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Sodium Selenite Improves In Vitro Maturation of *Bos primigenius taurus* Oocytes

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Abstract
Selenium (Se) is an essential trace element with important functions in animals and whose deficiency is associated with reproductive failures. The aim of this study was to investigate the effect of Se concentrations during in vitro maturation (IVM) of *Bos taurus* oocyte within the reference ranges for Se status in cattle. For this purpose, Aberdeen Angus cumulus–oocyte complexes (COCs) were matured in IVM medium supplemented with 0, 10, 50, and 100 ng/mL Se (control, deficient, marginal, and adequate, respectively). The results demonstrated that marginal and adequate Se concentrations added during IVM increased viability and non-apoptotic cumulus cells (CC). Moreover, the addition of Se to culture media decreased malondialdehyde level in COC with all studied concentrations and increased total glutathione content in CC and oocytes with 10 ng/mL Se. On the other hand, total antioxidant capacity of COC, nuclear maturation, and the developmental capacity of oocytes were not modified by Se supplementation. However, 10 ng/mL Se increased hatching rate. In conclusion, supplementation with 10 ng/mL Se during in vitro maturation of *Bos primigenius taurus* oocytes should be considered to improve embryo quality.

Keywords Selenium · MDA · TAC · Apoptosis · GSH · Embryo development

Introduction

Selenium (Se) is an essential trace element with important functions in animals, including reproductive activity in both sexes [1–3]. In males, Se deficiency may affect testosterone and spermatozoa synthesis, leading to infertility [4]. Besides, Se influences the gross and histological morphology of testes, as well as scrotal length and circumference [5–8]. In cows, Se deficiency has been associated with reduced fertility, placental retentions, and increased incidence of mastitis and metritis [9–11]. Komisrud et al. [12] demonstrated that Se supplementation improved the first-service conception rate in Se-deficient dairy cows. Moreover, Se increased fertility by reducing embryonic death during the first month of gestation [2].

Selenium is absorbed mainly in the jejunum and transported bound to α- and β-globulins, lipoproteins, and albumin [13]. In cattle, Se status is defined in terms of reference plasma Se concentrations as deficient, marginal, and adequate (< 50, 50–83, and > 83 ng/mL, respectively) [14–17]. Selenium plays an important role in cellular antioxidant defenses because it is a structural component of selenoproteins, many of which have antioxidant activity, such as the glutathione peroxidase family (GPx) and thioredoxin reductases (TrxRs) [18]. The GPx oxidizes the glutathione reduced form (GSH) to oxidized glutathione (GSSG) in the GSH/GSSG antioxidant system, regulating hydrogen peroxide and other hydroperoxides [19]. Selenium also plays a key role in cell cycle and apoptosis, inhibiting genetic damage [20]. In addition, several studies have revealed that Se regulates intracellular signaling, including protein kinase C, nuclear factor-kappa B, and the insulin pathway [21, 22].

Selenium has been used as a supplement in culture media, usually combined with other compounds such as insulin-transferrin [23–25] or calcium-calcium ionophore [26].

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According to Baker et al. [27], cell culture media are routinely deficient in Se and should therefore be supplemented with physiological concentrations of this trace element. Most researchers have reported the addition of lower or supraphysiological Se concentrations to IVM medium [25, 28]. In previous studies, we demonstrated that the addition of adequate concentrations of trace elements such as copper, manganese, or zinc to IVM media improved the developmental capacity of bovine oocytes [29–31]. Although several studies have shown the importance of Se in in vitro embryo production [23–26], the effect of Se concentrations during IVM within the reference ranges for Se status on bovine has not yet been quantified. Therefore, the objective of this study was to investigate the effects of Se supplementation (as sodium selenite) during IVM of *Bos taurus* oocytes within the reference ranges for Se status in cattle. Experiments were designed to evaluate the effect of various Se concentrations added to the IVM medium on viability and apoptosis of cumulus cells (CC), and total antioxidant capacity (TAC), GSH-GSSG intracellular concentrations, and malondialdehyde (MDA) levels in cumulus–oocyte complexes (COCs). In addition, Se concentrations in follicular fluid, nuclear maturation, and developmental capacity of oocytes matured with various Se concentrations were evaluated.

