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Meta-Analysis of the Addition of Exogenous Antioxidants to *in vitro* Maturation Medium: Improved *in vitro* Nuclear Maturation of Animal Oocyte

Review Article

M. Khazaei[1], A. Vaisi-Raygani[1,2] and F. Aghaz[1*]

[1] Fertility and Infertility Research Center, Health Technology Institute, Kermanshah University of Medical Science, Kermanshah, Iran
[2] Department of Clinical Biochemistry, Medical School, Kermanshah University of Medical Science, Kermanshah, Iran

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*Correspondence E-mail: faranak.aghaz@kums.ac.ir

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ABSTRACT

The use of antioxidants in *in vitro* maturation (IVM) media has been widely suggested, although this practice's evidence base is uncertain. The purpose of this study was to examine whether supplementing the IVM media with exogenous antioxidants would enhance the nuclear maturation and growth of the oocytes of domestic animals. A literature search was conducted using the databases published up to April 2019 in the PubMed, Scopus, and Web of Science. The primary outcome was the full oocyte maturation (MII %) rate (cytoplasmic and nuclear). In the final assessment, a total of 19 published experimental researches were included. There was a general statistically significant impact on MII oocyte in domestic animals by adding antioxidant supplements to the IVM medium. A meta-analysis was performed using a random effects model to predict odd ratio (OR) and 95% confidence interval (CI). The MII % in maturation medium supplemented with antioxidant was significantly better than maturation medium without antioxidant, (OR=0.59; 95% CI 0.48–0.72; I-square=40%; P=0.034). These results demonstrated that antioxidant supplements in domestic animals improved the MII percentage.

KEY WORDS exogenous antioxidants, *in vitro* maturation media, meta-analysis, nuclear maturation.

INTRODUCTION

In vitro embryo production (IVEP) technologies, including (i) *in vitro* maturation (IVM) of oocytes harvested directly from follicles (ii) *in vitro* fertilization (IVF) or co-incubation of capable spermatozoa with mature oocytes *in vitro*, and zygote *in vitro* culture (IVC) up to the blastocyst stage, which considered as the key technologies in animals' reproduction and biomedical fields (Shi *et al.* 2009). Fallowing reports, IVM is the key factor in determine a number of oocytes that develop to the blastocyst stage. IVM involves the *in vitro* culture of immature oocytes, from the germinal vesicle or the germinal vesicle breakdown stage, in special laboratory conditions until the complete maturation (nuclear and cytoplasmic maturation or MII stage), when the oocyte is considered being matured and prepared to undergo fertilization (Cha and Chian, 1998). Since its inception, numerous advances have occurred for improvement of IVM medium. However, compared to *in vivo*, the success rate of regular *in vitro* matured oocytes is not high. As one of the most important factors associated with poor success rates in the complete maturation or MII % rate of the domestic animals' oocyte, it is related to the generation of pro-oxidants such as reactive oxygen species (ROS) in the culture condition (Deleuze and Goudet, 2010a; Rocha *et al.* 2016b). It is well-known that high levels of ROS beyond the physiological range could motivate maturation promoting factor (MPF) destabilization, suppress survival factors, and trigger apoptosis in oocytes of several mammalian species (Pandey *et al.* 2010; Tiwari and Chaube, 2016).

Antioxidants are the main factors of protection against ROS-induced oxidative stress (OS). Numerous publications suggest that IVM media supplementation with exogenous antioxidants improves meiosis spontaneously by relieving ROS during oocyte maturation through increased storage of glutathione (GSH) and contributes to further protect the embryo against oxidative aggressions during its early developmental stages. Antioxidant supplementation during IVM also improves the quality of oocytes by reducing levels of ROS and apoptotic factors. Oocyte defense against ROS can play significant roles in pre-implantation embryonic development. Antioxidants, are ROS scavengers, thereby helping to maintain the oxidant/antioxidant balance of the oocyte.

A number of studies have been evaluated the effect of antioxidant supplements added to the IVM media in various domestic animals, however, the inconsistency was observed in the results (Deleuze and Goudet, 2010a; Aghaz *et al*. 2015; Rodrigues-Cunha *et al*. 2016). Improvements in MII rate, an increase in the rate of cleavage, and blastocyst rates in porcine (Ozawa *et al*. 2006), bovines (Ali *et al*. 2003) and sheep (Dash *et al*. 2008; Isobe *et al*. 2012) have been demonstrated. You *et al*. (2010) showed that GSH is one of the master synthesized antioxidants that safe keeps cells from ROS toxicity and manages the intracellular redox balance. Even, the intracellular level of GSH has been reported to increase during oocyte maturation in hamster (Perreault *et al*. 1988), pig (Yoshida *et al*. 1993), ovine (de Matos *et al*. 2002), and bovine (De Matos *et al*. 1996). Recent reports have indicated that the expansion of low submolecular weight thiol mixes, such as cysteamine and β-mercaptoethanol to IVM media improved the cytoplasmic maturation of oocytes and early embryonic development by expanding GSH synthesis (Choe *et al*. 2010; You *et al*. 2010; Nakamura *et al*. 2011).

