2022 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 12, Issue 6: 324-332; November 25, 2022 ISSN 2228-7701

DOI: https://dx.doi.org/10.51227/ojafr.2022.43

THE ROLE OF ANTIOXIDANTS IN IMPROVING THE QUALITY OF BOVINE EMBRYOS PRODUCED IN VITRO

Erni DAMAYANTI[1], Herry SONJAYA[2], Sudirman BACO[2], Hasbi HASBI[2]

[1]*Graduate School of agriculture Science, Hasanuddin University, Makassar 90245, Indonesia*
[2]*Department of Animal Production, Faculty of Animal Science, Hasanuddin University, Makassar 90245, Indonesia*

Email: hasbi_fapetunhas@yahoo.com

Supporting Information

REVIEW ARTICLE
PII: S222877012200043-12
Received: August 11, 2022
Revised: November 10, 2022
Accepted: November 11, 2022

ABSTRACT: Antioxidants are molecular compounds that can give their electron structure to free radical molecules without disturbing them and can break the chain of free radical compounds. Antioxidants that can be used include enzymatic and non-enzymatic antioxidants. Supplementation of antioxidants into maturation mediums or cultures with the right concentration can efficiently improves oocyte maturation, cell division, and embryo quality in bovine. Enzymatic and non-enzymatic antioxidant supplementation of the maturation medium increase the number of oocytes that reach metaphase II (MII). Furthermore, the supplementation of both antioxidants in maturation and culture mediums are also able to increase cell division and embryo that reaches blastocyst. Non-enzymatic antioxidant supplementation is more effective than enzymatic antioxidants in improving the maturation and division of cells in the production of bovine embryos in vitro. In conclusion, non-enzymatic antioxidant supplementation is more effective in supporting embryonic development in vitro.

Keywords: Bovine, Embryo, Enzymatic antioxidants, Non-enzymatic antioxidants

INTRODUCTION

Researches on in vitro embryo production (IVEP) in bovine continue to develop with improvements to support their success. Factors that often lead to unsuccessful embryo production include the quality of oocytes, sperm, and suboptimal culture conditions, which lead to oxidative stress. Cellular oxidative stress can cause a variety of cell damage, including lipid peroxidases on the membrane, oxidation of amino acids, death, and cell necrosis, thereby reducing embryo viability (Kitagawa et al., 2004; Su et al., 2019). Reactive oxidative species (ROS) at normal limits play a role in aiding in the process of proliferation and differentiation (Valko et al., 2007; Sharifi-Rad et al., 2020). A deeper number of cells, however, can result in cell damage and death (Agarwal et al., 2005; Pizzino et al., 2017). Oocytes or embryos cultured in vitro undergo manipulation of environmental conditions which may lead to the increase of ROS level, causing a decrease in cell regulation (Yu et al., 2014; Agarwal et al., 2022). Most ROS is formed from the process of degradation of long-chain fatty acids resulting from the process of respiration of mitochondria and peroxisomes (Ganguli et al., 2019). To prevent excessive ROS formation during the period of oocyte or embryo culture, several studies have been carried out including the addition of antioxidants to the medium used. Antioxidants are compounds with a molecular structure that can give their electron structure to free radical molecules without being disturbed and can break the chain of free radical compounds (Budani and Tiboni, 2020). Antioxidants play a vital role in reducing the activity of hydrogen peroxide (H_2O_2) levels and reactive oxygen species (ROS) (Kurutas, 2016). Antioxidant substances in cells present at low concentrations and significantly reduce or prevent oxidation of oxidizable substrates (Kurutas, 2016). The antioxidants used are divided into two, namely enzymatic and non-enzymatic antioxidants. Antioxidant enzymes include superoxide dismutase (SOD) (Lee et al., 2011), katalase (Ali et al., 2003) and glutathione ethyl ester (GSH-OEt) (García-Martínez et al., 2020), while non-enzyme antioxidants are sericin (Satrio et al., 2022), vitamin K2 (Baldoceda-Baldeon et al., 2014) and vitamin C (Sovernigo et al., 2017).

Enzymatic and non-enzymatic antioxidants work synergistically to support each other in protecting cells and organ systems of the body against free radical damage (Walczak-Jedrzejowska et al., 2013; Kurutas, 2016). Researches on the addition of media antioxidant, such as catalase are still regarded as controversial (Lane et al., 2001; Ali et al., 2003). Some reports, however, stated that the addition of catalase to embryo produced in vitro can reduce intracellular ROS levels and the rate of apoptosis in bovine embryos (Rocha-Frigoni et al., 2016). Cells naturally have physiological mechanisms to inhibit the formation of excessive free radicals including specific enzymes that control their intracellular levels (Liu et al., 2016). Several studies have been conducted regarding the use of antioxidants in maturation media or cultures to improve the quality of oocytes and embryos produced. The primary thing to know about the use of antioxidants is the right concentration of supplements so that it can effectively support embryonic development. This paper will outline the use of some enzymatic and non-enzymatic antioxidants in maturation media and bovine cultured in vitro.

Citation: Damayanti E, Sonjaya H, Baco S, Hasbi H (2022). The role of antioxidants in improving the quality of bovine embryos produced in vitro. *Online J. Anim. Feed Res.*, 12(6): 324-332. DOI: https://dx.doi.org/10.51227/ojafr.2022.43

Antioxidant supplementation to the maturation medium towards the degree of maturity of bovine oocytes

The first stage in the in vitro production of embryos is the maturation of oocytes (Strączyńska et al., 2022). Oocyte maturation is very important as an early stage and it is crucial to obtain quality oocytes before fermentation (Sirait et al., 2021). Mature oocytes are oocytes that reach the stage of metaphase II (MII) (De Vos et al., 1999; Parrella et al., 2019). Proper use of antioxidants can improve oocytes maturation and embryonic development (Budani and Tiboni, 2020; Rodríguez-Varela and Labarta, 2020). One of the important things to know is the proper concentration of antioxidants in the in vitro maturation medium to increase the number of oocytes that reach MII. Some studies that have been conducted regarding antioxidant supplementation on in vitro maturation media of bovine are presented in Table 1.

Table 1 - Antioxidant supplementation towards the degree of in vitro maturation of bovine oocytes

Name of antioxidant	Concentration	Number of Oocytes	Maturation Rate (MII) (%)	References
Non-antioxidant	-	154	75.3±0.8	(Satrio et al., 2022)
	-	120	76.7±1.7	(Rocha-Frigoni et al., 2016)
	-	59	86.4±2.7	(Sovernigo et al., 2017)
	-	101	84.2±2.3	(Wang et al., 2007)
	-	165	76.04±1.22	(Huang et al., 2018)
Average		119.8±38.11	79.73±4.62	
Enzymatic antioxidants				
Catalase	100 UI	104	80.6±5.2	(Rocha-Frigoni et al., 2016)
Serisin	0.1%	120	87.0±3.1	(Satrio et al., 2022)
Average		112±8	83.8±3.2	
Non-Enzymatic antioxidants				
Quercetin	2 µM	60	90.01±5.0	(Sovernigo et al., 2017)
Cysteamine	100 µM	62	91.9±2.9	(Sovernigo et al., 2017)
Carnitine	0.5 mg/mL	61	88.5±2.5	(Sovernigo et al., 2017)
Vitamin C	50 mg/mL	61	90.2±0.7	(Sovernigo et al., 2017)
Resveratrol	2 µM	61	91.8±3.0	(Sovernigo et al., 2017)
Green tea polyphenols (GTP)	10 µM	107	87.5 ±2.1	(Wang et al., 2007)
Epigallocatechin-3-gallate (EGCG)	50 µM	180	88.04±2.49	(Huang et al., 2018)
Average		84.57±42.07	89.71±1.63	