Materials and Methods

Reagents and Media

All reagents for media preparation were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The maturation medium was bicarbonate-buffered TCM-199 supplemented with 10% (v/v) fetal calf serum (FCS), 0.2 mM sodium pyruvate, 1 mM glutamine, 10 μg/mL LH (NIHoLH- S1), 1 μg/mL FSH (Folltropin, Bioniche Animal Health, USA), and 1 μg/mL 17b-estradiol. The fertilization medium consisted of TALP supplemented with 2% (v/v) MEM-essential amino acids, 1% (v/v) MEM-nonessential amino acids, 6 mg/mL bovine BSA-FAF, 20 μM penicillamine, 10 μM hypotaurine, and 10 μg/mL heparin sulfate [32]. The culture medium for embryo development consisted of modified synthetic oviduct fluid (SOFm), which was composed of SOF [33] supplemented with 1 mM glutamine, 2% (v/v) MEM-essential amino acids, 1% (v/v) MEM-nonessential amino acids, and 8 mg/mL bovine serum albumin fatty acid free (BSA-FAF) [34].

In Vitro Maturation, Fertilization, and Culture

For IVM, in vitro fertilization (IVF) and in vitro culture (IVC) of embryos, previously described protocols, were used [35]. Briefly, Aberdeen Angus ovaries were obtained from an abattoir and transported to the laboratory in sterile NaCl solution (9 g/L) with antibiotics at 37 °C within 3 h after slaughter. Ovaries were pooled, regardless of the estrous cycle stage of the donor. COCs were aspirated from 3- to 8-mm follicles using an 18-G needle connected to a sterile syringe. Only cumulus–intact complexes with evenly granulated cytoplasm were selected for IVM, using a low-power (× 20–30) stereomicroscope. For this purpose, COCs were washed twice in TCM-199 buffered with 15 mM Hepes and twice in IVM medium. Groups of 10 COCs were transferred into 50 μL of IVM medium under mineral oil (Squibb, Princeton, NJ, USA) pre-equilibrated in a CO_2 incubator. The COCs were matured in IVM medium supplemented with 0, 10, 50, and 100 ng/mL Se concentrations, which were established according to Se status in cattle (control, deficient, marginal, and adequate, respectively). Incubations were performed at 39 °C in an atmosphere of 5% CO_2 in air with saturated humidity for 24 h. For IVF, oocytes were washed twice in Hepes-TALP supplemented with 3 mg/mL BSA-FAF and placed into 50 μL drops of IVF medium under mineral oil. In all experiments, frozen semen from the same bull and batch was used. Two straws, each containing 40×10^6 spermatozoa, were thawed in a 37 °C water bath. Spermatozoa were washed in a discontinuous Percoll gradient prepared by layering 2 mL of 45% Percoll on top of 2 mL of 90% Percoll in a 15-mL centrifuge tube. Semen samples were deposited on top of the Percoll gradient and centrifuged for 20 min at 500×*g*. The pellet was removed and resuspended in 300 μL of Hepes-TALP solution and centrifuged at 300×*g* for 10 min. After removal of the supernatant, spermatozoa were resuspended in IVF medium, counted in a hemocytometer chamber, and further diluted. The final sperm concentration in IVF was 2×10^6 sperm/mL. Incubations were conducted at 39 °C in 5% CO_2 in air with saturated humidity for 24 h. After IVF, presumptive zygotes were stripped off CC by passing through a drawn pipette; they were then washed twice in Hepes-TALP and cultured in SOFm. Embryo culture was carried out in 40-μL drops of medium under mineral oil (10 zygotes per drop) at 39 °C in an atmosphere of 7% O_2, 5% CO_2, and 88% N_2 with saturated humidity. All embryos were cultured in the absence of glucose during the first 24 h and further cultured for 9 days in the presence of 1.5 mM glucose. The medium was changed every 48 h, and embryos were incubated for 10 days (day 0 = day of fertilization). Cleavage rates were recorded 48 h after insemination. At the end of incubations, embryos were evaluated for the morphological stages of development with an inverted microscope (Diaphot, Nikon, Tokyo, Japan).