Cysteamine supplementation during IVM reportedly improved the complete maturation rates of oocytes in canines (Hossein *et al*. 2007), mice (Chen *et al*. 2005), goats (Urdaneta *et al*. 2003), and porcine (Bing *et al*. 2001). Protecting spindle structures of MII mouse oocytes and chromosomal alignment against oxidant (hydrogen peroxide) damage recorded a beneficial function for vitamin C. It has been proposed that vitamin C's influence is primarily associated with its ability during IVM to facilitate ooplasmic maturation Choi *et al*.(2007). Conversely, other reports in goats (Zhou *et al*. 2008), pigs (Song and Lee, 2007), horses (Deleuze and Goudet, 2010a), buffalos (Singhal *et al*. 2009), and cattle (Balasubramanian and Rho, 2007) have demonstrated no statistically significant improvement in nuclear maturation rates (Wani *et al*. 2012); (Takami *et al*. 1999). Conflicting results have also been obtained even with the well-controlled studies.

Which of them was presented in this study. Tatemoto *et al*. (2001), Kere *et al*. (2012), and Córdova *et al*. (2010) discovered that vitamin C similar to vitamin E and trolox antioxidant supplements added to the oocyte maturing media, had no beneficial or negative effect on oocyte maturation levels, while other antioxidants such as propyl gallate and 2, 4, 5 trihydroxybutrophenone inhibited the spontaneous resumption of meiosis. Consequently, a clear interpretation of the current body of literature on antioxidant supplementation not only is difficult, but also the role of antioxidant supplementation in domestic animals IVM is controversial.

To reach a more definitive conclusion regarding the predictive value of antioxidant supplementations on maturation rates and pre-embryo development and to further examine why there are discrepancies between the various studies, a meta-analysis of published experimented studies was performed according to various factors such as the type of domestic animals, and the type of antioxidant supplements. Furthermore, we conducted additional sub analyses to evaluate the effect of antioxidant supplements added to the IVM media on MII rate, and subsequent embryo development in domestic animals.

MATERIALS AND METHODS

Identification of antioxidant supplements and their modes of action

This study reviewed substances with recognized antioxidant properties. These were: (i); cysteamine is a low-molecular weight amino acid that contains thiol (Uysal and Bucak, 2007). The addition of cysteamine not only improves the content of GSH in MII oocytes but the membrane lipids and proteins are also covered by indirect radical scavenging properties (Hendin *et al*. 1999). (ii); The concentrations of many amino acids, including taurine and hypotaurine, and non-enzymatic antioxidants, including (resveratrol, carnitine, quercetin, anethole and EOSA) help to maintain the redox status in oocytes (Premkumar and Chaube, 2014) (Alali *et al*. 2007); Resveratrol (3,4,5-trihydroxy-trans-stilbene) is a small polyphenol synthesized by several plants, such as nuts, mulberry and grapes. This phytoalexin is a potent antioxidant that, by activation of SIRT1 (a sensor of the redox state in oocytes and granulosa cells) induces the upregulation of the endogen antioxidant system (Piras *et al*. 2019). Recent studies highlighted that, resveratrol supplementation during IVM positively affected oocyte quality, fertilization and embryo development outcomes in goats, cattle, and pigs (Galeati and Spinaci, 2015). Carnitine is a naturally occurring quaternary amine with the L-stereoisomer (3R)-3-hydroxy-4- (trimethylazaniumyl) butanoate having potent bioactivity.

