Egyptian J. Anim. Prod. (2020) 57(2):89-94

BAICALEIN IMPROVES *IN VITRO* DEVELOPMENT RATE AND QUALITY OF PREIMPLANTATION BOVINE EMBRYOS WHEN SUPPLEMENTED TO MATURATION MEDIUM

M. Fakruzzaman[1], S. Yasmine[2] and N. Ghanem[3,*]

1- ***Department of Genetics and Animal Breeding, Faculty of Animal Science and Veterinary Medicine, Patuakhali Science and Technology University, Out Campus, Khanpura, Babuganj, Barishal-8210, Bangladesh, 2- Department of Pharmacy, Faculty of Life Science, University of Development Alternative, Dhanmondi, Dhaka-1209, Bangladesh ,3- Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt,*** **[*]Correspnding author E-mail: (nassergo@agr.cu.edu.eg)**

Received: 21/4/2020 Accepted: 17/6/2020

SUMMARY

Baicalein (5,6,7-trihydroxyflavone) is one of the flavonoid, that is traditionally used in Chinese herbal medicine. It has an antioxidant properties and acts as free radical scavengers. However, the effect of baicalein on bovine oocyte maturation and subsequent embryo development is unknown. For this, good quality bovine oocytes recovered from abattoir ovaries were cultured in IVM medium supplemented with various concentrations of baicalein (0, 0.1, 1.0 and 10 µM) followed by in vitro fertilization and embryo development. The cleavage and blastocyst development rates were recorded at days three and eight after fertilization, respectively. In addition, total cell number and total dead cells (apoptotic) were counted using TUNEL-Hoechst assay. The results indicate that the proportion of blastocysts derived from oocytes treated with baicalein of 1 µM (38.3%) was greater (P < 0.05) than those of control group (28.7%). In addition, the percentage of Day-eight blastocysts was not significantly different namong the 0.1 µM (31.5%), 1 µM (38.3%) and 10 µM (32.5%) embryo groups. The percentage of hatched blastocyst on day eight were significantly higher in the group supplemented with 1 µM (40.5%) baicalein than those in the control and 0.1 µM (33.3% and 32.4, respectively). Total cell number per blastocyst was increased (P < 0.05) in embryos treated with baicalein at the rate of 1 µM (150.3±5.0) compared with the control group (0 µM) and 10 µM (122.9±8.9 and 128.1±6.2, respectively). However, there were no significant differences between 1 µM (150.3±5.0) and 0.1 µM (139.4±5.7). Moreover, the number of apoptotic cells was lower (P < 0.05) in blastocysts derived from oocytes treated with baicalein of 1 µM (3.6±0.6) than in control (6.4±1.2) and 10 µM embryos (7.1±1.7). In conclusion, this study demonstrates that baicalein is a potent antioxidant that improves the maturation environment on the way to promote the developmental competence of bovine oocytes in vitro and increases hatching rate and the total blastocyst cell numbers by suppressing incidence of apoptosis when supplemented at the concentration of 1 µM.

Keywords: Baicalein, bovine embryos, in vitro maturation, development, apoptosis

INTRODUCTION

During *in vitro* production of bovine embryos, occytes maturation, fertilization and zygote culture play crucial roles to achieve the target goal (Absalón-Medina *et al*., 2014). For *in vitro* culture of mammalian embryos, it is widly used in *in vitro* environment consisting of 5% CO_2 and 95% air (~20% O_2 total) (Kitagawa *et al*., 2004). Moreover, high concentration of O_2 throughout *in vitro* culture obstructs embryonic development, due to, created additional reactive oxygen species (ROS) from the cytoplasm of developing embryos (Guérin *et* al., 2001). These ROS are highly reflect with intracellular macromolecules, like proteins, lipids and DNA, and may cause significant dysfunction including inactivation of enzyme, abnormalities in mitochondria or DNA fragmentation (Guérin *et* al., 2001). Living organisms have the natural protective equivalents/ROS scavengers, which are intracellular antioxidants that counter balance the negative effects of ROS (Wang *et al*., 2007). Still during the process of *in vitro*, antioxidants levels are lower than those *in vivo*; therefore, several antioxidant supplementation of the medium might improve developmental capability (Ali *et al*., 2003 and Livingston *et al*., 2004).