Analysis of Oocyte Nuclear Maturation

After IVM, oocytes were placed in TCM-199 medium with 0.2% hyaluronidase at room temperature (RT) and then pipetted to remove CC. Oocyte nuclear maturation was assessed by mounting and staining the denuded oocytes with Hoechst

33342, a fluorescent DNA-specific dye. Oocytes were then examined under an Olympus BX40 epifluorescence microscope (Olympus, Tokyo, Japan) equipped with an appropriate filter combination and classified as germinal vesicle (GV), metaphase I (MI), anaphase-telophase (A-T), or metaphase II + polar body (MII + PB) stage of maturation [36, 37]. Oocytes with abnormal or no chromatin configuration were classified as degenerates (D). Results were expressed as oocyte percentages with different status of nuclear maturation.

Assessment of CC Viability

After maturation, viability was evaluated by incubating CC for 10 min at 37 °C in phosphate-buffered saline (PBS) medium with 2.5 g/L trypan blue stain. Then, cells were washed in PBS medium and observed under an Olympus BX40 microscope at × 200 magnification. Dead CC showed a characteristic blue staining under white light.

Assessment of CC Apoptosis by Annexin V Staining Assay

Annexin V is a calcium-dependent phospholipid-binding protein with high affinity for phosphatidylserine (PS) [38, 39]. Early apoptosis was evaluated by membrane redistribution of PS with the Annexin-V-Fluos Staining Kit (Roche, cat no. 11-858-777-001). The assay involves simultaneous staining with both Annexin-V-Fluos (green) and the DNA stain propidium iodide (PI, red). While normal cells exclude PI and Annexin V, early-apoptotic cells are visible in green and can be differentiated from late-apoptotic and necrotic cells by PI staining. Necrotic cells take up PI and stain red, while late-apoptotic apoptotic cells stain orange/green and early-apoptotic stain green only. Briefly, cells (1×10^6) were washed twice with PBS and centrifuged at 200×*g* for 5 min. The pellet was resuspended in 100 mL of Annexin-V-Fluos labelling solution (Annexin V + fluorescein, Hepes and PI) and incubated in the dark for 10–15 min at 15–25 °C. The cell suspension (50 μL) was layered onto the slides, which were immediately covered with cover slips. A total of 200 CC per treatment were analyzed under a fluorescence microscope. Scoring was made at × 400 magnification using an Olympus BX40 epifluorescence microscope equipped with a 515–560-nm excitation filter.

Total GSH Assay

After completion of IVM, all oocytes from each treatment (n = 20) in a batch were combined and stripped of surrounding CC by repeated pipetting with a narrow-bore glass pipette in Hepes-TCM 199. For each replicate, pools of oocytes from each treatment were placed in microtubes containing 10 μL PBS, frozen at − 20 °C and thawed at RT. This procedure was repeated three times. Complete oocyte disruption was achieved by repeated aspiration using a narrow-bore pipette. Cumulus cells from at least 20 COCs were placed in Eppendorf tubes and washed twice by resuspension in PBS and centrifugation at 14,000×*g* for 10 s. The pellets were resuspended in 500 μL PBS and counted in a hemocytometer chamber. After centrifugation at 14,000×*g* for 10 s, pellets were resuspended in 40 μL PBS, frozen at − 20 °C, and thawed at RT. Complete cell disruption was performed by addition of 400 μL of distilled water and repeated aspiration with a 26-G needle. Distilled water was added to increase the volume of samples to 1.2 mL, and they were then mixed with 1.2 mL of 0.2 M phosphate buffer containing 10 mM EDTA. The increase in absorbance was measured at 412 nm every 30 s up to 5 min using a double-beam spectrophotometer, after rapid addition of 100 μL of 10 mM 5,5′-dithiobis 2-nitrobenzoic acid (DTNB), 1 unit of glutathione reductase (in 50 μL), and 50 μL of 4.3 mM NADPH. Blanks consisted of 10 μL PBS or 10 μL aliquots of wash medium. Total GSH (GSSG-GSH) content in CC and oocytes was calculated from a standard GSH curve [40].

Thiobarbituric-Acid-Reactive Substance Method

Lipid peroxidation levels were measured using the thiobarbituric-acid-reactive substance (TBARS) method. The TBARS concentration in COC was measured spectrophotometrically and expressed as the MDA level (nmol MDA/20 COCs) using tetramethoxypropane (TMP) as a standard. After completion of IVM, all COCs from each treatment (n = 20) in a batch were grouped and placed in microtubes containing 100 μL PBS, frozen at − 20 °C, and thawed at RT. This procedure was repeated three times and then the supernatant was mixed with 100 μL of 8.1% SDS solution and 750 μL of 20% acetic acid solution. After adding 750 μL of 0.8% TBA solution and 2-mL distilled water, the mixture was heated in a boiling water bath for 1 h at 90 °C. It was then cooled at RT and centrifuged at 4220×*g* for 15 min. Finally, the absorbance of the supernatant was measured at 532 nm using a spectrophotometer. The value was subsequently determined based on comparison with a TMP standard curve.