Additionally, L-carnitine has been used during IVM studies as a scavenger of free radicals, in turn protecting antioxidant enzymes from oxidant injury (Dunning and Robker, 2017). Quercetin (3,5,7,3′-4′-pentahiroxyflavone) is a natural flavonoid widely found in vegetables, grains, fruits, flowers, teas. It has therapeutics properties and antioxidant potential (Behling *et al*. 2004). The presence of quercetin promoted excellent oocyte maturation rates and blastocyst development in bovine (Guemra *et al*. 2013) and swine (Orlovschi *et al*. 2014) by reduction of ROS intracellular levels. Anethole has been proposed to act as a synergistic antioxidant increasing the activity of primary antioxidants (eg, SOD, CAT, and GPX), and the levels of tripeptide glutathione (GSH) (Sá *et al*. 2019). The plant *Syzygium aromaticum*, known as clove, has antioxidant activity associated with the presence of phenolic compounds (Viuda Martos *et al*. 2010). In during *in vitro* bovine oocyte maturation studies, EOSA at 20 µg/mL improves parthenogenetic embryonic development (De Oliveira Santos *et al*. 2019). The hormone melatonin (N-acetyl-5-metoxy tryptamine) is an antioxidant that, unlike GSH and vitamins C and E, is produced by mammals. In contrast to other antioxidants, however, melatonin cannot undergo redox cycling. Once oxidized, it is unable to return to its reduced state because of the formation of a stable end-products after the reaction (Deleuze and Goudet, 2010a; Aghaz *et al*. 2015; Rodrigues-Cunha *et al*. 2016). (iv); As an antioxidant, green tea has been shown to improve IVM and embryo development of sheep COCs to the blastocyst stage in IVM media (Cabrera *et al*. 2006). (v); sericin a water-soluble globular protein (protein hydrolysate) is derived from the silkworm *Bombyx mori*. This protein represents a family of proteins whose molecular mass ranges from 10 to 310 kDa (Tao *et al*. 2005). Dash *et al*. (2008) have reported that sericin might provide a protective effect on fibroblasts by promoting endogenous antioxidant enzyme *in vitro*. (vi); catalase (CAT) is a central enzymes capable of converting hydrogen peroxide into water and oxygen. (vii); cysteine or (viii); β-mercaptoethanol addition to culture media for lymphocytes has been shown to increase intracellular GSH levels and prevent its decrease during proliferation (Zmuda and Friedenson, 1983).

Search methods for identification of studies

This review was restricted to published research articles that compared the oocyte nuclear maturation outcome in IVM media supplemented with antioxidant to no antioxidant, in domestic animals. Several strategies were adopted to identify articles published on the effect of antioxidant supplementation in maturation medium on oocyte nuclear maturation in domestic animals.

The primary search was conducted with PubMed, Scopus, and Web of Science databases published up to April 2019 were identified.

Two subsets of search terms were used, one describing the antioxidants (antioxidant supplements, cysteamine+cysteine, CAT, cysteamine+cysteine+CAT, resveratrol, carnitine, EOSA, sericin, melatonin, β-mercaptoethanol, hypotaurine, taxifolin, quercetin, anethole (antioxidant flavonoids), and green tea) and the other describing oocyte, MII, nuclear maturation, IVM, *in vitro* maturation, *in vitro* maturation and domestic animals (bovine, ovine, porcine, goat), which were combined in the search using the 'OR' operator). The two subsets of terms were then combined using the 'AND' operator. Only those publications written in English were included. All pertinent articles were retrieved, and the relative reference lists were systematically reviewed in order to identify further reports that could be included in the meta-analysis. The full search strategy may be found in (Figure 1).

Study selection

Three of the authors independently performed an initial screening of the title and abstract of all articles to exclude citations deemed irrelevant by both observers. Authors of included studies were contacted for any additional information about their study when is necessary. Disagreement as to study eligibility was resolved after discussion by both reviewers. We thoroughly reviewed the full texts that were selected from the first screening and included the studies meeting the selection criteria in the final analysis (Figure1). Also, we searched for grey literature such as information obtained from sources other than published, peer-reviewed articles=unpublished data in the Open Grey (www. opengrey.eu/).

Data extraction and eligibility criteria

Based on the pre-determined selection criteria, data extraction was performed independently by both of the evaluators. Any disagreement between the two reviewers was, resolved by discussion. Study characteristics, participant features, study inclusion and exclusion criteria were extracted from each study. Only studies reporting these two variables as mean ± SEM were included (we excluded these data from those reporting only the median range). Exploration of special heterogeneity was conducted using variation in features of the types of animals, and the antioxidant supplements. The main outcome measure chosen for the current meta-analysis were polar body (PB) and oocyte complete maturation rates (MII). Prespecified inclusion criteria were as follows: (i) use of an experimental design, (ii) experimental studies that reported effects on MII % or MII %

+ PB %, (Alali *et al*. 2007) experimental studies that used antioxidant supplements and concurrent control groups.

Exclusion criteria were as follows: (i) experimental studies that enrolled antioxidant supplements on IVF/IVC outcome, (ii) experimental studies that enrolled antioxidant supplements on MII % outcome in other animals.

We thoroughly reviewed the full texts that were selected from the first screening and included the studies meeting the selection criteria in the final analysis (Figure 1).