Table 1 shows that antioxidant supplementation of both enzymatic and non-enzymatic antioxidants increased the number of oocytes that reach MII. The average oocyte that reached the MII stage without antioxidant supplementation was 79%, while the enzymatic and non-enzymatic antioxidants were 83% and 89% respectively. The addition of enzymatic antioxidants increased the MII stage by 4%, while the non-enzymatic antioxidants could increase the MII stage by 10%. This suggests that non-enzymatic antioxidants have more potential to preserve oocytes during maturation than enzymatic antioxidants. Based on Table 1, the addition of antioxidants of both enzymatic and non-enzymatic could inhibit the formation of free radicals. Antioxidants are formed as a defense mechanism against toxic reactive oxygen species (ROS). These pathways include photorespiration pathways, enzymatic and non-enzymatic pathways, regulation of corresponding response genes, and anatomical pathways (Kumar et al., 2014). Antioxidant system to ward off free radicals is naturally formed by the body itself (Vona et al., 2021). If the amount of ROS exceeds the amount of antioxidants in the cell, the excess will attack the lipid, protein, and DNA components, resulting in a damage called oxidative stress (Pham-Huy et al., 2008).

Some previous studies have reported enzymatic antioxidant supplementation such as catalase (Rocha-Frigoni et al., 2016) and sericin (Satrio et al., 2022). Catalase plays a role in reducing the production of H_2O_2 produced in extracellular environments (Nandi et al., 2019). The use of catalase becomes a strategy to prevent the formation of intracellular ROS at the end of maturation (Circu and Aw, 2010). Espinosa-Diez et al. (2015) reported an evidence that ROS affects cellular function by controlling the production and activation of biologically active substances, while Isobe et al. (2012) stated that sericin has a high hydroxyl amino acid (serine) that possess a potential as an antioxidant. Furthermore, Dash et al. (2008) reported that sericin can lower ROS levels in cultures by preventing H_2O_2 induced oxidative stress.

Non-enzymatic antioxidant supplementation such as quercetin, cysteamine, carnitine, vitamin C and resveratrol has been reported by Sovernigo et al. (2017). In addition, there are also green tea polyphenols (GTP) (Wang et al., 2007) and epigallocatechin-3-gallate (EGCG) (Huang et al., 2018). Quercetin is one of flavonoids which has antioxidant capacity in reducing ROS levels, protects mitochondrial functions and regulates the defense system of enzymatic antioxidants (Sameni et al., 2018). Cysteamine is an amino thiol ($HSCH_2CH_2NH_2$) formed in the body from the degradation of coenzyme A during the formation of pantetheine, which is eventually hydrolyzed into cysteine and pantothenic acid

Citation: Damayanti E, Sonjaya H, Baco S, Hasbi H (2022). The role of antioxidants in improving the quality of bovine embryos produced in vitro. *Online J. Anim. Feed Res.*, 12(6): 324-332. DOI: https://dx.doi.org/10.51227/ojafr.2022.43

(Sameni et al., 2018). Cysteamine is able to increase glutathione (GSH) production during the maturation process by reducing cystine to cysteine (Gasparrini et al., 2003). Cysteamine may increase intracellular GSH levels in oocytes and embryos in the absence of cumulus cell monolayers (de Matos et al., 2002). Carnitine is a quaternary ammonium synthesized from the amino lysine and methionine acids and acts as an antioxidant that neutralizes ROS and protects cell organelles (Ye et al., 2010; Mishra et al., 2016). L-carnitine supplementation during maturation improves the development of bovine embryos from less competent meiotic oocytes and accelerates the formation of blastocysts of more competent oocytes (Knitlova et al., 2017). 50 mg/mL vitamin C in maturation media could increase oocytes to reach metaphase II up to 90.2% and the addition of 2 µM resveratrol could increase oocytes to reach metaphase II in bovine up to 91.8% (Sovernigo et al., 2017). Likewise, Kere et al., (2013) reported that the addition of 50 mg/mL vitamin C to swine maturation media can increase the maturation of oocytes to reach metaphase II up to 79.8%. Vitamin C is no longer only an enzymatic cofactor, antioxidant, and extracellular promoter of matrix formation by stabilizing collagen structures, but it also has the potential to modulate gene expression (Ivanyuk et al., 2015; Duran et al., 2019). Another study carried out on goats stated that the addition of 1 µM of resveratrol in maturation media could increase oocytes to reach metaphase II up to 93% (Piras et al., 2019). Wang et al. (2014) found that resveratrol triggers the maturation of oocyte nuclei due to its antioxidant properties and can induce progesterone secretion. Meanwhile, GTP supplementation on maturation media also improves the quality of oocytes at the maturation level (Wang et al., 2007). The antioxidant effects of tea polyphenols are thought to be related to their ability to stimulate antioxidant defense metabolism through cell cycle regulation that relies on redox-regulated transcription factors and mitogen-activated protein kinase (MAPK) (Jiao et al., 2003; Williams et al., 2004). Epigallocatechin-3-gallate (EGCG) also has the potential to increase MII in in vitro cultured bovine oocytes (Huang et al., 2018). EGCG is the main component of polyphenol catechins in green tea and is considered one of the components with the most bioactive compounds due to its powerful antioxidant properties (Roychoudhury et al., 2017). Reduction of intracellular ROS levels after EGCG supplementation can contribute to direct radical clearance activity, or increase EGCG-induced antioxidant enzyme activity (Huang et al., 2018).

Research by Sovernigo et al. (2017) revealed that cysteamine and resveratrol are non-enzymatic antioxidants that are best at increasing oocyte maturation in cattle, which is around 91%. This proves that antioxidant supplementation on maturation media is effective in reducing ROS and in increasing GSH in bovine oocytes in vitro. The right amount of antioxidant supplementation can increase cytoplasmic maturation during oocyte maturation and can contribute further to protect embryos from oxidative attacks in their early development.

Antioxidant supplementation in the medium on embryos ability to reach the blastocyst stage

The ability of embryo development after fertilization is influenced by various factors including oocyte competence, spermatozoa and the ability to divide to blastocyst (Colaco and Sakkas, 2018). The occurrence of deviations at the beginning of embryogenesis can cause disruptions in embryonic development and survival (Feugang et al., 2009). Improving the quality of embryos in the production of bovine embryos in vitro is very important (Demetrio et al., 2020). To prevent metabolic disorders that can occur during the culture process, it is necessary to add antioxidants to the media (Kurutas, 2016). Different types of antioxidants that can be used in embryo production can be seen in Table 2.

Table 2 shows that the addition of antioxidants, both enzymatic and non-enzymatic, was able to increase the number of splitting embryos and blastocyst in bovine cultured in vitro. Enzymatic antioxidant supplementation increased about 5% of splitting embryos and 4% of blastocyst of control. Likewise, the use of non-enzymatic antioxidants could increase about 10% of the control splitting embryo and about 16% of embryos reached blastocyst of control (without antioxidant suplementation.