Total Antioxidant Status

The determination of TAC was carried out with colorimetric method using the Randox total antioxidant status kit (cat no. NX2332, Randox Laboratories Ltd, Crumlin, UK), with slight modifications. Briefly, after completion of IVM, all COCs from each treatment (n = 20) in a batch were grouped and placed in microtubes containing 100 μL PBS, frozen at − 20 °C, and thawed at RT. This procedure was repeated three times and then 20 μL of supernatant was added to 1 mL of the chromogen 2,2'-Azino-di- (3-ethylbenzthiazoline sulfonate) (ABTS). Twenty microliters of 6-hydroxyl-2, 5, 7, 8-

tetramethylchroman-2-carboxylic acid (Trolox) at a concentration of 2.27 mmol/L was used as standard, whereas 20 μL of deionized water was used as blank. Chromogen (1 mL) was added to standard and blank samples. The absorbance was measured with a spectrophotometer at 600 nm 3 min after substrate addition. Results were expressed as mmol/L. Measurements in duplicate were used to calculate intra-assay variability.

Se Concentrations in Follicular Fluid and IVM Medium

Aberdeen Angus ovaries were wrapped with plastic film and taken to the laboratory in an icebox within 2 h after slaughter. Follicle diameter was measured with a vernier caliper and then only large follicles (the largest follicles were 11–12 mm, but most of them were ~ 10 mm) were aspirated. Follicular fluid was collected from each follicle by aspiration with an insulin syringe. Samples were kept on ice until centrifugation. Samples of IVM medium with 10% FCS were also collected ($n = 5$). Selenium concentration was measured by atomic absorption spectrophotometer (GBC 902) with graphite furnace through an internal quality control [31].

Experimental Design

Effect of Se on Viability

In experiment 1, the effect of Se on CC viability following the addition of 0, 10, 50, or 100 ng/mL Se to IVM medium was evaluated. The COCs were matured for 24 h (as described above), and viability was evaluated as already mentioned. For this purpose, 512 COCs were matured in five replicates and at least 200 CC per treatment were analyzed in each replicate.

Effect of Se on Apoptosis

In experiment 2, the effect of Se supplementation to IVM medium with 0, 10, 50, or 100 ng/mL Se on CC apoptosis was determined by Annexin V Staining Assay. The IVM was performed as described above. For this purpose, 400 COCs were matured in four replicates.

Effect of Se on Total GSH Concentration

In experiment 3, the effect of adding 0, 10, 50, or 100 ng/mL Se to maturation medium on intracellular GSH-GSSG concentrations in both oocytes and CC was evaluated. The COCs were matured for 24 h (as described above), and total GSH concentrations were evaluated as previously mentioned. For this purpose, 320 COCs were matured in four replicates.

Effect of Se on Lipid Peroxidation

In experiment 4, the effect of Se on MDA levels following the addition of 0, 10, 50, or 100 ng/mL Se to IVM medium was evaluated by TBARS. The COCs were matured for 24 h (as described above) and total MDA level was evaluated as previously mentioned. For this purpose, 320 COC were matured in four replicates.

Effect of Se on TAC

In experiment 5, the effect of adding 0, 10, 50, or 100 ng/mL Se to maturation medium on TAC in COC was evaluated. The COCs were matured for 24 h (as described above) and TAC was evaluated as previously mentioned. For this purpose, 320 COCs were matured in four replicates.

Effect of Se on Oocyte Nuclear Maturation

In experiment 6, the effect of Se supplementation to IVM medium with 0, 10, 50, or 100 ng/mL Se on oocyte nuclear maturation was determined. The IVM was performed as described above. For this purpose, 357 COCs were matured in five replicates.

Effect of Se on Subsequent Embryo Development

In experiment 7, the developmental capacity of oocytes matured in IVM medium supplemented with 0, 10, 50, or 100 ng/mL Se was investigated. IVM, IVF, and IVC were performed as described above. For this purpose, 1336 COCs were matured in eight replicates.