The developmental competences of mammalian embryos by *in vitro* are still lower compared with that of embryos developed *in vivo* (Pontes *et al*., 2009). It is also well documented that both oocytes and embryos are vulnerable to oxidative stress and to any kinds of adverse factors when they are cultured in an *in vitro* culture system (Feng *et al*., 2014). As a result, various antioxidants such as dihydroxyflavone (Keum *et al*., 2011), quercetin (Sovernigo *et al*., 2017), baicalin (Xiaonan *et al*., 2016 and Qing *et al*., 2019), resveratrol (Feng *et al*., 2014) and melatonin (Feng *et al*., 2014) have been added to *in vitro* culture medium to improve the maturation of oocytes and the developmental competence of preimplantation embryos.

Flavonoids are phenolic compounds and are widely present in plants, fruits and Chinese herbal medicine (Lin *et al*., 2007). The structures and functions of flavonoids have evoked considerable interest because of their antioxidant properties (Keum *et al*, 2011). Moreover, the antioxidant activities of flavonoids have been given much attention due to better antioxidant activities than vitamins C and E (Lin *et al*., 2007). Several flavonoids show potent antitumor properties and can induce apoptosis, differentiation and the cell cycle, probably by virtue of their antioxidant functions (Lee *et al*., 2007). Flavonoids may have the capacity to inhibit the generation of primary oxygen radicals and subsequent oxidation chains, because they are effective chelators of transition metal ions (Afanas'ev *et al*., 1989). The number of hydroxyl substitutions in a flavonoid is thought to be a critical factor in its ROS-scavenging ability (Areias *et al*., 2001).

Baicalein (5,6,7-trihydroxyflavone) is one of the flavonoid, and a major component of *Scutellaria baicalensis* (Kim *et al*., 2001). It was reported that baicalein had free radical scavenging an antioxidant activities (Shieh *et al*., 2000). Moreover, baicalein is an antioxidant (Chen *et al*., 2000), and an anti-inflammatory agent (Lin and Shieh, 1996).

The exact role of baicalein in the development of bovine pre-implantation stage embryos has not been elucidated. This is the first study to investigate the effect of baicalein supplementation during *in vitro* maturation on development of bovine oocytes. The embryos were then cultured without baicalein.

MATERIALS AND METHODS

Reagents:

Unless otherwise mentioned, all the chemicals, reagents, media, and media constituents were purchased from Sigma-Aldrich Chemicals, Germany.

Experimental design:

Briefly, COCs were cultured in 700 µL of IVM (*In vitro* maturation) medium supplemented with various concentrations of baicalein (0, 0.1, 1.0 and 10 µM) in an incubator under a moisture-saturated atmosphere of 5% CO_2 in air for 24 h at 38.5 °C. All embryo groups were evaluated on Day eight (Day 0 = IVF) to determine the proportion of embryos that had reached the blastocyst stage and hatched. Blastocysts originated from oocytes treated with different concentration of baicalein were used to assess embryo quality. Non-treated blastocysts were used as the control (0 µM).

Oocyte collection and in vitro maturation:

Ovaries were collected from a local slaughterhouse and transported in normal saline solution at 35-37 °C to the lab. within 2 h. Cumulus-oocyte complexes (COCs) were aspirated from 3-8 mm follicles using an 18 gauge needle fixed to 5 ml syringe. Oocytes enclosed with 3-5 layers of cumulus cells and homogenous granular cytoplasm were considered as a good quality and were utilized for *in vitro* maturation.

Cumulus-oocyte complexes were cultured in maturation medium, as described by Nasser *et al*., (2014). In brief, COCs (55–60 oocytes/group) were washed three-times in maturation medium (TCM-199) supplemented with 10% (v/v) fetal bovine serum (FBS), 1 µg/mL of estradiol-17β, 10 µg/mL of FSH, 0.6 mM of cystein, and 0.2 mM of sodium pyruvate and transferred into a well of a 4-well dish containing 700 µL of IVM medium for 23 to 24 h at 38.5 °C in a humidified atmosphere of 5% CO_2 in air.