Quality assessment

There were at least 11 studies in our comparative analysis. In the number of articles reported the data of two or three antioxidant supplements on the complete maturation rates or MII %, we calculated each antioxidant supplement equal to one study (Table 1). Four of the included studies were in bovine (El Raey *et al*. 2011; Rocha Frigoni *et al*. 2016a), and two were studies in ovine (Barakat *et al*. 2014; Aghaz *et al*. 2015). Two of the included studied (Lv *et al*. 2010) were in goat, and three were studied in porcine (Kang *et al*. 2009; Do *et al*. 2014; Kang *et al*. 2016).

Statistical analysis

Dichotomous variables were summarized as the different ratio (DR), and the precision of the estimates evaluated by the 95% confidence interval (CI). Stata/SE version 11 software package (Stata Corp., College Station, TX, USA) was used for all statistical analyses. OR and 95% CI were calculated for each outcome. A random effect model was used for the meta-analysis. Heterogeneity was assessed with the use of the I-square test.

RESULTS AND DISCUSSION

Results of search and characteristics of studies

Figure 1 summarizes the processes of literature identification. In total, 65 studies were identified in the english literature, of which 19 matched the inclusion criteria, all of which were published as full in peer-reviewed journals published up to April 2019 (Kang *et al*. 2009; Lv *et al*. 2010; El Raey *et al*. 2011; Barakat *et al*. 2014; Do *et al*. 2014; Aghaz *et al*. 2015; Kang *et al*. 2016; Rocha-Frigoni *et al*. 2016a; Sovernigo *et al*. 2017; De Oliveira *et al*. 2019; Sá *et al*. 2019; Zabihi *et al*. 2019). Detailed characteristics of the selected studies are shown in (Table 1). Six (31%) was published in Reproduction in Domestic Animals (Do *et al*. 2014; Sovernigo *et al*. 2017), five of the 19 studies (26%) were published in the Theriogenology journal (Aghaz *et al*. 2015; Rocha-Frigoni *et al*. 2016a; De Oliveira *et al*. 2019), two (10%) were published in Italian Journal of Animal Science, one (5%) was published in Molecular Reproduction and Development journal (El Raey *et al*. 2011), one (5%) was published in Reproductive Sciences, journal one (5%) was published in Domestic Animal Endocrinology, journal one (5%) in Journal of Pineal Research (Kang *et al*. 2009), one (5%) was published in Asian-Australasian Journal of Animal Sciences (Kang *et al*. 2016), and one (5%) in Pakistan Journal of Zoology (Barakat *et al*. 2014). They were conducted in the following countries: Brazil (n=10), USA (n=2), Japan (n=2), Korea (n=2), Iran (n=2), and Egypt (n=1). The types of antioxidant supplements were as follows: cysteamine + cysteine (n=1), CAT (n=1), cysteamine + cysteine + CAT (n=1), cysteamine (n=1), sericin (n=2), melatonin (n=3), b-mercaptoethanol (n=1), hypotaurine (n=1), resveratrol (n=1), carnitine (n=1), EOSA (n=1), quercetin (n=1), anethole (n=1), taxifolin (antioxidant flavonoids) (n=1), and green tea (n=1). Because of that, the at least need article for meta-analysis is two articles, but we have just one article for some of antioxidant supplements, we cannot sub-group meta-analysis by types of antioxidant supplements.

Main and subgroup analyses

We investigated the association between the use of antioxidant supplements, and the MII rate. When we tested heterogeneity by I-square, variation in OR attribution showed heterogeneity (Figure 2). It has been proposed that influence of different domestic animals is associated with the MII rate. Additionally, we performed subgroup meta-analyses according to the type of domestic animals; (group 1=bovine, group 2=ovine, group 3=porcine, group 4=goat).

The random-effect meta-analysis of all 19 articles (Kang *et al*. 2009; Lv *et al*. 2010; El Raey *et al*. 2011; Barakat *et al*. 2014; Do *et al*. 2014; Aghaz *et al*. 2015; Kang *et al*. 2016; Rocha-Frigoni *et al*. 2016a; Sovernigo *et al*. 2017; De Oliveira *et al*. 2019; Sá *et al*. 2019; Zabihi *et al*. 2019), showed a statistically significant increase in MII % in oocyte matured with antioxidant supplementation compared with matured without antioxidant supplementation (OR=0.59; 95% CI 0.48–0.72; I-square=40%; P=0.034). Considering the I-square value (40%), the result clearly indicates moderate heterogeneity (Figure 2).

Figure 2 presents the findings from the subgroup meta-analyses according to domestic animals. Regarding types of domestic animals, eleven of the included studies reported the mean MII% in bovine cumulus–oocyte complexes matured with and without antioxidants supplementation (Alali *et al*. 2007; El Raey *et al*. 2011; Rocha-Frigoni *et al*. 2016a; Sovernigo *et al*. 2017; De Oliveira *et al*. 2019; Sá *et al*. 2019). Meta-analysis demonstrated statistically significant increase in MII% in bovine oocyte matured with antioxidant supplementation compared with matured without antioxidant supplementation (OR=0.68; 95% CI [0.54–0.86]; P=0.593).