Statistical Analysis

A completely randomized block design was used. The statistical model included the random effects of block (replicate; $n = 4–8$) and the fixed effect of treatment (0 vs 10 vs 50 vs 100 ng/mL Se). The analysis of TAC was performed with linear models using the MIXED procedure of SAS (SAS Institute, Cary, NC, USA). Oocyte nuclear maturation; CC viability; CC apoptosis; and cleavage, blastocyst, and hatching rates were analyzed by logistic regression using the GENMOD procedure (SAS Institute), whereas TBARS and total GSH were analyzed using the GLIMIX procedure (SAS Institute) with gamma distribution. TAC, TBARS, and total GSH are expressed as mean ± standard error of the mean (SEM). Oocyte nuclear maturation; CC viability; CC apoptosis; and the rates of cleavage, blastocyst, and hatching are expressed as percentage. Statistical significance was set at $p < 0.05$, while a trend for statistical significance was set between $p > 0.05$ and ≤ 0.10.

Results

Selenium determination in follicular fluid and IVM medium

Selenium concentration was 128.8 ± 7.9 ng/mL Se in FF and was not detected in IVM medium.

Effect of Se on Viability

In experiment 1, CC viability was increased when Se was added to IVM medium ($p < 0.05$), but the difference was highest with the addition of 100 ng/mL Se (60.1, 64.1, 66.5, and 69.0 % for 0, 10, 50, and 100 ng/mL Se, respectively; $p < 0.05$).

Effect of Se on Apoptosis

In experiment 2, results revealed a decrease of late-apoptotic cells when COCs were matured in the presence of Se ($p < 0.05$). The percentage of necrotic cells after IVM was lower in CC matured with 100 ng/mL Se than in CC matured without Se ($p < 0.05$). In addition, the percentage of non-apoptotic cells was higher when 50 and 100 ng/mL Se were added to IVM medium ($p < 0.05$; Table 1).

Effect of Se on Total GSH Concentration

In experiment 3, intracellular GSH-GSSG concentrations did not differ significantly in oocytes and CC in the presence of 0, 50, or 100 ng/mL Se during IVM. However, GSH-GSSG concentration was increased by 10 ng/mL Se ($p < 0.05$; Table 2).

Effect of Se on Lipid Peroxidation

In experiment 4, COC incubated with Se showed a significant decrease in MDA level with respect to the control ($p < 0.05$). The lowest MDA concentration was observed with 10 and 50 ng/mL Se ($p < 0.05$; Fig. 1).

Effect of Se on TAC

In experiment 5, the mean TAC of COC matured with Se was similar to that of the control ($p > 0.05$). However, COCs matured in 100 ng/mL Se showed lower TAC than those matured in 10 and 50 ng/mL Se ($p < 0.05$; Fig. 2).

Effect of Se on Oocyte Nuclear Maturation

In experiment 6, nuclear maturation was not significantly different in oocytes matured with 0, 10, 50, and 100 ng/mL Se concentrations (Table 3; $p > 0.05$).

Effect of Se on Subsequent Embryo Development

In experiment 7, no differences were detected in cleavage and blastocyst rates when Se was added to IVM medium ($p > 0.05$). However, 10 ng/mL Se in IVM medium improved the hatching rate ($p < 0.05$; Table 4).

Discussion

The objective of this study was to investigate the effects of Se supplementation during IVM of *Bos taurus* oocytes within the reference ranges for Se status in cattle. Our results demonstrated that Se concentration in FF was 128.8 ± 7.9 ng/mL. The addition of Se during IVM at marginal and adequate concentrations increased viability and non-apoptotic CC. Moreover, the addition of Se to culture media decreased MDA level in COC with all studied concentrations and increased GSH-GSSG content in CC and oocytes with 10 ng/mL Se. On the other hand, TAC of COC, nuclear maturation, and the developmental capacity of oocytes were not modified by Se

Table 1 Apoptosis in cumulus cells matured in vitro with various Se concentrations

