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Comparative efficacy of antioxidant retinol, melatonin, and zinc during in vitro maturation of bovine oocytes under induced heat stress

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Abstract: Both A and B grade cumulus-oocyte complexes (COCs) aspirated from cattle ovaries at slaughter were matured in vitro under normal (38.5 °C) and elevated temperatures (41 °C). Maturation competence based on cumulus expansion, COC diameter, and nuclear maturation were compared with and without antioxidant supplementation incorporating 7.5 µM retinol, 1 nM melatonin, and 1.5 µg/mL zinc chloride in an oocyte maturation medium. Heat stress significantly reduced cumulus expansion by approximately 20%, while only retinol could bring a significant ($P \leq 0.05$) increase. Heat stress also decreased expansion of A (approximately 50%) and B (approximately 40%) grade COC diameter. All antioxidants significantly increased COC diameter at 38.5 °C in grade A COCs, but only retinol could significantly increase grade B COCs. At 41 °C, only retinol in grade A COCs significantly enhanced diameter. Elevated temperature also decreased the metaphase II stage of nuclear maturation by approximately 75%, and no antioxidant was protective, except retinol, which was only marginally so. Retinol (7.5 µM) was further supplemented in maturation and culture medium for in vitro embryonic development at 38.5 °C and demonstrated significantly higher ($P \leq 0.01$) maturation, fertilization, and a 2–4 cell cleavage rate. Retinol supplementation not only showed better maturation results, but also was a better antioxidant in overcoming the deleterious effects of elevated temperature.

Key words: Bovine, heat stress, in vitro maturation, melatonin, retinol, zinc

1. Introduction

Elevated ambient temperature is a major factor responsible for reduced fertility in farm animals. Although the normal body temperature of a cow is 38.7 °C, during temperature extremes rectal temperatures may reach or exceed 41 °C (1). High ambient temperatures negatively impact reproductive processes, which has been well documented in the literature (2). Production of free radicals had been suspected during heat shock, which has now been well established and was found to promote cellular oxidation events (3). Incorporation of antioxidants, on the other hand, has been reported to moderate the deleterious effects of heat stress on oocytes (4). Supplementation with exogenous antioxidants has been found to increase the chance of embryos, even those of fair quality, to develop to blastocysts, and not only during heat stress (5,6).

Retinol as an antioxidant supplement has been attributed with many beneficial effects including improvement of nuclear competence and cytoplasmic competence of in vitro maturation (IVM) oocytes (7). The antioxidant role of melatonin is also well known and its metabolites have been shown to be potent scavengers of free radicals (8). Likewise, zinc also has antioxidant properties reducing intracellular reactive oxygen metabolites in oocytes (9).

The effect of the antioxidants retinol, melatonin, and zinc on in vitro fertilization and embryo development have been variously documented in the literature; however, to our knowledge, their comparative assessment in regards to oocyte maturation competence is lacking. Besides, their role in protecting against deleterious heat stress effects warrants investigation. As such, the present study was designed to compare these antioxidants, probably for the first time, during in vitro maturation of bovine oocytes under normal (38.5 °C) and elevated (41.0 °C) temperature. It was also intended to find the best antioxidant for embryonic development of in vitro matured oocytes under normal temperature (38.5 °C).

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2. Materials and methods

2.1. Collection of oocytes and in vitro maturation

Aspiration medium, oocyte maturation medium (OMM), and IVM medium were prepared according to Dutta et al. (10). Ovaries from cows were collected from local abattoirs immediately after slaughter and transported to the laboratory in sterile prewarmed normal saline containing antibiotics (penicillin G at 0.06 g/1000 mL) at 37 °C. The connective tissues covering the ovaries were removed and they were washed 3 times with normal saline containing antibiotics. Cumulus-oocyte complexes (COCs) were collected by aspiration of surface follicles with a sterile 18-gauge needle attached to a 10-mL syringe containing the aspiration medium. Only follicles of 2–8 mm in diameter were selected among those present on the surface. The COCs were separated from the debris, picked out individually under a stereo-zoom microscope, placed into another petri dish with washing medium, and graded according to Hafez and Hafez (11), while only grade A and B COCs were selected for in vitro maturation.

IVM droplets were prepared by taking 50 µL of in vitro OMM into a 35-mm petri dish and covering it with 0.2 µm of sterile filtered mineral oil and incubating for 1 h in a CO_2 incubator at 38.5 °C with 5% CO_2 and humidified air. Selected COCs (A and B grade) were washed 6 times in washing medium and twice in OMM medium.

Approximately 10–12 washed COCs were then transferred into each OMM droplet for maturation and incubated for 24 h under the same conditions. For heat stress studies COCs were exposed to a temperature of 41 °C during the first 12 h of IVM as described by Roth and Hansen (12).