Antioxidants can be added to maturation media, sperm washing and culture, and have been shown to have an effect on improving embryo quality. The use of antioxidant enzymes had an average of about 30% of embryos reaching blastocyst from splitting embryos, while the use of non-enzymatic antioxidants had an average of about 42% of embryo reaching blastocyst. The use of enzymatic antioxidants, namely superoxide dismutase (SOD), in bovine culture media (Lee et al., 2011) and the use of enzymatic antioxidant catalase (CAT) in maturation media (Ali et al., 2003) have been done. Superoxide dismutase (SOD) is responsible for converting oxygen free radicals into H_2O_2, which is highly toxic and immediately discarded (Kürüm et al., 2019). In the in vivo culture, sperm and oocyte spasms in the oviduct ducts are involved in the regulation of SOD (Yan et al., 2014). Supplementation of 300 U/mL SOD in the culture medium increased blastocyst production by 33.6% (Lee et al., 2011). Superoxide dismutase (SOD) catalyzes the distillation of superoxide into oxygen and hydrogen peroxide. Antioxidant is important for every cell exposed to oxygen as an antioxidant chelator which is effective in transferring metal ions and can reduce free radicals such as oxygen and nitrogen and inhibit the generation of primary oxygen radicals and oxidation (Kostyuk et al., 2004). Catalase (CAT) can be the dominant antioxidant in the early stages of bovine folliculogenesis (Gupta et al., 2011).

Non-enzymatic antioxidants used in an effort to improve embryonic development in bovine are glutathione (Itahashi, 2022), quercetin, cysteamine, carnitine, vitamin C, resveratrol (Sovernigo et al., 2017), anethole (Anjos et al., 2019), *Phellodendron amurense* extract and melatonin (Do et al., 2017), β-mercaptoethanol (β-ME) (Hosseini et al., 2009), vitamin K2 (Baldoceda-Baldeon et al., 2014), flavonoid 3,4-dihydroxy flavone (3,4-DHF) (Lee et al., 2011), and green tea polyphenols (GTP) (Wang et al., 2007). The use of such antioxidants has been shown to be able to increase embryos to reach blastocyst by 42% of the dividing cells.

Citation: Damayanti E, Sonjaya H, Baco S, Hasbi H (2022). The role of antioxidants in improving the quality of bovine embryos produced in vitro. *Online J. Anim. Feed Res.*, 12(6): 324-332. DOI: https://dx.doi.org/10.51227/ojafr.2022.43

Table 2 - Antioxidant supplementation on the development of bovine embryos in vitro

Name of antioxidant	Concentration	Media	Number of oocytes	Cleavage rate (%)	Blastocyst (%)	References
	-		114	47.4±4.0	15.8±1.3	(Itahashi, 2022)
	-		142	85.9±4.1	47.2±2.7	(Sovernigo et al., 2017)
	-		130	-	31.5	(Lee et al., 2011)
Non-antioxidant	-		-	69	20	(Ali et al., 2003)
	-		-	87.4±1.9	25.4±2.8	(Anjos et al., 2019)
	-		36	89.4±6.2	25.0±0.0	(Do et al., 2017)
	-				23	(Baldoceda-Baldeon et al., 2014)
Average				75.82	26.84	
Enzymatic antioxidants						
Superoxide dismutase (SOD)	300 U/mL	Culture	124	-	33.6	(Lee et al., 2011)
Catalase	127 U/mL	Maturation	-	81	28	(Ali et al., 2003)
Average			-	81	30.8	
Non-enzymatic antioxidants						
Glutathione	1 µM	Sperm washing	119	82.4 ±3.7	39.5±2.3	(Itahashi, 2022)
Quercetin	2 µM	Maturation	142	85.9±4.5	53.5±3.9	(Sovernigo et al., 2017)
Cysteamine	100 µM	Maturation	143	86.7±7.2	52.4±2.7	(Sovernigo et al., 2017)
Carnitine	0.5 mg/mL	Maturation	144	84.7±6.5	54.2±3.1	(Sovernigo et al., 2017)
Vitamin C	50 mg/mL	Maturation	142	85.9±7.1	52.1±3.1	(Sovernigo et al., 2017)
Resveratrol	2 µM	Maturation	142	88.7±8.4	54.2±4.0	(Sovernigo et al., 2017)
Anethole	30 µg/ml	Maturation	-	86.2±5.8	32.1±4.7	(Anjos et al., 2019)
Phellodendron amurense extract	0.01 µL	Culture	36	85.6±6.2	30.6±0.9	(Do et al., 2017)
Melatonin	0.01 µL	Culture	114	84.2±7.4	35.7±5.1	(Do et al., 2017)
β-mercaptoethanol (β-ME)	100 µL	Culture	1250	92.0 ± 3.1	40.1±1.1	(Hosseini et al., 2009)
Vitamin K2	0.50 mM	Culture	-	-	31	(Baldoceda-Baldeon et al., 2014)
3,4-dihydroxyflavone (3,4-DHF)	10 µM	Culture	129	-	44.2	(Lee et al., 2011)
Green tea polyphenols (GTP)	15 µM	Maturation	-	76	38.1	(Wang et al., 2007)
Average				85.3	42.9	

Citation: Damayanti E, Sonjaya H, Baco S, Hasbi H (2022). The role of antioxidants in improving the quality of bovine embryos produced in vitro. *Online J. Anim. Feed Res*., 12(6): 324-332. DOI: https://dx.doi.org/10.51227/ojafr.2022.43

1 µM GSH supplementation on sperm washing media caused the number of the dividing cell to reach about 89% and was able to reach blastocyst of about 39% of the dividing cells. GSH not only reduces the formation of ROS, but also reduces the formation of disulfide bonds (Oikawa et al., 2018). The stimulating effect of GSH on embryonic development is mainly on the reduction of effective disulfide bonds in spermatozoa (Mayor et al., 2001). Itahashi et al. (2022) explained that GSH is important for decondensation of sperm chromatin after sperm penetration in oocytes, destabilization and replacement of protamines by oocyte-derived histones (Caglar et al., 2005), and the development of sperm nuclei into the male pronucleus (Canel et al., 2017).

Resveratrol and quercetin are secondary plant metabolites found in vegetables, fruits, flowers and seeds, and are flavonoid compounds produced by the interaction of plants and microorganisms to protect themselves against fungal and bacterial infections (Kwak et al., 2012; Kang et al., 2013). Cysteamine (CYS) supplementation in maturation media increases intracellular levels of GSH in mature oocytes and embryonic development rate (de Matos et al., 2002; Alsalim, 2020). Cysteamine (CYS) is one of the thiol compounds which has been successfully used in the maturation of oocytes in vitro by acting as a ROS collector and maintaining cellular redox balance for proper conditions in embryonic development (Gasparrini et al., 2000).

Vitamin C supplementation in maturation medium has a positive impact on embryo division by about 85% and also increases blastocyst by about 54% of the dividing cells (Sovernigo et al., 2017). Vitamin C (ascorbic acid) by virtue of its chemical structure is an electron donor and therefore a reducing agent with two different biochemical roles, namely as antioxidant and enzymatic cofactor (Belin et al., 2009; Khazaei and Aghaz, 2017). Due to its antioxidant properties, vitamin C is able to protect cells from ROS (Padayatty et al., 2003).