Se supplementation		Non-apoptotic	Early-apoptotic	Late-apoptotic	Necrotic
(ng/mL)	(*n*)	(%)	(%)	(%)	(%)
0 (control)	600	68.6 [a]	1.8 [a]	19.5 [a]	10.0 [a]
10	600	67.5 [a]	2.3 [a]	14.6 [b]	15.5 [b]
50	597	74.3 [b]	1.3 [a]	15.5 [b]	8.7 [a]
100	599	76.7 [b]	2.5 [a]	15.0 [b]	5.6 [c]

Bovine COCs were incubated in IVM medium with 0, 10, 50, and 100 ng/mL Se during 24 h. Apoptosis was evaluated by Annexin-V-FITC - propidium iodide (PI). CCs (cumulus cells) were classified as non-apoptotic (Annexin V–negative/PI-negative); early-apoptotic (Annexin V–positive/PI-negative); late-apoptotic (Annexin V–positive/PI-positive), and necrotic (Annexin V–negative/PI-positive). Data are expressed as percentage. For this purpose, 400 COCs were matured in four replicates

[a–c] Values with different superscript within each column differed ($p < 0.05$)

Table 2 Total intracellular glutathione concentration in bovine oocytes and cumulus cells matured with various Se concentrations

	Se supplementation (ng/mL)			
	0	10	50	100
Oocyte GSH-GSSG (pmol/oocytes)	5.98 ± 0.05 [a]	8.41 ± 0.04 [b]	5.99 ± 0.05 [a]	6.35 ± 0.04 [a]
Cumulus cells GSH-GSSG (nmol/106 cells)	0.76 ± 0.07 [a]	0.94 ± 0.07 [b]	0.73 ± 0.09 [a]	0.66 ± 0.09 [a]

Bovine COCs were incubated in IVM medium with 0, 10, 50, and 100 ng/mL Se during 24 h. Data are expressed as mean ± SEM. For this purpose, 320 COCs were matured in four replicates

[a,b] Values with different superscript within each row differed ($p < 0.05$)

supplementation to IVM medium, but an increase in hatching rate was observed with 10 ng/mL Se.

Recently, Xiong et al. [28] reported that Se content in FF of *Bos grunniens* (a wild bovine species) was 69 ± 3 ng/mL, whereas in the present study, the Se concentration was twice as high. Selenium concentration found in FF of *Bos taurus* was similar to the concentration considered as adequate in plasma. In the present study, IVM medium without Se or with a deficient Se concentration had a detrimental effect on CC viability after maturation. Indeed, although apoptosis in CC was significantly decreased when Se was added to IVM medium, only an adequate Se concentration was able to reduce the percentage of necrotic cells. This finding is in disagreement with Mauro et al. [41], who observed that the presence of 5, 50, or 500 ng/mL Se in the culture medium did not modify viability or apoptosis of HT29 cells with respect to the control. However, our results are consistent with that observed by Zou et al. [42] in bovine mammary epithelial cells (BMEC). These researchers demonstrated that sodium selenite increased viability and reduced apoptosis and necrosis of BMEC exposed to heat stress [42]. The antioxidant role of Se may be an important mechanism in maintaining cell viability and preventing apoptosis. It has been demonstrated that sodium selenite protects porcine embryo cells against oxidants and apoptosis [43].

The importance of Se in cellular antioxidant defense has been widely studied. It has been shown that Se may exert its beneficial effects through selenoproteins such as GPx and TrxR [18]. Glutathione peroxidase enzymes eliminate hydrogen peroxide and lipid peroxides generated by free radicals and other oxygen-derived species [44]. Malondialdehyde is considered the major breakdown product split off from lipid peroxides. An inverse correlation between plasma Se and plasma MDA contents has been reported in pregnant women [45]. In rats with hepatic damage induced by silver nanoparticles, sodium selenite elevated the level of GSH and the activity of GPx, catalase, and superoxide dismutase in liver [46]. Recently, Ceko et al. [47] demonstrated that Se and GPx1 are present in bovine granulosa cells of large follicles and play a critical role as antioxidants during late follicular development. In addition, culture of mouse preantral follicles in the presence of sodium selenite increased GPx activity and TAC,

