVM and development rates of buffalo oocytes

Buffalo ovaries were collected and transported to the laboratory in thermos containing warm (32-35°C) saline solution. Cumulus oocytes complexes (COCs) were retrieved and classified into four categories based on number of cumulus cell layers and homogenous of the cytoplasm [21], and Grade 1 and 2 COCs were used for IVM. After selection, COCs were washed at least 3 times in phosphate-buffered saline supplemented with 4 mg/mL bovine serum albumin (BSA) + 50 µg/mL gentamicin, then washed at least 2 times in IVM basic medium (BMM), which consists of TCM-199 medium supplemented with 10% fetal calf serum + 10 µg/mL follicle-stimulating hormone + 50 IU equine chorionic gonadotropin (Merck, Germany) + 50 µg/mL gentamicin. Twenty-five to 50 COCs were cultured in 500 µL of BMM in 4-well culture plate (Nunc, Denmark). COCs were IVM at 38.5°C for 24 h in a standard CO₂ incubator (Contherm, New Zealand) under 5% CO₂, and 20% O₂ (OS group) or in Multi-gas incubator (Binder, Germany) under 5% CO₂, 5% O₂, and 90% N₂ (control group) in humidified air. This experiment was conducted at least 3 times.

For the assessment of nuclear maturation, cumulus cells were removed by gentle pipetting and oocytes were examined under an inverted microscope (Axio Vert, Zeiss, Germany). Oocytes showing the 1st polar body were considered as matured at metaphase II (M II) (Figure-1a) and oocytes without polar body were considered as non-matured oocytes (Figure-1b).

After IVM, matured oocytes were subjected to in vitro fertilization (IVF). Frozen buffalo semen was purchased from Animal Reproduction Research Institute; the same batch of the same bull with proven fertility was used throughout the study. One 0.25 mL straw of frozen semen was thawed in a water bath at 37°C for 30 s. Spermatozoa were washed twice by centrifugation at 180 g for 5 min in Sperm Tyrode’s albumin lactate pyruvate (Sp-TALP) medium supplemented with 4 mg/mL BSA + 50 µg/mL gentamicin. After washing, the sperm pellet was suspended in 200 µL Fert-TALP medium and the sperm number was counted using a hemocytometer. The final sperm concentration was adjusted to 2×10⁶/mL using Fert-TAPL medium supplemented 10 µg/mL heparin, 5 mg/mL BSA (fatty acid free) + 2.5 mM caffeine sodium benzoate + 50 µg/mL gentamicin. The sperm suspension (300 µL) was placed into a 4-well culture plate and covered with warm 200 µL mineral oil. Maturated buffalo oocytes were washed 3 times in Fert-TALP medium, and then, 15-20 oocytes were transferred into the sperm suspension droplet and cultured either under 5% CO₂ and 20% O₂ in standard CO₂ incubator (OS group) or 5% CO₂, 5% O₂, and 90% N₂ (Multi-gas incubator, control group) at 38.5°C for 18-20 h in humidified air.

The fertilization rate was checked by counting the number of oocytes extruding the second polar body (Figure-2). Then, the presumptive zygotes were washed twice and cultured in modified synthetic oviduct fluid (mSOF) medium supplemented with 5 mg/mL BSA + 50 µg/mL gentamicin for 8 days. Zygotes were culture under the same incubation conditions during IVM and IVF (5% or 20% O₂ tension). Cleavage and embryo development rates were checked on days 2, 5, 7, and 8 using an inverted microscope.

Experiment 2: Effect of addition of 50 µM ascorbic acid, 3.0 mM GSH, or 10⁻⁵ M melatonin in IVM medium on maturation rate and development of buffalo oocytes

In six replicates, buffalo COCs were collected, evaluated and IVM in 500 µL BMM as in Experiment 1. Buffalo COCs were divided into four groups: (1) Control group, COCs were IVM in BMM; (2) ascorbic acid group, in which buffalo COCs were IVM in BMM supplemented with 50 µM ascorbic acid; (3) glutathione group, in which buffalo COCs were IVM in BMM supplemented with 3.0 mM GSH; and (4) melatonin group, in which buffalo COCs were IVM in BMM supplemented with 10⁻⁵ M melatonin. In all groups, buffalo COCs were incubated at 38.5°C for
Evaluation of oocytes cytoplasmic and nuclear maturation rates