The rate of cell division with the use of anethole antioxidants was quite high at around 86.2%. It becomes an alternative because anethole has the opportunity to be used as a fairly good antioxidant in supporting embryonic development. Furthermore, supplementation of 30 µg/mL anethole in maturation medium increased blastocyst and decreased ROS concentration (Anjos et al., 2019). Anethone compounds can support glutathione (GSH) synthesis to fight or suppress oxidative and can also simultaneously increase GSH which is considered to be a homocysteine-lowering agent (Giustarini et al., 2014). Do et al. (2017) reported that the utilization of plants extraction containing antioxidants can be done to improve the quality of blastocyst. 0.01 µg/mL of *Phellodendron amurense* extract in the culture medium improved the quality of embryos produced, cell division and blastocyst as well as allows the repair of poor quality embryo (Do et al., 2017).

Melatonin 0.01 µL supplementation in culture media was able to achieve cleavage by 84% and blastocyst by 35% (Do et al., 2017). Exogenous melatonin increases the speed of blastocysts in fresh embryos and vitrification by various mechanisms improves embryonic development (Wang et al., 2014). Other mechanisms may use melatonin to induce changes in the production of antioxidant enzymes and oxidative substrates, as well as regulate gene expression associated with oxidative stress responses. The use of melatonin can be a supporting tool in embryo production in vitro to reduce negative influences during culture (Mehaisen et al., 2015; Truong and Gardner, 2020). Melatonin is a unique antioxidant compared to other antioxidants not only because of its prevalence and the various mechanisms involved in the transmission of signals it carries, but also because of its anti-oxidant activity (Chrustek and Olszewska-Słonina, 2021).

β-mercaptoethanol (β-ME) is an antioxidant that can be added to the culture medium to increase the embryo to reach blastocyst on the eighth day of the culture. Hosseini et al (2009) stated that β-mercaptoethanol (βME) has a low molecular weight thiol compound, and is often used to increase antioxidant capacity in embryos through increased levels of intracellular reactive such as glutathione (GSH) (Takahashi et al., 2002).

Vitamin K2 is known to have antioxidant and anti-inflammatory properties that can act as a powerful protective molecule (Shandilya et al., 2021). Baldoceda-Baldeon et al. (2014) reported that the addition of 0.5 mM of vitamin K2 into the culture medium could increase the production of blastocyst percentage by 8.6% in cattle. Vitamin K2 plays a role of an electron carrier in the mitochondrial electron transport chain complex, resulting in more efficient use of oxygen and ATP production (Vos et al., 2012). Other studies have also reported that vitamin K2 supplementation after geonomics geonomic activation of day 3 and 7 may improve embryo development competence (Sefid et al., 2017).

3,4-dihydroxyflavone (3,4-DHF) is a group of flavonoids reported to have anti-apoptotic and anti-oxidant activity (Hossain et al., 2014). 3,4-DHF 10 µM supplementation in culture media could increase the number of blastocyst about 44% of the dividing cells (Lee et al., 2011). Another study also revealed that 3,4-DHF significantly reduced ROS content and apoptotic cell count as well as increased expression of antioxidant and anti-apoptotic genes to increase yak's in vitro development capacity (Xiong et al., 2014).

Green tea polyphenol (GTP) is a source of polyphenols from green tea that acts as a source of antioxidants and has a molocular mechanism that plays a role in preventing various diseases (Ding et al., 2017). The addition of 15 µM of green tea polyphenol (GTP) in maturation medium efficiently improves the fertilization competence and development of bovine embryos, and this increase correlates with an increase in intracellular GSH concentrations of embryos (Wang et al., 2007). The antioxidant effects of tea polyphenols are also thought to be related to their ability to stimulate antioxidant defense metabolism through redox-regulated transcription factors and protein kinase activated by mitogen cellular cycle regulation (Yan et al., 2020).

Some studies have revealed that non-enzymatic antioxidant supplementation in embryo production in vitro is more effective than enzymatic antioxidants. Enzymatic antioxidants increased the embryo to reach blastocyst by about 50% of the dividing cells while the enzymatic antioxidant was about 30%.

Citation: Damayanti E, Sonjaya H, Baco S, Hasbi H (2022). The role of antioxidants in improving the quality of bovine embryos produced in vitro. *Online J. Anim. Feed Res.*, 12(6): 324-332. DOI: https://dx.doi.org/10.51227/ojafr.2022.43

Antioxidant mechanisms in cells

High level of ROS which exceed the physological range causes cell imbalances and can decrease the survival rate, triggering apoptosis in oocytes (Khazaei and Aghaz, 2017). Antioxidants are defensive factor against Oxidative Species (OS) induced by ROS (Bhattacharyya et al., 2014). During the embryo culture process, the addition of antioxidants to the culture medium can reduce ROS so that the quality of oocytes increases and the apoptotic factor decreases (Khazaei and Aghaz, 2017).

Antioxidants in cells are distinguished into 2 groups, namely enzymatic (primary) and non-enzymatic (secondary) antioxidants. Enzymatic antioxidants are also referred to as antioxidants that work by preventing the formation of new free radical compounds (Zulaikhah, 2017). Enzymatic antioxidants include the enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). The mechanism of action of enzymatic antioxidants is to catalyze the extermination of free radicals in cells (Azat Aziz et al., 2019). Chain-breaking antioxidants are small molecules that can receive or give electrons to or from free radical so as to form stable compounds (Lü et al., 2010).

Non-enzymatic antioxidants are also called preventive defense antioxidants that work by cutting or capturing chain oxidation reactions from free radicals so that they will not react (Nimse and Pal, 2015). Non-enzymatic mechanisms consist of: 1) glutathione which is a very important antioxidant and is abundant in the cytoplasm (Lushchak, 2012); 2) bilirubin which is an antioxidant found in the blood (Sedlak et al., 2009); 3) melatonin which is a type of powerful antioxidant (Tarocco et al., 2019); and 4) coenzyme Q which acts as an antioxidant that dissolves inside the fat membrane (Littarru and Tiano, 2007). In addition, vitamin C, β-carotene, flavonoids and albumin are found in plants (Kumar and Pandey, 2013). One of the flavonoid components of plants that can function as antioxidants is a natural coloring agent called anthocyanins (He and Monica Giusti, 2010). Non-enzymatic antioxidants have great value as additives for pharmaceuticals and others (Kumar et al., 2014).

CONCLUSION

Enzymatic and non-enzymatic antioxidant supplementation on the medium increases the number of oocytes that reach metaphase II (MII), cell division and blastocyst. Non-enzymatic antioxidant supplementation is more effective than enzymatic antioxidants in supporting the development of bovine embryos produced in vitro. As a suggestion, antioxidant supplementation in maturation media or culture media with the use of non-enzymatic antioxidants

DECLARATIONS

Corresponding author

E-mail: hasbi_fapetunhas@yahoo.com

Author's contribution

E. Damayanti collected and drafted the manuscript, formatted it, and approved the final manuscript. H. Sonjaya, S. Baco and H. Hasbi approved the final manuscript.