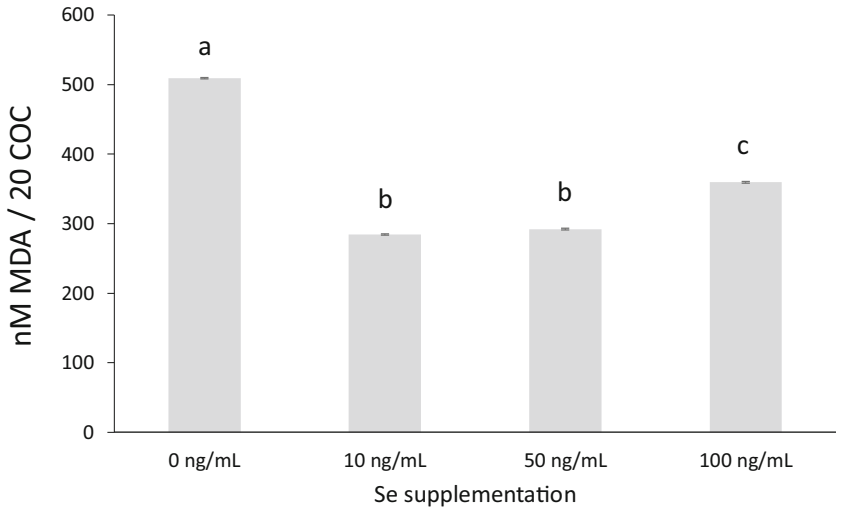

Fig. 1 Lipid peroxidation in cumulus–oocyte complexes matured in vitro with various Se concentrations. Bovine COCs were incubated in IVM medium with 0, 10, 50, and 100 ng/mL Se during 24 h. Lipid peroxidation levels were measured using the thiobarbituric-acid-reactive substances (TBARS) method and expressed as nmol MDA/20 COC. Values are expressed as mean ± SEM. For this purpose, 320 COCs were matured in four replicates. MDA, malondialdehyde. (a–c) Bars with different letters differed statistically ($p < 0.05$)

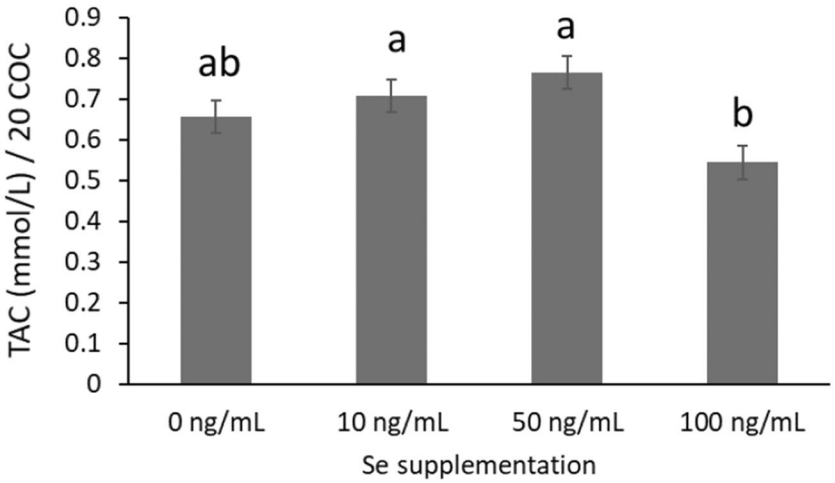

Fig. 2 Total antioxidant capacity in cumulus–oocyte complexes matured in vitro with various Se concentrations. Bovine COCs were incubated in IVM medium with 0, 10, 50, and 100 ng/mL Se during 24 h. Total antioxidant capacity is expressed as mmol/L. Data are expressed as mean ± SEM. For this purpose, 320 COCs were matured in four replicates. TAC, total antioxidant capacity. (a–c) Bars with different letters differed statistically ($p < 0.05$)

improving follicular development in vitro [48]. In the present study, addition of Se to IVM medium did not modify COC TAC, but MDA level was decreased with all the concentrations tested, and GSH-GSSG content was increased with 10 ng/mL Se supplementation.

Although we did not examine GPx activity in this study, this could be increased by addition of Se to IVM medium.