After IVM, cytoplasmic maturation was checked in all groups based on the degree of cumulus cell expansion. Cumulus oocyte complexes were classified according to their degree of cumulus cell expansion into four grades. Grade 0 (G0), COCs with no cumulus cell expansion. Grade 1 (G1), COCs with slight expansion of the outer layer of cumulus cells, Grade 2 (G2), COCs with moderate expansion of cumulus cells, and Grade 3 (G3), COCs with full expansion of cumulus cells (Figure-3a-d). Assessment of nuclear maturation was performed as in Experiment 1. After evaluation of IVM rate, GSH group was excluded out from in vitro fertilization and in vitro culture due to the low maturation rate compared with the control group. In control, ascorbic acid, and melatonin groups, IVM matured oocytes were subjected to IVF as in Experiment 1, and fertilized oocytes were transferred into the sperm suspension droplet (300 µL Fert-TALP medium) and incubated for 18-20 h at 38.5°C under 5% CO₂ and 20% O₂ (OS) in humidified air.

Embryo development rates were checked on days 2, 5, 7, and 8 using an inverted microscope.

Statistical analysis

Data were expressed as the Mean ± SEM. Statistical analysis was performed using Student’s “t” test and analysis of variance (ANOVA) with the aid of SPSS 20.0 statistical software (SPSS Inc., Chicago, IL, USA). Duncan’s multiple range tests were used to differentiate between significant means at p<0.05.

Results

Experiment 1: Effect of OS condition on the developmental competence of buffalo oocytes

The effect of OS (20% vs. 5% oxygen) on maturation rate and development of buffalo oocytes is illustrated in Table-1. Data revealed that IVM under 20% oxygen tension significantly (p<0.05) decreased maturation rate of buffalo oocytes. After IVF, cleavage rate was higher (p<0.05) for buffalo oocytes cultured under 5% CO₂ + 5% O₂ and 90% N₂ than that cultured under 5% CO₂ + 20% O₂ (OS). Moreover, the percentage of 8-16 cell stage and morula stages did not vary for buffalo oocytes cultured under 5% or 20% O₂ (Figure-4b). Meanwhile, the percentage of 2-4 cell stage was lower (p<0.05) and blastocyst rate (Figure-4a) was higher (p<0.05) for oocytes cultured under 5% CO₂ + 5% O₂ and 90% N₂ than that cultured under OS conditions of 5% CO₂ + 20% O₂. These results indicated that 5% CO₂ + 5% O₂ support the development of in vitro produced buffalo embryos to the blastocyst stage better than 20% O₂ conditions.

Experiment 2: Effect of addition of 50 µM ascorbic acid, 3.0 mM GSH, or 10⁻⁵ M melatonin in IVM medium on maturation and development of buffalo oocytes

The effect of addition of 50 µM ascorbic acid, 3.0 mM GSH, or 10⁻⁵ M melatonin to IVM medium on cumulus cell expansion and nuclear maturation rate of IVM buffalo oocytes is demonstrated in Table-2. Results showed that the addition of 10⁻³ M melatonin to maturation medium of buffalo oocytes significantly (p<0.05) increased the percentage of oocytes with Grade 3 cumulus cell expansion (G3, full cumulus cell expansion). Melatonin also increased oocyte nuclear maturation (MII stage) compared to control oocytes.
or those that were matured in the presence of 50 µM ascorbic acid or 3.0 mM GSH (p<0.05). Addition of 3.0 mM GSH to IVM medium of buffalo oocytes significantly (p<0.05) decreased the percentage of G3 cumulus cell expansion and nuclear maturation rate compared with the control group.

The effect of addition of 50 µM ascorbic acid or 10⁻⁵ M melatonin to IVM medium of buffalo oocytes on fertilization, cleavage, and blastocyst rates is illustrated in Table-3. Results indicated that the addition of 10⁻⁵ M melatonin in IVM medium significantly (p<0.05) increased fertilization, cleavage
and blastocysts rates when compared with control or 50 µM ascorbic acid groups (Figure 5b). Meanwhile, fertilization, cleavage, (Figure 5a), and blastocyst rates were significantly (p<0.05) increased after addition of 50 µM ascorbic acid to IVM medium compared with the control group. Overall, addition of 10⁻³ M melatonin or 50 µM ascorbic acid to IVM medium beneficially improves the developmental competence of buffalo oocytes cultured under OS condition (20% O₂).