Conflict of interests

The authors declared that they have no conflict of interest.

REFERENCES

Agarwal A, Gupta S, and Sharma RK (2005). Role of oxidative stress in female reproduction. Reproductive Biology and Endocrinology, 3: 1-21. DOI: https://doi.org/10.1186/1477-7827-3-28

Agarwal A, Maldonado Rosas I, Anagnostopoulou C, Cannarella R, Boitrelle F, Munoz LV, et al. (2022). Oxidative stress and assisted reproduction: a comprehensive review of its pathophysiological role and strategies for optimizing embryo culture environment. Antioxidants. 11(3): 477. DOI: https://doi.org/10.3390/antiox11030477

Ali AA, Bilodeau JF, and Sirard MA (2003). Antioxidant requirements for bovine oocytes varies during in vitro maturation, fertilization and development. Theriogenology, 59:939-949. DOI: https://doi.org/10.1016/S0093-691X(02)01125-1

Alsalim H (2020). The effect of antioxidant cysteamine with ascorbic acid on in vitro fertilization in cows. International Journal of Pharmaceutical Research, 12. DOI: https://doi.org/10.31838/ijpr/2020.sp2.459

Anjos JC, Aguiar FLN, Sá NAR, Souza JF, Cibin FWS, Alves BG, et al. (2019). Anethole improves blastocysts rates together with antioxidant capacity when added during bovine embryo culture rather than in the in vitro maturation medium. Zygote, 16-19. DOI: https://doi.org/10.1017/S0967199419000443

Azat Aziz M, Shehab DA, and Abdulrazak MA (2019). Antioxidant categories and mode of action. Antioxidants. DOI: https://doi.org/10.5772/intechopen.8354

Baldoceda-Baldeon LM, Gagné D, Vigneault C, Blondin P, and Robert C (2014). Improvement of bovine in vitro embryo production by vitamin k2 supplementation. Reproduction, 5:489-497. DOI: https://doi.org/10.1530/REP-14-0324

Belin S, Kaya F, Burtey S, and Fontes M (2009). Ascorbic acid and gene expression: another example of regulation of gene expression by small molecules? Current genomics, 1:52-57. DOI: https://doi.org/10.2174/138920210790217936

Bhattacharyya A, Chattopadhyay R, Mitra S, and Crowe SE (2014). Oxidative stress: An essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiological Reviews, 2:329-354. DOI: https://doi.org/10.1152/physrev.00040.2012

Budani MC. and Tiboni GM (2020). Effects of supplementation with natural antioxidants on oocytes and preimplantation embryos. Antioxidants, 7:1-25. DOI: https://doi.org/10.3390/antiox9070612

Caglar GS, Hammadeh M, Asimakopoulos B, Nikolettos N, Diedrich K, and Al-Hassani S (2005). In vivo and in vitro decondensation of human sperm and assisted reproduction technologies. In Vivo, 3:623-630.

Canel NG, Bevacqua RJ, Hiriart MI, Rabelo NC, de Almeida CLS, Romanato M, et al (2017). Sperm pretreatment with heparin and L-glutathione, sex-sorting, and double cryopreservation to improve intracytoplasmic sperm injection in bovine. Theriogenology, 93:62-70. DOI: https://doi.org/10.1016/j.theriogenology.2016.12.018

Chrustek A and Olszewska-Słonina D (2021). Melatonin as a powerful antioxidant. Acta Pharmaceutica, 3:335-354. DOI: https://doi.org/10.2478/acph-2021-0027

Circu ML and Aw TY (2010). Reactive oxygen species, cellular redox systems, and apoptosis. Free Radical Biology and Medicine. 48(6):749-62. DOI: https://10.1016/j.freeradbiomed.2009.12.022

Colaco S. and Sakkas D (2018). Paternal factors contributing to embryo quality. Journal of Assisted Reproduction and Genetics, 11:1953-1968. DOI: https://doi.org/10.1007/s10815-018-1304-4

Dash R, Acharya C, Bindu PC, and Kundu SC (2008). Antioxidant potential of silk protein sericin against hydrogen peroxide-induced oxidative stress in skin fibroblasts. Journal of Biochemistry and Molecular Biology, 41, 3:236-24. DOI: https://doi.org/10.5483/bmbrep.2008.41.3.236

De Matos DG, Herrera C, Cortvrindt R, Smitz J, Van Soom A, Nogueira D, et al (2002). Cysteamine supplementation during in vitro maturation and embryo culture: A useful tool for increasing the efficiency of bovine in vitro embryo production. Molecular Reproduction and Development, 2:203-209. DOI: https://doi.org/10.1002/mrd.10087

De Vos A, Van De Velde, H, Joris H, and Van Steirteghem A (1999). In-vitro matured metaphase-I oocytes have a lower fertilization rate but similar embryo quality as mature metaphase-II oocytes after intracytoplasmic sperm injection. Human Reproduction, 7:1859-1863. DOI: https://doi.org/10.1093/humrep/14.7.1859

Demetrio DGB, Benedetti E, Demetrio CGB, Fonseca J, Oliveira M, Magalhaes A, et al (2020). How can we improve embryo production and pregnancy outcomes of Holstein embryos produced in vitro? (12 years of practical results at a California dairy farm). Animal Reproduction, 3:1-13. DOI: https://doi.org/10.1590/1984-3143-AR2020-0053

Ding S, Jiang J, Yu P, Zhang G, Zhang G, and Liu X (2017). Green tea polyphenol treatment attenuates atherosclerosis in high-fat diet-fed apolipoprotein E-knockout mice via alleviating dyslipidemia and up-regulating autophagy. PLoS ONE, 8:1-12. DOI: https://doi.org/10.1371/journal.pone.0181666

Do GY, Kim JW, Park HJ, Yoon SB, Park JY, Yang SG, et al (2017). Native plants (*Phellodendron amurense* and *Humulus japonicus*) extracts act as antioxidants to support developmental competence of bovine blastocysts. Asian-Australasian Journal of Animal Sciences, 9:1245-1252. DOI: https://doi.org/10.5713/ajas.16.0985

Duran BO, Góes GA, Zanella BTT, Freire PP, Valente JS, Salomão RAS, et al (2019). Ascorbic acid stimulates the in vitro myoblast proliferation and migration of pacu (*Piaractus mesopotamicus*). Scientific Reports, 9:1-13. DOI: https://doi.org/10.1038/s41598-019-38536-4

Espinosa-Diez C, Miguel V, Mennerich D, Kietzmann T, Sánchez-Pérez P, Cadenas S, et al (2015). Antioxidant responses and cellular adjustments to oxidative stress. Redox Biology, 6:183-197. DOI: https://doi.org/10.1016/j.redox.2015.07.008

Feugang J. M, Camargo-Rodríguez O, and Memili E (2009). Culture systems for bovine embryos. Livestock Science, 121:141-149. DOI: https://doi.org/10.1016/j.livsci.2008.06.019

Ganguli G, Mukherjee U, and Sonawane A (2019). Peroxisomes and oxidative stress: Their implications in the modulation of cellular immunity during mycobacterial infection. Frontiers in Microbiology, 10:1-17. DOI: https://doi.org/10.3389/fmicb.2019.01121