In vitro fertilization and embryo culture:

Samples of frozen semen were thawed at 37°C for 30 seconds and sperms were washed twice with Sperm Tyrod's Albumin Lactate Pyruvate medium (Sperm-TALP) containing 10 µg/ml heparin, 2.2 mg/ml sodium pyruvate and BSA F-V (6 mg/ml) + 50 µg/ml gentamycin at 500 g for 10 min. After washing, a sperm pellet was suspended in 0.5 ml of fresh Fert-TALP medium supplemented with six mg/ml BSA (fatty acid free) + 10 µg/ml heparin + 3 µl PHE and 50 µg/ml gentamycin. Sperm concentration was adjusted to $1x10^6$ spermatozoa/ml. A total of 500 µl of motile sperm suspension was placed in 4-well culture plate. *In vitro* matured oocytes were washed in Fert-TALP media three times and placed into the sperm suspension and kept at 39 °C in an incubator under a moisture-saturated atmosphere of 5% CO_2 in air for 18-20 h. After co-incubation, cumulus cells were removed by pipetting and the presumptive zygotes were washed and transferred to 700 µL of CR1-aa medium (Nasser *et al*., 2014) supplemented with 44 µg/mL Na-pyruvate, 14.6 µg/mL glutamine, 10 µl/mL penicillin-streptomycin, 3 mg/mL BSA, and 310 µg/mL glutathione (IVC-I) for three days. Medium was then replaced with fresh one. Embryos (eight-cell stage) were cultured until Day eight of embryonic development (Day 0 = day of IVF) in medium with the same composition as IVC-I, except that BSA was replaced with 10% (v/v) FBS (IVC-II). Day eight blastocysts were washed three times in TL-HEPES, transferred to fixative (4% [v/v] paraformaldehyde prepared in one M PBS), and stored at 4 °C until the total cell number and total cells dead were counted.

Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL):

The TUNEL was performed according to the manufacturer's protocol using an In Situ Cell Death Detection Kit (Roche Diagnostics Corp., Indianapolis, IN, USA). Briefly, fixed blastocysts (n =75) were washed twice with 0.3% (w/v) polyvinylpyrrolidine (PVP) prepared in one M PBS (PVP-PBS) and then incubated in permeabilization buffer (0.5% [v/v] Triton X-100 and 0.1% [w/v] sodium citrate) for 30 min at room temperature. After permeabilization, embryos were washed twice in PVP-PBS and incubated in the dark with fluorescently-conjugated terminal deoxynucleotide

transferase dUTP for one h at 37°C. TUNEL-stained embryos were washed with PVP-PBS and incubated in PVP-PBS containing 10 µg/mL Hoechst 33342 for 10 min. After being washed twice with PVP-PBS, blastocysts were mounted onto glass slides and their nuclear configuration was analyzed. The number of cells per blastocyst was determined by counting Hoechst-stained cells under an epifluorescence microscope (Olympus IX71, Tokyo, Japan) equipped with a mercury lamp. TUNEL-positive cells fluoresced red, indicating they were apoptotic, whereas the total number of cells was determined by the extent of blue fluorescence.

Statistical analyses

All data were analyzed using the Statistical Package for the Social Sciences (SPSS) software package for Windows (SPSS v.18; SPSS Inc., Chicago, IL, USA). Data of at least five replicates of each treatment were analyzed with ANOVA, using the general linear model procedure. Results were expressed as the percentage (%), mean ± SEM (standard error of the mean). Data on blastocysts development rate was analyzed by one-way ANOVA followed by multiple pairwise comparisons (Tukey's Test). Differences with $P<0.05$ were considered as significant.

RESULTS

Embryo development rate

Embryo development rates after culturing in IVM medium supplemented with baicalein are shown in Table 1. The percentage of embryo cleavage was not significantly different among all experimental groups (Table 1). The proportion of blastocysts derived from oocytes treated with baicalein of one µM (38.3%) was greater (P < 0.05) than those of control (0 µM) group (28.7%). In addition, the percentage of Day-8 blastocysts was not significantly different among 0 µM, 0.1 µM and 10 µM embryo groups.

Embryo hatching rate

The percentage of hatched blastocyst on day 8 (Table 1) was significantly higher in the group supplemented with one µM (40.5%) baicalein than those in the control and 0.1 µM (33.3% and 32.4, respectively). In addition, there was no significant difference on embryo hatching rate when baicalein was supplemented at the level of one µM (40.5%) and 10 µM (36.6%).

Table 1. Effect of different concentrations of baicalein on the development of bovine embryos *in vitro*

Concentration of Baicalein (µM)	Oocytes (N)	Number of embryos that underwent cleavage rate % (n)	Number of embryos that developed to the blastocyst stage % (n)	Hatched/blastocyst % (n)
0 (Control)	408	81.6 ± 2.4 (333)^{a}	28.7 ± 1.8 (117)^{b}	33.3 ± 0.8 (39)^{b}
0.1	420	83.6 ± 2.0 (351)^{a}	31.5 ± 2.9 (130)^{ab}	32.4 ± 1.1 (42)^{b}
1.0	413	87.4 ± 2.7 (361)^{a}	38.3 ± 1.2 (158)^{a}	40.5 ± 1.0 (64)^{a}
10	410	81.7 ± 2.9 (335)^{a}	32.5 ± 3.7 (131)^{ab}	36.6 ± 0.9 (48)^{ab}

[1] N: total number of cumulus oocytes complexes.