Discussion

In vitro production of embryos at oxygen tension close to normal physiological level present in utero (5-7% oxygen tension) produced higher cleavage and blastocyst rates [22]. The present data indicated that maturation rate was significantly (p<0.05) higher for buffalo oocytes IVM under 5% O₂ than that matured under OS condition of 20% oxygen tension. After IVF, cleaved rate was higher (p<0.05) and the percentage of 2-4 cell stage was lower (p<0.05) for buffalo oocytes IVM and IVF under 5% CO₂ + 5% O₂ compared to that cultured under OS condition of 5% CO₂ + 20% O₂. Furthermore, culture of the presumptive zygotes under 5% CO₂ + 5% O₂ increased (p<0.05) the development of buffalo embryos to the blastocyst stage than that cultured under 5% CO₂ + 20% O₂. These results indicated that 5% CO₂ + 5% O₂ support the development of in vitro produced buffalo embryos to the blastocyst stage better than 20% O₂ conditions. These results are concomitant with those previously reported in cattle [9,23] and pigs [24]. Embryos cultured under OS showed higher ROS accumulation and higher abundance of transcripts related to OS response, which, in turn, decreases cell proliferation and number. In addition, it affects mitochondrial and endoplasmic reticulum functions, resulting in metabolic alterations, DNA fragmentation, and other detrimental effects [9,22]. Low oxygen tension during maturation alters the expression of genes related to bovine oocyte competence and glucose metabolism and significantly improves embryo development, but not blastocyst quality [4,25]. In contrast, other studies reported that oxygen tension did not affect the proportion of one-cell embryos undergoing cleavage or progressing to bovine morula and blastocyst stages [26], sheep and deer [27], or human [28]. This difference could be attributed to species difference or to the conditions under which oocytes or embryos were cultured.

According to our knowledge, this is the first study to address the effect of ascorbic acid, GSH, and melatonin on cumulus cell expansion, nuclear maturation, cleavage, and development of buffalo oocytes in vitro. The present investigation revealed that IVM of buffalo oocytes in basic maturation medium supplemented with 10⁻³ M melatonin or 50 µM ascorbic acid significantly (p<0.05) increased cumulus cell expansion and nuclear maturation of buffalo oocytes reaching to the MII stage compared to the control oocytes or those matured in basic medium supplemented with 3.0 mM GSH. Moreover, addition of 10⁻³ M melatonin in IVM medium of buffalo oocytes cultured under 20% O₂ tension significantly (p<0.05) increased fertilization, cleavage, and blastocyst rates compared with control or 50 µM ascorbic acid groups. The beneficial effects of melatonin on in vitro embryo development have been recorded in mice [29], buffalo [30,31], bovine [32,33], ovine [34], porcine [35], and humans [36]. Melatonin improves cytoplasmic maturation of bovine oocytes by improving the normal distribution of organelles and increasing intracellular GSH, ATP levels, and upregulates genes regulating oocytes maturation [33,37], and mitigates mitochondrial DNA damage [17], and increase total antioxidant capacity in mice oocytes [35]. In contrast, in bovine, melatonin supplementation of IVM media did not improve the cleavage and blastocyst rates [38]. This discrepancy could be attributed to the dose or source or batch of melatonin used for IVM.

Moreover, the present work revealed that addition of 50 µM ascorbic acid into IVM of buffalo oocytes cultured under 20% O₂ tension did not affect cumulus cell expansion, while it significantly (p<0.05) increased nuclear maturation rate compared with control or GSH groups. It also increased (p<0.05) the blastocyst rate compared with the control one. Addition of Vitamin C to the oocyte maturation medium improves maturation.
rates of porcine oocytes [39]. The beneficial role for Vitamin C was to protect the spindle structures of MI mouse oocytes and chromosomal alignment against an oxidant (hydrogen peroxide) induced damage [40]. It is suggested that the effect of Vitamin C was associated mainly with its capability to promote ooplasmic maturation during IVM. In contrast, addition of Vitamin C to the oocyte maturation medium has no beneficial effect on the maturation rates of oocytes [41]. This difference could be due to the concentration or culture conditions during IVM. Furthermore, in the present work, the addition of 3.0 mM GSH to IVM of buffalo oocytes adversely affects both cumulus cell expansion and nuclear maturation of IVM buffalo oocytes, and therefore, it is excluded from the study of in vitro fertilization and embryo development. This could be in part due to the dose or batch of GSH used and different concentrations of GSH should be tested to identify the proper dose for IVM of buffalo oocytes.