García-Martínez T, Vendrell-Flotats M, Martínez-Rodero I, Ordóñez-León E.A, Álvarez-Rodríguez M, López-Béjar M, et al (2020). Glutathione ethyl ester protects in vitro-maturing bovine oocytes against oxidative stress induced by subsequent vitrification/warming. International Journal of Molecular Sciences, 20:1-26. DOI: https://doi.org/10.3390/ijms21207547

Gasparrini B, Neglia G, Di Palo R, Campanile G, and Zicarelli L (2000). Effect of cysteamine during in vitro maturation on buffalo embryo development. Theriogenology, 9:1537-1542. DOI: https://doi.org/10.1016/S0093-691X(00)00473-8

Gasparrini B, Sayoud H, Neglia G, De Matos DG, Donnay I, and Zicarelli L (2003). Glutathione synthesis during in vitro maturation of buffalo (*Bubalus bubalis*) oocytes: Effects of cysteamine on embryo development. Theriogenology, 60:943-952. DOI: https://doi.org/10.1016/S0093-691X(03)00098-0

Giustarini D, Paolo F, Anna S, Elena M, and Rossi R (2014). Anethole dithiolethione lowers the homocysteine and raises the glutathone levels in solid tissues and plasma of rats: a novel non-vitamin homocysteine-lowering agent. Biochemechial Pharmacology, 89:246-254. DOI: https://doi.10.1016/j.bcp.2014.03.005

Gupta S, Choi A, Hope YY, Czerniak SM, Holick EA, Paolella LJ, et al (2011). Fluctuations in total antioxidant capacity, catalase activity and hydrogen peroxide levels of follicular fluid during bovine folliculogenesis. Reproduction, Fertility and Development. 23(5):673-80. DOI: https://doi.org/10.1071/RD10270.Fluctuations

He J and Monica Giusti M (2010). Anthocyanins: Natural colorants with health-promoting properties. Annual Review of Food Science and Technology, 1:163-187. DOI: https://doi.org/10.1146/annurev.food.080708.100754

Hossain MK, Choi HY, Hwang JS, Dayem AA, Kim JH, Kim YB, et al (2014). Antiviral activity of 3,4'-dihydroxyflavone on influenza a virus. Journal of Microbiology, 52:521-526. DOI: https://doi.org/10.1007/s12275-014-4212-z

Hosseini SM, Forouzanfar M, Hajian M, Asgari V, Abedi P, Hosseini L, et al (2009). Antioxidant supplementation of culture medium during embryo development and/or after vitrification-warming; Which is the most important? Journal of Assisted Reproduction and Genetics, 26:355-364. DOI: https://doi.org/10.1007/s10815-009-9317-7

Huang Z, Pang Y, Hao H, Du W, Zhao X, and Zhu H (2018). Effects of epigallocatechin-3-gallate on bovine oocytes matured in vitro. Asian-Australasian Journal of Animal Sciences, 31:1420-1430. DOI: https://doi.org/10.5713/ajas.17.0880

Isobe T, Ikebata Y, Onitsuka T, Wittayarat M, Sato Y, Taniguchi M, et al (2012). Effect of sericin on preimplantation development of bovine embryos cultured individually. Theriogenology, 78:747-752. DOI: https://doi.org/10.1016/j.theriogenology.2012.03.021

Itahashi M, Oikawa W, and Numabe T (2022). Effects of glutathione treatments during sperm washing and in vitro fertilization on the in vitro early development of embryos of Japanese Black cattle. Journal of Reproduction and Development. 68:1-14. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8872747/pdf/jrd-68-074.pdf

Ivanyuk D, Budash G, Zheng Y, Gaspar JA, Chaudhari U, Fatima A, et al (2015). Ascorbic acid-induced cardiac differentiation of murine pluripotent stem cells: Transcriptional profiling and effect of a small molecule synergist of Wnt/β-catenin signaling pathway. Cellular Physiology and Biochemistry, 36:810-830. DOI: https://doi.org/10.1159/000430140

Jiao H, Ye P, and Zhao BL (2003). Protective effects of green tea polyphenols on human HepG2 cells against oxidative damage of fenofibrate. Free Radical Biology and Medicine, 35:1121-1128. DOI: https://doi.org/10.1016/S0891-5849(03)00506-9

Kang JT, Kwon D, Park SJ, Kim SJ, Moon JH, Saadeldin IM, et al (2013). Quercetin improves the in vitro development of porcine oocytes by decreasing reactive oxygen species levels. Journal of Veterinary Science, 14:15-20. DOI: https://doi.org/http://dx.doi.org/10.4142/jvs.2013.14.1.15

Citation: Damayanti E, Sonjaya H, Baco S, Hasbi H (2022). The role of antioxidants in improving the quality of bovine embryos produced in vitro. *Online J. Anim. Feed Res.*, 12(6): 324-332. DOI: https://dx.doi.org/10.51227/ojafr.2022.43

Kasote DM, Katyare SS, Hegde MV, and Bae H (2015). Significance of antioxidant potential of plants and its relevance to therapeutic applications. International Journal of Biological Sciences, 11:982-991. DOI: https://doi.org/10.7150/ijbs.12096

Kere M, Siriboon C, Lo NW, Nguyen NT and Ju JC (2013). Ascorbic acid improves the developmental competence of porcine oocytes after parthenogenetic activation and somatic cell nuclear transplantation. Journal of Reproduction and Development, 59:78–84. DOI: https://doi.org/10.1262/jrd.2012-114

Khazaei M and Aghaz F (2017). Reactive oxygen species generation and use of antioxidants during in vitro maturation of oocytes. International Journal of Fertility and Sterility, 11:63-70. DOI: https://doi.org/10.22074/ijfs.2017.4995

Kitagawa Y, Suzuki K, Yoneda A, and Watanabe T (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, 62:1186-1197. DOI: https://doi.org/10.1016/j.theriogenology.2004.01.011

Knitlova D, Hulinska P, Jeseta M, Hanzalova K, Kempisty B, and Machatkova M (2017). Supplementation of L-carnitine during in vitro maturation improves embryo development from less competent bovine oocytes. Theriogenology, 102:16-22. DOI: https://doi.org/10.1016/j.theriogenology.2017.06.025

Kostyuk VA, Potapovich AI, Strigunova EN, Kostyuk TV, and Afanas'ev IB (2004). Experimental evidence that flavonoid metal complexes may act as mimics of superoxide dismutase. Archives of Biochemistry and Biophysics, 428:204-208. DOI: https://doi.org/10.1016/j.abb.2004.06.008

Kumar RR, Rao PH, Subramanian VV, and Sivasubramanian V (2014). Enzymatic and non-enzymatic antioxidant potentials of Chlorella vulgaris grown in effluent of a confectionery industry. Journal of Food Science and Technology, 51:322-328. DOI: https://doi.org/10.1007/s13197-011-0501-2

Kumar S and Pandey AD (2013). Flavonoids chemistry and biological activities of flavonoids: an overview shashank. The Scientific World Journal. 1-17. DOI: https://doi.org/https://downloads.hindawi.com/journals/tswj/2013/162750.pdf