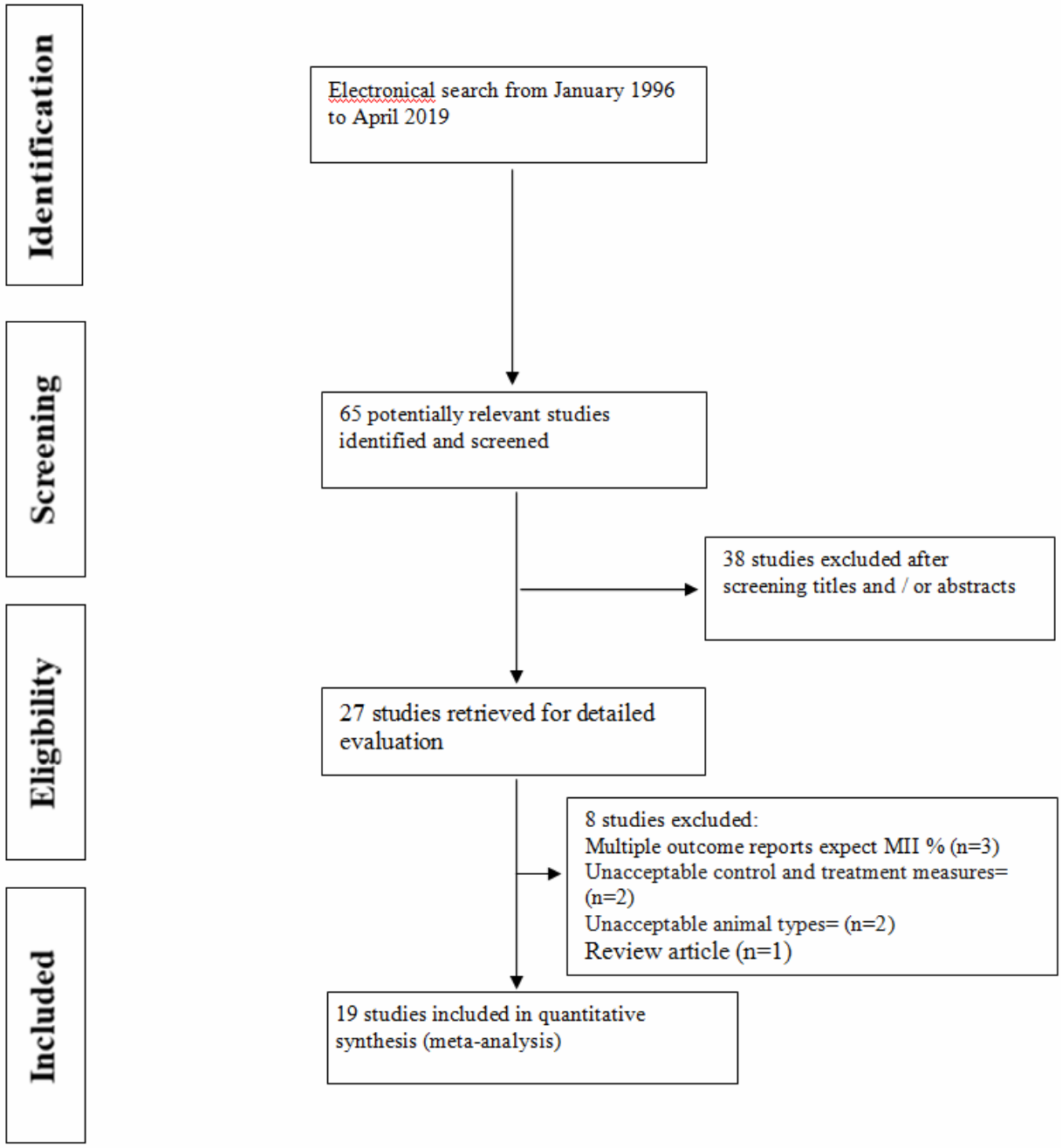

Figure 1 Flowchart for systematic review and meta-analysis

The I-square value was 0.0% indicating the absence of heterogeneity. Three studies evaluated the association between ovine oocyte matured with and without antioxidant supplementation (Barakat *et al.* 2014; Aghaz *et al.* 2015; Zabihi *et al.* 2019). Our result not showed statistically significant increase in MII % in ovine oocyte matured with antioxidant supplementation compared with matured without antioxidant supplementation (OR=0.27; 95% CI [0.19–0.40]; P= 0.503). The I-square value was 0.00% indicating absent of heterogeneity.