[a,b] Within a column, means without a common superscript differed (P < 0.05).

Assessment of total cell and apoptotic cell numbers in blastocysts

The total cell number per blastocyst was higher (P < 0.05) in embryos originated from oocytes treated with baicalein at the rate of one µM (150.3±5.0) compared with the control (0 µM) group (122.9±8.9) and 10 µM (128.1±6.2), but there were no significant differences between one µM and 0.1 µM (139.4±5.7) groups (Table 2 and Figure1). The number of apoptotic cells (Table 2, Fig. 1) was lower (P < 0.05) in 1 µM-treated blastocysts (3.6±0.6) than controls (6.4±1.2) and 10 µM (7.1±1.7). In addition, there were no significant differences in apoptosis rate between one µM and 0.1 µM groups.

Table 2. Effect of baicalein on the quality of Day eight blastocysts (mean ± SEM)

Concentration of Baicalein (µM)	Number of blastocysts examined	Total number of cells per blastocyst	Number of apoptotic cells per blastocyst
0 (Control)	18	122.9±8.9^{c}	6.4±1.2^{c}
0.1	19	139.4±5.7^{ab}	5.3±1.0^{abc}
1.0	19	150.3±5.0^{a}	3.6±0.6^{a}
10	19	128.1±6.2^{bc}	7.1±1.7^{bc}

[a,b,c] Within a column, means without a common superscript differed (P < 0.05).