**Conclusion**

About 5% O₂ tension is the optimum gas condition for in vitro production of buffalo embryos. However, addition of 10⁻⁵ M melatonin to IVM medium of buffalo oocytes can alleviate the adverse effect of OS produced by culture of buffalo oocytes under 20% O₂ tension.

**Authors’ Contributions**

ASSA designed the experiments. AME, ASSA, OMK conducted the experiments. ASSA, HIHA, AMA performed the statistical analysis. ASSA, OMK, HHA, AME, NEE and AMA drafted and revised the manuscript. All authors have read and approved the final manuscript.

**Acknowledgments**

The authors gratefully acknowledge the financial support from National Research Centre (Proj. 12/1/7). Prof. Dr. Omaima M. Kandil for the facilities provided to conduct this work at the Embryo and Genetic Resource Conservation Bank at National Research Centre (financially supported by STDF, CB Grant ID: 2339) and Egyptian Academy for Science and Technology for supporting the project “Assessment the viability of cryopreserved buffalo embryo and semen” (Egyptian – Bulgarian cooperation bilateral agreement).

**Competing Interests**

The authors declare that they have no competing interests.

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**References**

1. An, Q., Peng, W., Cheng, Y., Lu, Z., Zhou, C., Zhang, Y. and Su, J. (2019) Melatonin supplementation during in vitro maturation of oocyte enhances subsequent development of bovine cloned embryos. J. Cell Physiol., 234(10): 17370-17381.
2. Soto-Heras, S. and Paramio, M.T. (2020) Impact of oxidative stress on oocyte competence for in vitro embryo production programs. Res. Vet. Sci., 132: 342-350.
3. Li, W., Goossens, K., Van Poucke, M., Forier, K., Braeckmans, K., Van Soom, A. and Peelman, L.J. (2016) High oxygen tension increases global methylation in bovine 4-cell embryos and blastocysts but does not affect general retrotransposon expression. Reprod. Fertil. Dev., 28(7): 948-959.
4. Marin, D.F.D., Costa, N.N., Santana, P.P.B. Souza, E.B. and Ohashi, O.M. (2019) Importance of lipid metabolism on oocyte maturation and early embryo development: Can we apply what we know to buffalo? Anim. Reprod. Sci., 211: 106220.
5. Goto, Y., Noda, Y., Mori, T. and Nakano, M. (1993) Increased generation of reactive oxygen species in embryos cultured in vitro. Free Radic. Biol. Med., 15(1): 69-75.
6. Lim, J., Reggio, B., Godke, R. and Hansel, W. (1999) Development of in-vitro-derived bovine embryos cultured in 5% CO₂ in air or in 5% O₂, 5% CO₂, and 90% N₂. Hum. Reprod., 14(2): 458-464.
7. Elamran, G., Singh, K., Singh, M., Singla, S., Chauhan, M., Manik, R. and Palta, P. (2012) Oxygen concentration and cysteamine supplementation during in vitro production of buffalo (Bubalus bubalis) embryos affect mRNA expression of BCL-2, BCL-XL, MCL-1, BAX and BID. Reprod. Dom. Anim., 47(6): 1027-1036.
8. Correa, G., Rumpf, R., Mundim, T., Franco, M. and Dode, M. (2008) Oxygen tension during in vitro culture of bovine embryos: Effect in production and expression of genes related to oxidative stress. Anim. Reprod. Sci., 104(2-4): 132-142.
9. Leite, R.F., Annes, K., Ispada, J., de Lima, C.B., Dos Santos, E.C., Fontes, P.K., Gouveia Nogueira, M.F. and Milazzoott, M.P. (2018) Oxidative stress alters the profile of transcription factors related to early development on in vitro produced embryos. Oxid. Med. Cell Longev., 2018: 6730857.
10. Ashibe, S., Miyamoto, R., Kato, Y. and Nagao, Y. (2019) Detrimental effects of oxidative stress in bovine oocytes during intracytoplasmic sperm injection (ICSI). Theriogenology, 133: 71-78.
11. Beheshti, R., Mohammadi-Roshandeh, A., Giasi Ghalakand, J., Ghaemmaghami, S. and Houshangi, A. (2011) Effect of antioxidant supplements on in vitro maturation of bovine oocyte. Adv. Environ. Biol., 5(7): 1473-1475.
12. Tao, Y., Chen, H., Tian, N.N., Huo, B.T., Li, G., Zhang, Y.H., Liu, Y., Fang, F.G., Ding, J.P. and Zhang, X.R. (2010) Effects of L-ascorbic acid, alpha-tocopherol and co-culture in vitro developmental potential of porcine cumulus cells free oocytes. Reprod. Domest. Anim., 45(1): 19-25.
13. Tian, X., Wang, F., He, C., Zhang, L., Tan, D.X., Reiter, R.J., Xu, J., Ji, P.Y. and Liu, G.S. (2014) Beneficial effects of melatonin on bovine oocytes maturation: A mechanistic approach. J. Fimal. Res., 57(3): 239-247.
14. Liu, T., Lee, J.E., Jeong, J.W., Oqani, R.K., Cho, E.S., Kim, S.B. and Jin, D.I. (2018) Melatonin supplementation during prolonged in vitro maturation improves the quality and development of poor-quality porcine oocytes via anti-oxidative and anti-apoptotic effects. Mol. Reprod. Dev., 85(8-9): 665-681.
15. Zhang, Y., Wang, T., Lan, M., Zhang, X.W., Li, Y.L., Cui, X.S., Kim, N.H. and Sun, S.C. (2018) Melatonin protects oocytes from MEHP exposure-induced meiosis defects in porcine. Biol. Reprod., 98(3): 286-298.
16. Lan, K.C., Lin, Y.C., Chang, Y.C., Lin, H.J., Tsai, Y.R. and Kang, H.Y. (2019) Limited relationships between reactive oxygen species levels in culture media and zygote and embryo development. J. Assist. Reprod. Genet., 36(2):