Three studies (Kang *et al.* 2009; Do *et al.* 2014; Kang *et al.* 2016) compared OR in matured porcine oocytes with and without antioxidant supplementation. Meta-analysis showed statistically significant increase between matured porcine oocytes with and without antioxidant supplementation. (OR=0.64; 95% CI [0.48–0.86]; P=0.687) (Figure 2). The I-square value was 0.00% indicating the absence of heterogeneity. Two studies evaluated the association between goat oocyte matured with and without antioxidant supplementation.

Table 1 Characteristics of the preclinical studies included in the meta-analysis

Authors (year)	Location	Study design	Domestic animals	Antioxidant supplementation	IVM media	No. of oocyte (control)	No. of oocyte (study)	Outcomes	Journal	Impact factor of journal
De Oliveira *et al.* (2019)	Brazil	Experimental study	Bovine	EOSA	TCM-199	95	84	MII %	*Theriogenology*	2.29; Q1
Sá *et al.* (2019)	Brazil	Experimental study	Bovine	Anethole	TCM-199	66	58	MII %	*Reprod. Sci.*	1.81; Q1
Zabihi *et al.* (2019)	Iran	Experimental study	Bovine	Resveratrol	TCM-199	132	120	MII %	*Domest. Anim. Endocrinol.*	2.32; Q1
Sovernigo *et al.* (2017)	Brazil	Experimental study	Bovine	Quercetin	TCM-199	59	60	MII %	*Reprod. Domest. Anim.*	1.81; Q1
Sovernigo *et al.* (2017)	Brazil	Experimental study	Bovine	Cysteamine	TCM-199	59	62	MII %	*Reprod. Domest. Anim.*	1.81; Q1
Sovernigo *et al.* (2017)	Brazil	Experimental study	Bovine	Carnitine	TCM-199	59	61	MII %	*Reprod. Domest. Anim.*	1.81; Q2
Sovernigo *et al.* (2017)	Brazil	Experimental study	Bovine	Vitamin C	TCM-199	59	61	MII %	*Reprod. Domest. Anim.*	1.81; Q2
Sovernigo *et al.* (2017)	Brazil	Experimental study	Bovine	Resveratrol	TCM-199	59	61	MII %	*Reprod. Domest. Anim.*	1.81; Q2
Rocha-Frigoni *et al.* (2016a)	Brazil	Experimental study	Bovine	Cysteamine + cysteine	TCM-199	120	110	MII %	*Theriogenology*	2.29; Q1
Rocha-Frigoni *et al.* (2016a)	Brazil	Experimental study	Bovine	CAT	TCM-199	120	104	MII %	*Theriogenology*	2.29; Q1
Rocha-Frigoni *et al.* (2016a)	Brazil	Experimental study	Bovine	Cysteamine + cysteine + CAT	TCM-199	120	102	MII %	*Theriogenology*	2.29; Q1
Aghaz *et al.* (2015)	Iran	Experimental study	Ovine	Sericin	TCM-199	25	25	MII %	*Theriogenology*	2.29; Q1
Do *et al.* (2014)	Japan	Experimental study	Porcine	Sericin	TCM-199	130	133	MII %	*Reprod. Domest. Anim.*	1.81; Q1
El Raey *et al.* (2011)	Japan	Experimental study	Bovine	Melatonin	TCM-199	114	127	MII %	*Mol. Reprod. Dev.*	3.11; Q2
Kang *et al.* (2009)	Korea	Experimental study	Porcine	Melatonin	TCM-199	221	227	MII and PB %	*J. Pineal. Res.*	15.22; Q1
Lv *et al.* (2010)	USA	Experimental study	Goat	B-mercaptoethanol	TCM-199	88	103	MII %	*Italian J. Anim. Sci.*	1.26; Q1
Lv *et al.* (2010)	USA	Experimental study	Goat	Hypotaurine	TCM-199	88	84	MII %	*Italian J. Anim. Sci.*	1.26; Q1
Kang *et al.* (2016)	Korea	Experimental study	Porcine	Taxifolin (antioxidant flavonoids)	TCM-199	181	192	MII and PB %	*Asian-Australasian J. Anim. Sci.*	1.27; Q1
Barakat *et al.* (2014)	Egypt	Experimental study	Ovine	Green tea	TCM-199	315	361	MII %	*Pakistan J. Zool.*	0.79; Q3

EOSA: *Syzygium aromaticum* essential oil supplementation; CAT: catalas (enzymatic antioxidant); TCM-199: IVM media; MII %: nuclear maturation rat and PB%: Polar body extrusion rate.