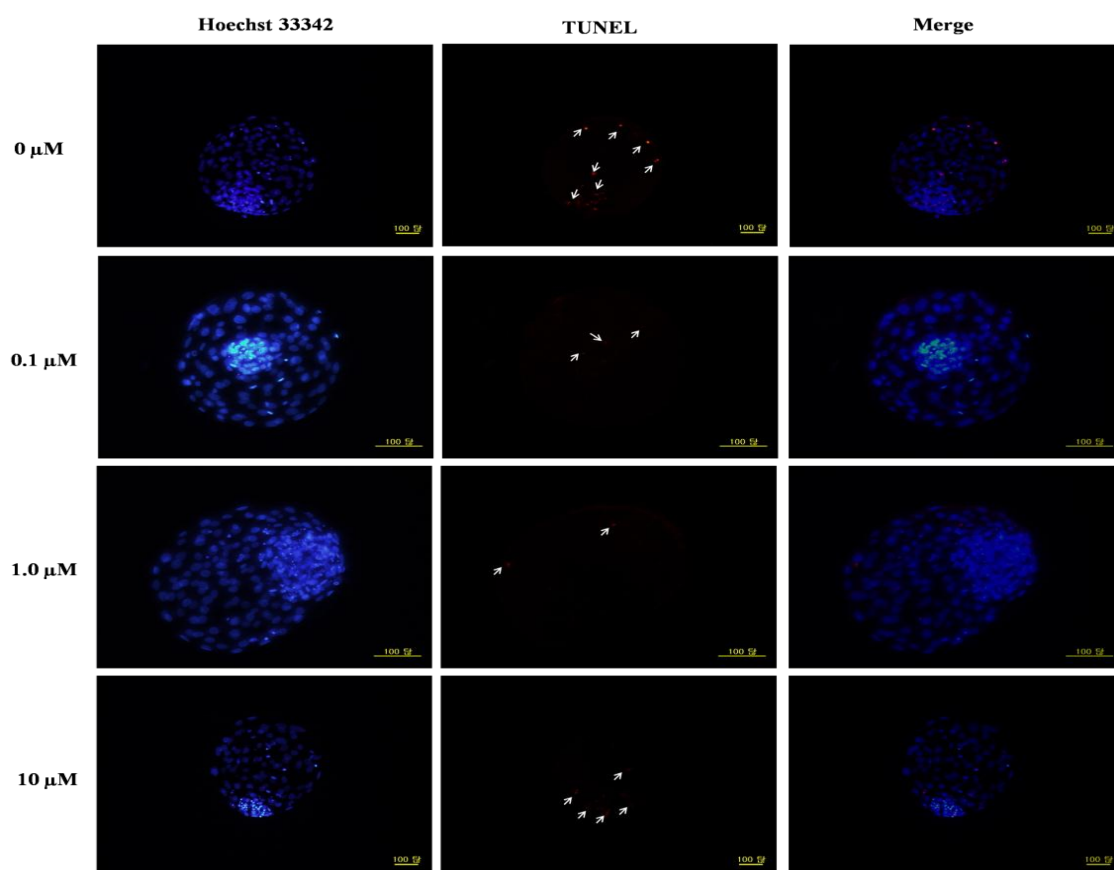

Figure 1. Representative images of bovine embryos stained with Hoechst 33342. Apoptotic cells were identified by terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL). Corresponding images were merged. Scale bar = 100 μm.

DISCUSSION

The results of the present study demonstrate that antioxidant flavonoid baicalein has positive effects on *in vitro* embryo development and increase the total cell numbers while significantly decrease the number of apoptotic cells in the blastocysts. The arrest of embryonic development during *in vitro* condition is caused by high abundance of ROS resulting from the higher ambient oxygen concentration and relatively lower free radicals scavengers than *in vivo* condition (Goto *et al*., 1993). Addition of several antioxidants, those acts as free radical scavengers, to the culture media under normal oxygen conditions may enhance embryo development rate as well as quality (Rocha-Frigoni *et al*., 2015). This study has demonstrated that baicalein supplementation during oocytes maturation increased blastocyst developmental rates, and promoted proliferation of bovine blastocysts cultured *in vitro*. Consequently, the percentage of bovine embryos that developed to the blastocyst stage increased compared to that reported for the control group. Several studies have investigated the several compounds of flavonoid groups except baicalein effects on embryonic development rate in bovine (Keum *et al*., 2011), mouse (Xiaonan *et al*., 2016), and pig (Qing *et al*., 2019).

Additionally, reports showed that baicalein has beneficial effects to inhibit of hydrogen peroxide-induced apoptosis in different cells (Shieh *et al*., 2000). None investigated the influence of supplementing of baicalein to the maturation medium on embryo development and quality. There was no significant difference in the percentage of embryos that underwent cleavage among the groups. However, the percentage of embryos that developed to the blastocyst stage was higher in the one μM treated than in the control (0 μM) group. Furthermore, blastocysts developed in the presence of one μM baicalein had higher hatching rates in the present study. These findings are in agreement with other studies, which showed that supplementation of *in vitro* maturation medium with flavonoids, and resveratrol increases the percentages of bovine, pig and mouse embryos that develop to the blastocyst stage (Feng *et al*., 2014; Jung *et al*., 2016, Xiaonan *et al*., 2016 and Qing *et al*., 2019). Based on the results above, the effects of baicalein when added to the maturation medium on bovine oocytes increased the developmental competence of embryos compared to the control group.

The supplementation of baicalein to IVM medium improves cells number per blastocyst by reducing the number of apoptotic cells. The total cell number and the apoptotic index are suggested to be important indicators of embryo quality; previous study demonstrated that embryos with a greater number of cells are more likely to implant and to develop into live offspring (Van Soom *et al*., 2007).

Furthermore, apoptosis is an important physiological process for eliminating mutated or damaged cells under stressed condition (Yang and Rajamahendran, 2002). Increased incidence of apoptosis in embryonic cells indicates the poor quality of IVC embryos (Fabian *et al*., 2005).

The treatment with flavonoid substances 3,4-Dihydroxyflavone improved the embryo quality when added to the culture medium (Keum *et al*, 2011). *In vitro* embryonic culture under high O_2 tension enhances to produce more free radicals that have detrimental effect on embryo development (Xiaonan *et al*., 2016). The addition of baicalein had a positive effect on bovine embryo development by increasing cell numbers in blastocysts. These results are in accordance with other studies that highlighted an increase in the number of total cells and reduction in apoptotic cells when embryos cultured in presence of flavonoids during different culture medium in bovine (Keum *et al*, 2011), mouse (Xiaonan *et al*., 2016), and pig (Jung *et al*., 2016; Qing *et al*., 2019).