Available at www.veterinaryworld.org/Vol.14/January-2021/10.pdf
21. Abdoon, A.S.S., Gabler, C., Holder, C., Kandil, O.M. and Sovernigo, T.C., Adona, P.R., Monzani, P.S., Guemra, S., de Matos, D.G., Gasparrini, B., Pasqualini, S.R. and Yoshida, M., Ishigaki, K., Nagai, T., Chikyu, M. and Viloria, T., Sonia Pérez, S., Romero, J.L. and Remohï, J. (2010) Low oxygen tension during IVM improves antioxidation and after fertilization in pig oocytes: Relevance to the ability oocytes to form male pronucleus. *Biol. Reprod.*, 82(8): 1055-1067.

22. Yoon, B.S., Choi, S.A., Sim, B.W., Kim, J.S., Mun, S.E., Chang, KT. (2014) Developmental competence of bovine early embryos depends on the coupled response between oxidative and endoplasmic reticulum stress. *Biol. Reprod.*, 90(5): 1-10.

23. Bennemann, J., Grothmann, H. and Wrenzycki, C. (2018) Reduced oxygen concentration during *in vitro* oocyte maturation alters global DNA methylation in the maternal pronucleus of subsequent zygotes in cattle. *Mol. Reprod. Dev.*, 85(11): 849-857.

24. Garcia-Martinez, S., Sánchez Hurtado, M.A., Gutiérrez, H., Sánchez Margallo, F.M., Romar, R., Latorre, R., Coy, P. and López Albors, O. (2018) Mimicking physiological O2 tension in the female reproductive tract improves assisted reproduction outcomes in pig. *Mol. Hum. Reprod.*, 24(5): 260-270.

25. Bermeno-Alvarez, P., Lonergan, P., Rizos, D. and Gutiérrez-Adan, A. (2010) Low oxygen tension during IVM improves bovine oocyte competence and enhances anaerobic glycolysis. *Reprod. Biomed. Online*, 20(3): 341-349.

26. Khurana, N.K. and Niemann, H. (2000) Effects of oocyte quality, oxygen tension, embryo density, cumulus cells and energy substrates on cleavage and morula/blastocyst formation of bovine embryos. *Theriogenology*, 54(5): 741-756.

27. Sánchez-Ajofrín, I., Iniesta-Cuerda, M., Sánchez-Calabuig, M.J., Peris-Fraru, P., Martín-Maestro, A., Ortiz, J.A., Fernández-Santos, M.D.R., Garde, J.J., Gutiérrez-Adán, A. and Soler, A.J. (2020) Oxygen tension during *in vitro* oocyte maturation and fertilization affects embryo quality in sheep and deer. *Anim. Reprod. Sci.*, 213: 106279.

28. de los Santos, M.J., Gámiz, P., Alberti, C., Galan, A., Viloría, T., Sonia Pérez, S., Romero, J.L. and Remohi, J. (2013) Reduced oxygen tension improves embryo quality but not clinical pregnancy rates: a randomized clinical study into ovum donation cycles. *Fertil. Steril.*, 100(2):402-407.