Meta-analysis indicated no association statistically significant between matured porcine oocytes with and without antioxidant supplementation (Lv *et al.* 2010). (OR=0.59; 95% CI [0.30–1.17]; P=0.109) (Figure 2). The I-square value was 0.61% indicating the moderate of heterogeneity.

Overall, there were significant effects of antioxidant supplements in the subgroup meta-analyses by domestic animals.

However, the MII % was marginally increased in domestic animals.

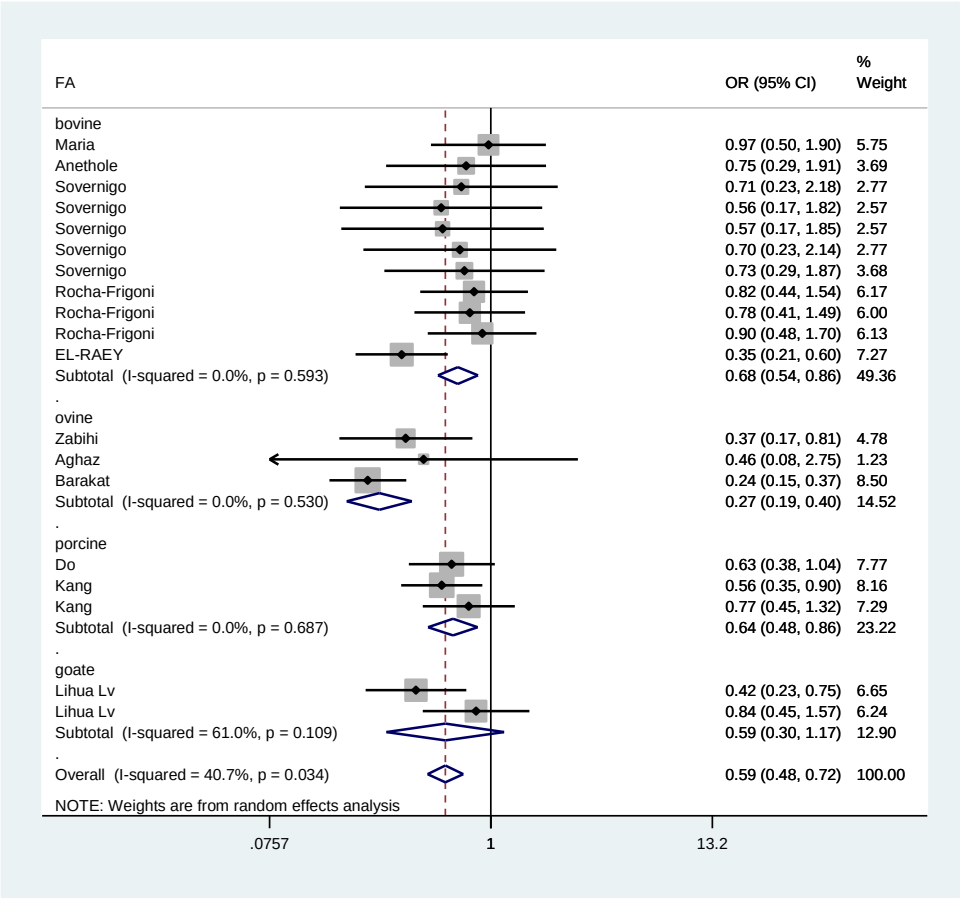

Figure 2 Beneficial impact of antioxidant supplementation on developmental competency of *in vitro* matured oocytes by a random-effect sub-groups meta-analysis model of published experimental studies on domestic animals
OR: odds ratio and CI: confidence interval

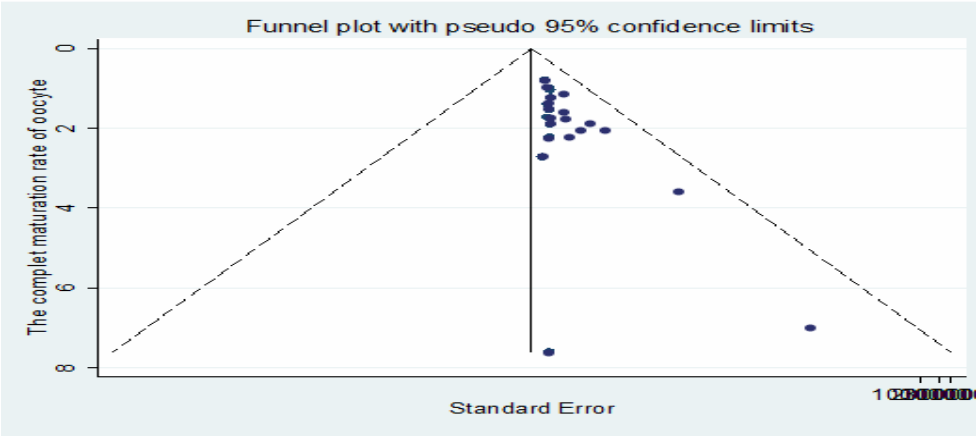

Figure 3 Funnel plot to assess publication bias

The success of IVEP is closely related to IVM success rate, which is one of the limiting steps. Consistently, % of MII rate (cytoplasmic and nuclear) of oocyte would dramatically improve the efficiency of animal reproduction (Aghaz *et al*. 2015).