CONCLUSION

In conclusion, the results of the present study suggest that supplementation of baicalein in culture medium has positive effects for the improvement of maturation environment that promotes developmental competence of bovine embryos and increases the total cell numbers while significantly reducing the apoptotic cell of embryos. Therefore, baicalein is a potent antioxidant that promotes the *in vitro* developmental capacity of bovine embryos by antioxidant effects. Although our data partially support this idea, further investigations need to identify the exact pathway(s) of baicalein that involved in pronounced improvement of embryo quality.

REFERENCES

Absalón-Medina, V.A., W.R. Butler, and R.O. Gilbert, 2014. Preimplantation embryo metabolism and culture systems: experience from domestic animals and clinical implications. J. Assist. Reprod. Genet., 31(4): 393-409.

Afanas'ev, I.B., A.I. Dorozhko, A.V. Brodskii, V.A. Kostyuk, and A.I. Potapovitch, 1989. Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. Biochem. Pharmacol., 38(11): 1763-1769.

Ali, A.A., J.F. Bilodeau, and M.A. Sirard, 2003. Antioxidant requirements for bovine oocytes varies during *in vitro* maturation, fertilization and development. Theriogenology, 59(3-4): 939-949.

Areias, F.M., A.C. Rego, C.R. Oliveira, and R.M. Seabra, 2001. Antioxidant effect of flavonoids after ascorbate/Fe(2+)-induced oxidative stress in cultured retinal cells. Biochem. Pharmacol., 62(1): 111-118.

Chen, Z.Y., Y.L. Su, Y.R. Bi, S.Y. Tsang, and Y. Huang, 2000. Effect of baicalein and acetone extract of *Scutellaria baicalensis* on canola oil oxidation. J. Am. Oil. Chem. Soc., 77: 73-78.

Fabian, D., J. Koppel, P. Maddox-Hyttel, 2005. Apoptotic processes during mammalian preimplantation development. Theriogenology, 64: 221-231.

Feng, W., X.Z. Tian, L. Zhang, C. Gao, C.J. He, Y. Fu, P.Y. Ji, Y. Li, N. Li, and G.S. Liu, 2014. Beneficial effects of melatonin on *in vitro* bovine embryonic development are mediated by melatonin receptor 1. J. Pineal Res., 56: 333–342.

Feng, W., X.Z. Tian, L. Zhang, C.J. He, P.Y. Ji, Y. Li, D.X. Tan, and G.S. Liu, 2014. Beneficial effect of resveratrol on bovine oocyte maturation and subsequent embryonic development after *in vitro* fertilization. Fertil. Steril., 101(2): 577-586.

Goto, Y., Y. Noda, T. Mori, and M. Nakano, 1993. Increased generation of reactive oxygen species in embryos cultured *in vitro*. Free Radic. Biol. Med., 15(1): 69-75.

Guérin, P., S. El Mouatassim, and Y. Ménézo, 2001. Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. Hum. Reprod. Update, 7(2): 175-189.

Jung, T.K., J.H. Moon, J.Y. Choi, S.J. Park, S.J. Kim, I.M. Saadeldin, and B.C. Lee, 2016. Effect of antioxidant flavonoids (quercetin and taxifolin) on *in vitro* maturation of porcine oocytes. Asian Australas. J. Anim. Sci., 29(3): 352-358.

Keum, S.L., E.Y. Kim, K. Jeon, S.G. Cho, Y.J. Han, B.C. Yang, S.S. Lee, M.S. Ko, K.J. Riu, H.T. Lee, and S.P. Park, 2011. 3,4-Dihydroxyflavone acts as an antioxidant and antiapoptotic agent to support bovine embryo development *in vitro*. J. Reprod. Dev., 57(1): 127-134.

Kim, Y.O., K. Leem, J. Park, P. Lee, D.K. Ahn, B.C. Lee, H.K. Park, K. Suk, S.Y. Kim, and H. Kim, 2001. Cytoprotective effect of Scutellaria baicalensis in CA1 hippocampal neurons of rats after global cerebral ischemia. J. Ethnopharmacol., 77: 183-188.

Kitagawa, Y., K. Suzuki, A. Yoneda, and T. Watanabe, 2004. Effects of oxygen concentration and antioxidants on the *in vitro* developmental ability, production of reactive oxygen species (ROS), and DNA fragmentation in porcine embryos. Theriogenology, 62(7): 1186-1197.

Lee, E.R., J.H. Kim, Y.J. Kang, and S.G. Cho, 2007. The anti-apoptotic and anti-oxidant effect of eriodictyol on UV-induced apoptosis in keratinocytes. Biol. Pharm. Bull., 30(1): 32-37.