Kürüm A, Karahan S, Kocamiş H, Çinar M, and Ergün E (2019). Determination of antioxidants in bovine oviduct epithelial cell culture isolated at different periods of the estrous cycle. Turkish Journal of Veterinary and Animal Sciences, 43:448-455. DOI: https://doi.org/10.3906/vet-1810-9

Kurutas EB (2016). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state. Nutrition Journal, 15: 1-22. DOI: https://doi.org/10.1186/s12937-016-0186-5

Kwak SS, Cheong SA, Jeon Y, Lee E, Choi KC, Jeung EB, et al (2012). The effects of resveratrol on porcine oocyte in vitro maturation and subsequent embryonic development after parthenogenetic activation and in vitro fertilization. Theriogenology, 78:86-101. DOI: https://doi.org/10.1016/j.theriogenology.2012.01.024

Lane M, O'Donovan MK, Squires EL, Seidel GE, and Gardner DK (2001). Protection against reactive oxygen species during mouse preimplantation embryo development: Role of EDTA, oxygen tension, catalase, superoxide dismutase and pyruvate. Molecular Reproduction and Development, 59:44-53. DOI: https://doi.org/10.1002/mrd.1006

Lee KS, Kim EY, Jeon K, Cho SG, Han YJ, Yang BC, et al (2011). 3,4-Dihydroxyflavone Acts as an Antioxidant and Antiapoptotic Agent to Support Bovine Embryo Development in Vitro. Journal of Reproduction and Development, 57: 127-134. DOI: https://doi.org/10.1262/jrd.10-029A

Littarru GP, and Tiano L (2007). Bioenergetic and antioxidant properties of coenzyme Q10: Recent developments. Molecular Biotechnology, 37:31-37. DOI: https://doi.org/10.1007/s12033-007-0052-y

Liu R, Xing L, Fu Q, Zhou GH, and Zhang WG (2016). A review of antioxidant peptides derived from meat muscle and by-products. Antioxidants, 5. DOI: https://doi.org/10.3390/antiox5030032

Lü JM, Lin PH, Yao Q, and Chen C (2010). Chemical and molecular mechanisms of antioxidants: Experimental approaches and model systems. Journal of Cellular and Molecular Medicine, 14: 840-860. DOI: https://doi.org/10.1111/j.1582-4934.2009.00897.x

Lushchak VI (2012). Glutathione homeostasis and functions: Potential targets for medical interventions. Journal of Amino Acids, 1-26. DOI: https://doi.org/10.1155/2012/736837

Mayor P, López-Béjar M, Rodríguez-González E, and Paramio MT (2001). Effects of the addition of glutathione during maturation on in vitro fertilisation of prepubertal goat oocytes. Zygote, 9:323-330. DOI: https://doi.org/10.1017/S096719940100137X

Mehaisen GMK, Saeed AM, Gad A, Abass AO, Arafa M, El-Sayed A, et al (2015). Antioxidant capacity of melatonin on preimplantation development of fresh and vitrified rabbit embryos: Morphological and molecular aspects. PLoS ONE, 10:1-16. DOI: https://doi.org/10.1371/journal.pone.0139814

Mishra A, Reddy IJ, Gupta PSP, and Mondal S (2016). l-carnitine mediated reduction in oxidative stress and alteration in transcript level of antioxidant enzymes in sheep embryos produced in vitro. Reproduction in Domestic Animals, 51:311-321. DOI: https://doi.org/10.1111/rda.12682

Nandi A, Yan LJ, Jana CK, and Das N (2019). Role of catalase in oxidative stress- and age-associated degenerative diseases. Oxidative Medicine and Cellular Longevity. DOI: https://doi.org/10.1155/2019/9613090

Nimse SB, and Pal D (2015). Free radicals, natural antioxidants, and their reaction mechanisms. RSC Advances, 5:27986-28006. DOI: https://doi.org/10.1039/c4ra13315c

Oikawa T, Itahashi T, Yajima R, and Numambe T (2018). Intracytoplasmic Sperm Injection Promotes Embryo Development. Journal of Reproduction and Development, 64:4.

Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee JH, Chen S, Corpe C, Levine M, Dutta A, and Dutta SK (2003). Vitamin c as an antioxidant: evaluation of its role in disease prevention. Journal of the american college of nutrition, 22:18-35. DOI: https://doi.org/10.1080/07315724.2003.10719272

Parrella A, Irani M, Keating D, Chow S, Rosenwaks Z, and Palermo GD (2019). High proportion of immature oocytes in a cohort reduces fertilization, embryo development, pregnancy and live birth rates following ICSI. Reproductive BioMedicine Online, 39: 580-587. DOI: https://doi.org/10.1016/j.rbmo.2019.06.005

Pham-Huy LA, He H and Pham-Huy C (2008). Free radicals, antioxidants in disease and health. International Journal of Biomedical Science, 4:89-96.

Piras A, Menéndez-blanco I, Soto-heras S, Catalá M, Izquierdo D, Bogliolo L, and Paramio M (2019). By Brilliant Cresyl Blue Staining. 65(2).

Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, et al (2017). Oxidative Stress: Harms and Benefits for Human Health. Oxidative Medicine and Cellular Longevity, 2017. DOI: https://doi.org/10.1155/2017/8416763

Rocha-Frigoni NAS, Leão BCS, Dall'Acqua PC, and Mingoti GZ (2016). Improving the cytoplasmic maturation of bovine oocytes matured in vitro with intracellular and/or extracellular antioxidants is not associated with increased rates of embryo development. Theriogenology, 86:1897-1905. DOI: https://doi.org/10.1016/j.theriogenology.2016.06.009

Rodríguez-Varela C and Labarta E (2020). Clinical application of antioxidants to improve human oocyte mitochondrial function: A review. Antioxidants, 9:1-30. DOI: https://doi.org/10.3390/antiox9121197

Citation: Damayanti E, Sonjaya H, Baco S, Hasbi H (2022). The role of antioxidants in improving the quality of bovine embryos produced in vitro. *Online J. Anim. Feed Res.*, 12(6): 324-332. DOI: https://dx.doi.org/10.51227/ojafr.2022.43

Roychoudhury S, Agarwal A, Virk G, and Cho CL (2017). Potential role of green tea catechins in the management of oxidative stress-associated infertility. Reproductive BioMedicine Online, 5:487-498. DOI: https://doi.org/10.1016/j.rbmo.2017.02.006

Sameni HR, Javadinia SS, Safari M, Amjad MHT, Khanmohammadi N, Parsaie H, and Zarbakhsh S (2018). Effect of quercetin on the number of blastomeres, zona pellucida thickness, and hatching rate of mouse embryos exposed to actinomycin D: An experimental study. International Journal of Reproductive BioMedicine, 2:101-108. DOI: https://doi.org/10.29252/ijrm.16.2.101

Satrio FA, Karja NWK, Setiadi MA, Kaiin EM, Gunawan M, Memili E, et al (2022). Improved maturation rate of bovine oocytes following sericin supplementation in collection and maturation media. Tropical Animal Science Journal, 1: 24-29. DOI: https://doi.org/10.5398/tasj.2022.45.1.24

Sedlak TW, Saleh M, Higginson DS, Paul BD, Juluri KR, and Snyder SH (2009). Bilirubin and glutathione have complementary antioxidant and cytoprotective roles. Proceedings of the National Academy of Sciences of the United States of America, 13:5171-5176. DOI: https://doi.org/10.1073/pnas.0813132106