Although the beneficial role of moderate levels of ROS on *in vitro* nuclear maturation is reported in several mammalian species (Pandey and Chaube, 2014; Premkumar and Chaube, 2016). But, high ROS level beyond the physiological range and oxidative stress have been implicated in

the trigger of granulosa cell apoptosis and thereby reducing the transfer of nutrients and survival factors to oocytes, which correlated with lower rates of the MII%, impaired fertilization and embryo development (Aghaz *et al*. 2016; Tiwari *et al*. 2016; Aghaz and Khazaei, 2017; Hajian *et al*. 2017).

Although the effects of antioxidant supplementation in IVM media have been studied in various domestic animals (Alali *et al*. 2007; Wang *et al*. 2014; Kang *et al*. 2016; Sovernigo *et al*. 2017; Wang *et al*. 2017; Mao *et al*. 2018; Marques *et al*. 2018; De Oliveira Santos *et al*. 2019; Sá *et al*. 2019; Zabihi *et al*. 2019), but the results were inconsistent in some of the published papers (Deleuze and Goudet, 2010b; Li *et al*. 2015; Rocha-Frigoni *et al*. 2016a; Rodrigues-Cunha *et al*. 2016). So, a variety of conclusions have been drawn until now. Consequently, this systematic review and meta-analysis study, for the first time, enabled us to address this question that, whether the effect of antioxidant supplementation in maturation medium is associated with improved MII % rate (cytoplasmic and nuclear) compared with IVM media without antioxidant supplements or not? In the present study, meta-analysis of all 19 articles , by the random-effect model, showed a statistically significant increase in MII % rate of domestic animal's oocyte matured in IVM media with antioxidant supplementation compared without antioxidant supplementation (OR=0.59; 95% CI [0.48–0.72]; P=0.034). One of the major confounding variables of this study is heterogeneous that caused by domestic animals include bovine, porcine, goats and ovine, and was controlled by the subgroup meta-analysis. This suggests a ubiquitous benefit of antioxidant supplementation independent of the domestic animals. This finding supports the combining/pooling of the various supplementations during *in vitro* maturation studies.

The present study has several limitations. First, the published experimental studies in the present meta-analysis, involved only types of domestic animals. Thus, we used a random-effect sub-groups meta-analysis model of these studies for specifying the effect of antioxidant supplements on MII % rate of oocyte in domestic animals. Other factors which warrant the interpretation of results in this systematic review with caution are the various types of antioxidant supplements. There are possible explanations for this discrepancy. It is well-know that, low glutathione levels decrease cytoplasmic maturation of animal's domestic oocytes *in vitro*. Embryo production was improved by increasing intracellular glutathione levels of mammalian oocytes induced by low-molecular-weight thiol compounds (cysteamine, cysteine, CAT (Sovernigo *et al*. 2017) and thiol derivatives such as, B-mercaptoethanol, hypotaurine, taxifolin, carnitine (Sovernigo *et al*. 2017), EOSA (De Oliveira Santos *et al*. 2019), quercetin (Sovernigo *et al*. 2017), anethole (Sá *et al*. 2019), and others antioxidant supplements such as sericin (Aghaz *et al*. 2015), melatonin (El Raey *et al*. 2011) green tea (Barakat *et al*. 2014), resveratrol (Sovernigo *et al*. 2017; Zabihi *et al*. 2019), during IVM.

Also, a meta-analysis requires at least 2 studies, but we have just one article for some of antioxidant supplements, so we cannot sub-group meta-analysis by types of antioxidant supplements. Second, we were unable to investigate whether the specific dose for each antioxidant supplements are beneficial in the increase of MII % in domestic animals, that is due to deficiency in a number of published experimental studies on domestic animals. Further studies are required to evaluate this association. Last, we haven't assessed the methodological quality of individual published experimental studies on domestic animals by only using the data shown in each article. Therefore, we might not have assessed the actual performance or biases in individual experimental studies.

CONCLUSION

This meta-analysis could show that the presence of exogenous antioxidants on IVM medium, in standard protocols and optimal concentrations, could prevent against ROS (high levels of ROS generated under *in vitro* cultured condition) and decreased deterioration in domestic oocyte quality.

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