Lin, C.C., and D.E. Shieh, 1996. The anti-inflammatory activity of *Scutellaria rivularis* extracts and its active components, baicalin, baicalein and wogonin. Am. J. Chin. Med., 24(1): 31-36.

Lin, H.Y., S.C. Shen, C.W. Lin, L.Y. Yang, and Y.C. Chen, 2007. Baicalein inhibition of hydrogen peroxide-induced apoptosis via ROS-dependent heme oxygenase 1 gene expression. Biochim. Biophys. Acta., 1773(7): 1073-1086.

Livingston, T., D. Eberhardt, J.L. Edwards, and J. Godkin, 2004. Retinol improves bovine embryonic development *in vitro*. Reprod. Biol. Endocrinol., 2: 83.

Nasser, G., A.N. Ha, M. Fakruzzaman, J.I. Bang, S.C. Lee, and I.K. Kong, 2014. Differential expression of selected candidate genes in bovine embryos produced *in vitro* and cultured with chemicals modulating lipid metabolism. Theriogenology, 82: 238-250.

Pontes, J.H., I. Nonato-Junior, B.V. Sanches, J.C. Ereno-Junior, S. Uvo, T.R. Barreiros, J.A. Oliveira, J.F. Hasler, and M.M. Seneda, 2009. Comparison of embryo yield and pregnancy rate between *in vivo* and *in vitro* methods in the same Nelore (Bos indicus) donor cows. Theriogenology, 71(4): 690-697.

Qing, G., M.F. Xuan, Z.B. Luo, J.X. Wang, S.S. Jin, X.J. Yin, and J.D. Kang, 2019. Baicalin improves IVM of pig oocytes and subsequent preimplantation embryo development by inhibiting apoptosis. Reprod. Fertil. Dev., 31(5): 983-992.

Rocha-Frigoni, N.A., B.C. Leão, É. Nogueira, M F. Accorsi, and G.Z. Mingoti, 2015. Effects of gaseous atmosphere and antioxidants on the development and cryotolerance of bovine embryos at different periods of *in vitro* culture. Zygote, 23(2): 159-168.

Shieh, D.E., L.T. Liu, and C.C. Lin, 2000. Antioxidant and free radical scavenging effects of baicalein, baicalin and wogonin. Anticancer. Res., 20: 2861-2865.

Sovernigo, T.C., P.R. Adona, P.S. Monzani, S. Guemra, F. Barros, F.G. Lopes, and C. Leal, 2017. Effects of supplementation of medium with different antioxidants during *in vitro* maturation of bovine oocytes on subsequent embryo production. Reprod. Domest. Anim., 52(4): 561-569.

Van Soom, A., L. Vandaele, K. Goossens, A. de Kruif, and L. Peelman, 2007. Gamete origin in relation to early embryo development. Theriogenology, 68 (Suppl. 1): S131–S137.

Wang, Z.G., S.D. Yu , and Z.R. Xu, 2007. Improvement in bovine embryo production *in vitro* by treatment with green tea polyphenols during *in vitro* maturation of oocytes. Anim. Reprod. Sci., 100(1-2): 22-31.

Xiaonan, Q.I., L.I. Huatao, C. Xia, W. Xin, J. Zhongling, C. Rongfeng, and T. Wenru, 2016. Baicalin increases developmental competence of mouse embryos *in vitro* by inhibiting cellular apoptosis and modulating *HSP70* and *DNMT* expression. Journal of Reproduction and Development. 62(6): 561-569.

Yang, M.Y., and R. Rajamahendran, 2002. Expression of *Bcl-2* and *Bax* proteins in relation to quality of bovine oocytes and embryos produced *in vitro*. Anim. Reprod. Sci., 70: 159-169.

البيسلاين يحسن من تطور وجودة أجنة الابقار اثناء التطور الجنيني عند اضافته لبيئة الانضاج

فاكروزمان مد[١]، ياسمين س[٢]، ناصر غانم[٣]

١ - قسم الوراثه وتربية الحيوان، كلية علم الحيوان والطب البيطري، جامعة باتوخالى للعلوم والتكنولوجيا، بنجلاديش، ٢ - قسم الصيدلة، كلية علوم الحياة، جامعة بدائل التنمية، بنجلاديش، ٣ - قسم الإنتاج الحيوانى، كلية الزراعة، جامعة القاهرة