29. Almohammed, N.H.Z., Moghanni-Ghoroghi, F., Ragerdi-Kashani, I., Fathi, R., Tahaei, L.S., Naji, M. and Pasbakhsh, P. (2020) The effect of melatonin on mitochondrial function and autophagy in *in vitro* matured oocytes of aged mice. *Cell J.*, 22(1): 9-16.

30. Nagine, G., Asima, A., Nemat, U. and Shamim, A. (2016) Effect of melatonin on maturation capacity and fertilization of Nili-Ravi buffalo (*Bubalus bubalis*) oocytes. *Open Vet. J.*, 6(2): 128-134.

31. Manjunatha, B.M., Devaraj, M., Gupta, P.S., Ravindra, P. and Nandi, S. (2009) Effect of taurine and melatonin in the culture medium on *in vitro* embryo development. *Reprod. Domest. Anim.*, 44(12): 1-16.

32. Dimitriadis, I., Paapanikolau, T., Vainas, E., Amiridis, G.S., Valasi, I., Samrzi, F. and Rekkas, C.A. (2005) Effects of Melatonin on *in Vitro* Maturation of Bovine Oocytes. Annual Conference of the European Society for Domestic Animal Reproduction (ESDAR) Murcia, Spain p.597.

33. Zhao, X.M., Wang, N., Hao, H.S., Li, C.Y., Zhao, Y.H., Yan, C.L., Wang, H.Y., Du, W.H., Wang, D., Liu, Y., Pang, Y.W. and Zhu, H.B. (2018) Melatonin improves the fertilization capacity and developmental ability of bovine oocytes by regulating cytoplasmatic maturation events. *J. Pineal Res.*, 64(1): 12445.

34. Barros, V.R.P., Monte, A.P.O., Santos, J.M.S., Lins, L.B.G., Cavalcante, A.Y.P., Gouveia, B.B., Müller, M.C., Oliveira Junior, J.L., Barberino, R.S., Donfack, N.J., Araújo, V.R. and Matos, M.H.T. (2020) Effects of melatonin on the *in vitro* growth of early antral follicles and maturation of ovine oocytes. *Domest. Anim. Endocrinol.*, 71: 106386.

35. Yang, L., Wang, Q., Cui, M., Li, Q., Mu, S. and Zhao, Z. (2020) Effect of melatonin on the *in vitro* maturation of porcine oocytes, development of parthenogenetically activated embryos, and expression of genes related to the oocyte developmental capability. *Animals (Basel)*, 10(2): 209.

36. Nishihara, T., Hashimoto, S., Ito, K., Nakaoka, Y., Matsumoto, K., Hosoi, Y. and Morimoto, Y. (2014) Oral melatonin supplementation improves oocyte and embryo quality in women undergoing *in vitro* fertilization-embryo transfer. *Gynecol. Endocrinol.*, 30(5): 359-362.

37. Pang, Y., Zhao, S., Sun, Y., Jiang, X., Hao, H., Du, W. and Zhu, H. (2018) Protective effects of melatonin on the in vitro developmental competence of bovine oocytes. *Anim. Sci. J.*, 89(4):648-660.

38. Tsantarliotou, M.P., Altanasio, L., Rosa, A.D., Boccia, L., Cavalcante, A.Y.P., Gouveia, B.B., Müller, M.C., Oliveira Junior, J.L., Barberino, R.S., Donfack, N.J., Araújo, V.R. and Matos, M.H.T. (2020) Effects of melatonin on the *in vitro* growth of early antral follicles and maturation of ovine oocytes. *Domest. Anim. Endocrinol.*, 71: 106386.

39. Kere, M., Siriboon, C., Lo, N.W., Nyuyen, N.T. and Pellerano, G. and Gasparrini, B. (2007) The effect of melatonin supplementation improves oocyte and embryo development of porcine oocytes by regulating cytoplasmatic maturation events. *J. Pineal Res.*, 46(1): 12445.

40. Pursel, V.G. (1993) Glutathione concentration during maturation of ovine oocytes. *Biol. Reprod.*, 52(4): 561-569.

41. Khazaei, M. and Aghaz, F. (2017) Reactive oxygen species and decreases excessive intracellular Ca2+ levels in immature human oocytes. *Life Sci.*, 235: 116810.