In the female reproductive system, although the target organ for Se action is currently unclear, it is well known that reduction in fertility is possibly related to Se deficiencies [49]. A significant decrease in follicular fluid Se levels was found in women with unexplained infertility [50]. According to Basini and Tamanini [49], Se not only prevents oxidative damage but might also influence the expression of the FSH receptor in granulosa cells. In addition, these researchers showed that Se modulates granulosa cell proliferation and estradiol synthesis [49]. In 2012, Makki et al. [26] demonstrated that addition of Se, calcium, and calcium ionophore to IVM medium increased the percentage of meiosis II human oocytes. In the present study, neither nuclear maturation nor the developmental capacity of oocytes was modified by Se supplementation. However, hatching rate was increased with 10 ng/mL Se in IVM medium. This is in agreement with Shamsuddin et al. [51] who reported that insulin, transferrin, and Se supplementation during bovine IVM and IVF did not modify cleavage and blastocyst rates, but increased blastocyst quality exhibiting better viability and post-thaw survivability. In pig, Uhm et al. [43] showed that addition of sodium selenite to IVC media increased the development rate and quality of parthenotes. In the present study, the Se concentration that improved embryo quality (hatching rate) was the same that increased GSH levels after IVM. In this sense, a strong relationship between GSH level in oocytes and embryo development has been reported in the literature. Intracellular GSH content in oocytes at the end of IVM reflects the degree of cytoplasmic maturation [40, 52, 53]. Therefore, an increase in GSH concentrations during IVM of cattle oocytes improves embryo development and quality [40, 53–55]. It is not clear why the Se concentration considered as deficient (10 ng/mL) was the only one that improved total GSH content in the present study. A plausible explanation is that, in vivo, Se is mainly contained in selenoproteins, whereas in vitro, Se was supplemented as a sodium selenite inorganic salt. Selenite undergoes a thiol-dependent reduction to form selenide before being incorporated into specific selenoproteins, oxidizing GSH to GSSG [56, 57]. This reaction can result in GSH depletion due to the export of cellular GSSG via a transporter [58]. Shalini and Bansal [59] showed that the addition of sodium selenite to testicular cells in vitro at the concentrations

Table 3 Meiotic maturation of bovine oocytes matured in vitro with various Se concentrations

	Se supplementation (ng/mL)			
	0 (control)	10	50	100
Oocyte (*n*)	77	72	91	117
GV	1.3	2.8	0.0	0.0
MI	7.8	6.9	11.0	6.0
A-T	0.0	0.0	0.0	0.9
MII+PB	88.3	87.5	85.7	88.9
D	2.6	2.8	3.3	4.3

Differences among treatments within each category were not significant ($p > 0.05$). Bovine COCs were incubated in IVM medium with 0, 10, 50, and 100 ng/mL Se during 24 h. Data are expressed as percentage. For this purpose, 357 COCs were matured in five replicates. *GV*, germinal vesicle; *MI*, metaphase I; *A-T*, anaphase-telophase; *M II+ PB*, metaphase II and the first polar body; *D*, degenerate

Table 4 Developmental capacity of cattle oocytes matured in vitro with various Se concentrations

Se supplementation (ng/mL)	Oocytes (*n*)	Cleaved (%)	Blastocyst/ oocytes (%)	Blastocyst/ cleaved (%)	Hatched (%)
0 (Control)	321	77.25[a]	25.54[a]	30.39[a]	47.56[a]
10	365	75.06[a]	27.67[a]	36.76[a]	64.35[b]
50	316	77.53[a]	20.56[a]	26.75[a]	50.76[ab]
100	334	75.74[a]	28.14[a]	36.69[a]	55.31[ab]

Bovine COCs were incubated in IVM medium with 0, 10, 50, and 100 ng/mL Se during 24 h. Cleavage rates were recorded 48 h after insemination. Data reported for development to the blastocyst stage included those embryos that progressed to the expanded or hatched blastocyst stages after 8 days in culture. Data are expressed as percentage. For this purpose, 1336 COCs were matured in eight replicates.

[a,b] Values with different superscript within each column differed ($p < 0.05$)

that here are considered as adequate (about 118 ng/mL) significantly decreased GSH content.

In conclusion, COC incubation with Se increased CC viability and reduced lipoperoxidation level in COC. However, only the Se concentration considered as deficient improved GSH-GSSG content in oocytes and CC, as well as hatching rate. Thus, supplementation with 10 ng/mL Se during in vitro maturation of *Bos primigenius taurus* oocytes should be considered to improve embryo quality.

Author contributions R.M.L. and J.M.A. designed the study, E.M.G., N.A.F., and A.C-M. assisted with data collection. C.C.F. and G.A.M analyzed the data and J.P.A. coordinated the experiments and revised the manuscript. All co-authors participated in lab work.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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