تعتبر مادة البيسلاين (Baicalein) أحد مركبات الفلافونويد (5,6,7-trihydroxyflavone) التي تستخدم بشكل تقليدي في طب الأعشاب الصيني. ويمتاز هذا المركب أن له خصائص مضادة للأكسدة ولذلك يعمل على التخلص من الشقوق الحرة الناتجة عن العمليات الحيوية بالخلية. ومع ذلك، فإن استخدام وتأثير البيسلاين على نضج بويضات الأبقار وتطور الأجنة معمليا غير معروف. ولذا الهدف من إجراء هذه الدراسة هو التعرف على تأثير اضافة هذه المادة أثناء عملية الإنضاج المعملي لبويضات الأبقار وذلك بتتبع التطور الجنيني اللاحق وجودة الاجنة المنتجة بعد إضافة هذه المادة اثناء عملية الإنضاج. حيث تم تجميع مبايض الابقار من المجزرفي محلول فسيولوجي دافئ على درجة حرارة ٣٧ مئوية، ثم تجميع البويضات بعد سحبها بالسرنجة وفحصها تحت الميكروسكوب. حيث تم إستخدام البويضات عالية الجودة مورفولوجيا في عملية الانضاج المعملي بعد إضافة البيسلاين بتركيزات متدرجة في أربع معاملات (٠، ٠.١، ١.٠ و١٠ ميكرو مولر). وتم تتبع التطور الجنيني بعد اجراء عملية الاخصاب وزراعة الاجنة حتى اليوم الثامن للإخصاب. بالإضافه الى ذلك تم عد العدد الكلى لخلايا الجنين كامل التطور(البلاستوسيست) وكذلك العدد الكلى للخلايا الميتة فيما يعرف باختبار TUNNEL-HOECHST assay. وقد أظهرت النتائج زيادة نسبة الجنين كامل التطور لمرحلة البلاستوسيست (٣٨.٣٪) بشكل معنوي ($P\leq0.05$) بالمجموعة التي تم إضافة البيسلاين بتركيز ١.٠ ميكرو مولر مقارنة بالمجموعة الغير معاملة (٢٨.٧٪)، وبشكل غير معنوي بالمجموعات التي تم إضافة هذه المادة بتركيزات ٠.١ و١٠ ميكرو مولر (٣١.٥٪ و٣٢.٥٪ على التوالي). أظهرت النتائج أيضا زيادة في معدل فقس الأجنة في اليوم الثامن من التطور الجنينى في المجموعة التي تم إضافة البيسلاين بتركيز ١.٠ ميكرو مولر(٤٠.٥٪) مقارنة بالمجموعة الغير معاملة (٣٣.٣٪)، و المجموعة المضاف لها هذه المادة بتركيز ٠.١ (٣٢.٤٪). كما أظهرت النتائج زيادة معنوية ($P\leq0.05$) العدد الكلى لخلايا الجنين بالمجموعة التي تم إضافة البيسلاين بتركيز ١.٠ ميكرو مولر (١٥٠.٣ ± ٥.٠) مقارنة بالمجموعة الغير معاملة (١٢٢.٩ ± ٨.٩) والمجموعة المضاف لها بتركيز ١٠(١٢٨.١ ± ٦.٢)، وبشكل غير معنوي بالمجموعة التي تم إضافة هذه المادة بتركيزات ٠.١ ميكرو مولر (١٣٩.٤ ± ٥.٧). هذا وقد إنخفض العدد الكلى للخلايا الميتة بشكل معنوي ($P\leq0.05$) بالمجموعة التي تم إضافة البيسلاين بتركيز١.٠ ميكرو مولر (٣.٦ ± ٠.٦) مقارنة بالمجموعة الغير معاملة (٦.٤ ± ١.٢) والمجموعة المضاف لها بتركيز ١٠ (٧.١ ± ١.٧)، وبشكل غير معنوي بالمجموعة التي تم إضافة هذه المادة بتركيزات ٠.١ ميكرو مولر (٥.٣ ± ١.٠). بناء على نتائج هذه الدراسة، يُوصى بإضافة مادة البيسلاين في بيئة الانضاج المعملي وذلك لتأثيرها الإيجابي في تعزيز نمو وتطور أجنة الأبقار وفقسها (خروج الجنين خارج الطبقة الشفافة) بالإضافة الى تأثيره المفيد في تحسين جودة الأجنة المنتجة معملياً من خلال تثبيط موت الخلايا الجنينية عند اضافته بتركيز١.٠ ميكرو مولر.