Sefid F, Ostadhosseini S, Hosseini SM, Ghazvini Zadegan F, Pezhman M, and Nasr Esfahani MH (2017). Vitamin K2 improves developmental competency and cryo-tolerance of in vitro derived ovine blastocyst. Cryobiology, 77:34-40. DOI: https://doi.org/10.1016/j.cryobiol.2017.06.001

Shandilya S, Kesari KK, and Ruokolainen J (2021). Vitamin K2 modulates organelle damage and tauopathy induced by streptozotocin and menadione in SH-SY5Y cells. Antioxidants, 10:6. DOI: https://doi.org/10.3390/antiox10060983

Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, et al (2020). Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. Frontiers in Physiology, 11:1–21. DOI: https://doi.org/10.3389/fphys.2020.00694

Sirait B, Wiweko B, Jusuf AA, Iftitah D, and Muharam R (2021). Oocyte competence biomarkers associated with oocyte maturation: a review. Frontiers in Cell and Developmental Biology, 9. DOI: https://doi.org/10.3389/fcell.2021.710292

Sovernigo TC, Adona PR, Monzani PS, Guemra S, Barros FDA, Lopes FG, et al (2017). Effects of supplementation of medium with different antioxidants during in vitro maturation of bovine oocytes on subsequent embryo production. Reproduction in Domestic Animals, 52:561-569. DOI: https://doi.org/10.1111/rda.12946

Strączyńska P, Papis K, Morawiec E, Czerwiński M, Gajewski Z, Olejek A, et al (2022). Signaling mechanisms and their regulation during in vivo or in vitro maturation of mammalian oocytes. Reproductive Biology and Endocrinology, 20:1-12. DOI: https://doi.org/10.1186/s12958-022-00906-5

Su LJ, Zhang JH, Gomez H, Murugan R, Hong X, Xu D, et al (2019). Reactive Oxygen Species-Induced Lipid Peroxidation in Apoptosis, Autophagy, and Ferroptosis. Oxidative Medicine and Cellular Longevity. DOI: https://doi.org/10.1155/2019/5080843

Takahashi M, Nagai T, Okamura N, Takahashi H, and Okano A (2002). Promoting effect of β-mercaptoethanol on in vitro development under oxidative stress and cystine uptake of bovine embryos. Biology of Reproduction, 66:562-567. DOI: https://doi.org/10.1095/biolreprod66.3.562

Tarocco A, Caroccia N, Morciano G, Wieckowski MR, Ancora G, Garani G, et al (2019). Melatonin as a master regulator of cell death and inflammation: molecular mechanisms and clinical implications for newborn care. Cell Death and Disease, 10:4. DOI: https://doi.org/10.1038/s41419-019-1556-7

Truong TT and Gardner DK (2020). Antioxidants increase blastocyst cryosurvival and viability post-vitrification. Human Reproduction, 35:12-23. DOI: https://doi.org/10.1093/humrep/dez243

Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, and Telser J (2007). Free radicals and antioxidants in normal physiological functions and human disease. International Journal of Biochemistry and Cell Biology, 39:44-84. DOI: https://doi.org/10.1016/j.biocel.2006.07.001

Vona R, Pallotta L, Cappelletti M, Severi C, and Matarrese P (2021). The impact of oxidative stress in human pathology: Focus on gastrointestinal disorders. Antioxidants, 2:1-26. DOI: https://doi.org/10.3390/antiox10020201

Vos M, Esposito G, Edirisinghe JN, Vilain S, Haddad DM, Slabbaert JR, et al (2012). Vitamin K2 is a mitochondrial electron carrier that rescues pink1 deficiency. Science, 336:1306-1310. DOI: https://doi.org/10.1126/science.1218632

Walczak-Jedrzejowska R, Wolski J K, and Slowikowska-Hilczer J (2013). The role of oxidative stress and antioxidants in male fertility. Central European Journal of Urology, 66: 60-67. DOI: https://doi.org/10.5173/ceju.2013.01.art19

Wang F, Tian X, Zhang L, He C, Ji P, Li Y, et al (2014). Beneficial effect of resveratrol on bovine oocyte maturation and subsequent embryonic development after in vitro fertilization. Fertility and Sterility, 101. 2. DOI: https://doi.org/10.1016/j.fertnstert.2013.10.041

Wang F, Tian XZ, Zhou YH, Tan DX, Zhu SE, Dai YP, et al (2014). Melatonin improves the quality of in vitro produced (IVP) bovine embryos: Implications for blastocyst development, cryotolerance, and modifications of relevant gene expression. PLoS ONE, 9: 1-7. DOI: https://doi.org/10.1371/journal.pone.0093641

Wang Z. guang, Yu S dong, and Xu Z rong (2007). Improvement in bovine embryo production in vitro by treatment with green tea polyphenols during in vitro maturation of oocytes. Animal Reproduction Science, 100: 22-31. DOI: https://doi.org/10.1016/j.anireprosci.2006.06.014

Williams RJ, Spencer JPE, and Rice-Evans C (2004). Flavonoids: Antioxidants or signalling molecules? Free Radical Biology and Medicine, 7:838-849. DOI: https://doi.org/10.1016/j.freeradbiomed.2004.01.001

Xiong X, Li J, Wang L, Zhong J, Zi X, and Wang, Y (2014). Low oxygen tension and relative defined culture medium with 3, 4-dihydroxyflavone are beneficial for yak-bovine interspecies somatic cell nuclear transfer embryo. Reproduction in Domestic Animals, 49: 26-133. DOI: https://doi.org/10.1111/rda.12240

Yan L, Liu J, Wu S, Zhang S, Ji G, and Gu A (2014). Seminal superoxide dismutase activity and its relationship with semen quality and SOD gene polymorphism. Journal of Assisted Reproduction and Genetics, 31:549-554. DOI: https://doi.org/10.1007/s10815-014-0215-2

Yan Z, Zhong Y, Duan Y, Chen Q, and Li F (2020). Antioxidant mechanism of tea polyphenols and its impact on health benefits. Animal Nutrition, 6:115-123. DOI: https://doi.org/10.1016/j.aninu.2020.01.001

Ye J, Li J, Yu Y, Wei Q, Deng W, and Yu L (2010). L-carnitine attenuates oxidant injury in HK-2 cells via ROS-mitochondria pathway. Regulatory Peptides, 161: 58-66. DOI: https://doi.org/10.1016/j.regpep.2009.12.024

Yu S, Long H, Lyu QF, Zhang QH, Yan ZG, Liang HX, et al (2014). Protective effect of quercetin on the development of preimplantation mouse embryos against hydrogen peroxide-induced oxidative injury. PLoS ONE, 9.2. DOI: https://doi.org/10.1371/journal.pone.0089520

Zulaikhah ST (2017). The role of antioxidant to prevent free radicals in the body. Sains Medika, 8:39. DOI: https://doi.org/10.26532/sainsmed.v8i1.1012

Citation: Damayanti E, Sonjaya H, Baco S, Hasbi H (2022). The role of antioxidants in improving the quality of bovine embryos produced in vitro. *Online J. Anim. Feed Res.*, 12(6): 324-332. DOI: https://dx.doi.org/10.51227/ojafr.2022.43