2.1.1. Antioxidant supplementation

OMM was supplemented with 7.5 µM retinol (Sigma, India) modified according to the procedure of Livingston et al. (7), with 1 nM melatonin (Sigma, India) modified according to the procedure of Jang et al. (13) and prepared according to Farahavar et al. (14), or with 1.5 µg/mL (approximately 11 mM) zinc modified according to the procedure of Picco et al. (15) as zinc chloride (Sigma, India).

2.1.2. Fixation and staining of oocytes

After the completion of IVM, the oocytes were prepared for fixation and subsequent staining. Briefly, in vitro matured COCs were exposed to 0.2 % hyaluronidase for 1 min to loosen the cumulus cells and vortexed for 2–3 min. Cumulus-free oocytes were washed 3 times in oocyte maturation medium and fixed with acetic alcohol (acetic acid and ethyl alcohol, 1:3 parts) and stained with either acetoorcein or with the nuclear fluorescent stain DAPI (4',6-diamidino-2-phenylindole, NucBlue Fixed Cell ReadyProbes Reagent, Life Technology, India).

2.2. In vitro fertilization (IVF) of oocytes

Commercially available good quality frozen bull semen straws stored in liquid nitrogen were used and sperm capacitation was done by the swim-up technique using Bracket and Oliphant (BO) medium (16).

IVF droplets were prepared by taking 50 µL of IVF medium covered with sterile mineral oil and incubated for 1 h in a CO_2 incubator at 38.5 °C with 5% CO_2 and humidified air. Then 25 µL of fertilization medium was discarded from the IVF droplets.

In vitro matured oocytes were taken out of OMM droplets and washed 6 times in a washing medium while the IVM droplets containing the cumulus remnants were kept as such in the CO_2 incubator for later use and for culture of the presumed zygote. About 12 in vitro matured oocytes were transferred to each preincubated IVF droplet of 25 µL, to which 25 µL of processed semen at a concentration of 2×10^6/mL was added for fertilization. The IVF droplets were further incubated for 18 h in a CO_2 incubator at 38.5 °C with 5% CO_2 and humidified air.

2.2.1. In vitro culture of presumed zygote

After 18 h of incubation, presumed zygotes were taken out of IVF droplets and washed 3 times in 2–3 mL of in vitro culture (IVC) medium. Simultaneously, the maturation medium was replaced by IVC medium from the OMM droplets containing the cumulus remnants. The washed presumed zygotes were vortexed for 2–3 min in washing medium for removal of dead sperm and cumulus cells that adhered to the surface of the zygotes. About 12 presumed zygotes were transferred to the OMM droplets and replaced with IVC medium and further incubated for 48 h in a CO_2 incubator at 38.5 °C with 5% CO_2 and humidified air. After every 24 h the droplets were observed for cell division/zygote formation, while approximately 60%–65% of the medium was replaced with fresh IVC medium after every 48 h of incubation.

2.3. Experimental design

In vitro maturation of bovine COCs was determined microscopically under a phase contrast microscope (Inverted Laboratory Microscope, DM IL LED, Leica) with LAS-EZ software (Leica Application Suit) after 24 h of incubation in OMM medium through experiments 1–3 and replicated many times.

2.3.1. Experiment 1: effect of antioxidants on cumulus expansion of COCs

Bovine COCs were matured with and without retinol, melatonin, and zinc supplementation and assessed visually for the extent of cumulus expansion under normal (38.5 °C) and elevated temperature (41.0 °C).

2.3.2. Experiment 2: effect of antioxidants on COC diameter

Oocyte diameter with cumulus cells before and after maturation was recorded with and without retinol,

melatonin, and zinc supplementation under normal (38.5 °C) and elevated temperature (41.0 °C).

2.3.3. Experiment 3: effect of antioxidants on nuclear maturation

Effects of retinol, melatonin, and zinc supplementation, along with a nonsupplemented control, on in vitro maturation of bovine COCs were assessed by demonstration of metaphase I and metaphase II nuclear stages by staining of oocytes.

2.3.4. Experiment 4: effect of best antioxidant on embryonic development

The antioxidant showing the best results during maturation was used as a supplement for in vitro bovine embryonic development under normal temperature (38.5 °C). The experiment was replicated 3 times.

3. Results

3.1. Experiment 1: effect of antioxidants on cumulus expansion of COCs

The results of percentage of bovine COCs undergoing cumulus expansion during IVM in different maturation medium supplementations and temperatures of incubation are summarized in Figure 1, while comparative pairwise chi-square values are depicted in Table 1.

3.1.1. Without heat stress (38.5 °C)

The best cumulus expansion was observed when the COCs were matured with retinol supplementation in the medium, with a significant increase ($P < 0.01$) compared with nonsupplemented OMM as well as with melatonin and zinc supplementation.

3.1.2. With heat stress (41 °C)

The best maturation performance was seen when the COCs were matured with retinol-supplemented medium. Within nonsupplemented groups, heat stress significantly ($P < 0.01$) reduced the maturation percentage of COCs by approximately 25%. The expansion of cumulus cells in heat-stressed, nonsupplemented oocytes was significantly ($P < 0.01$) poorer than those of the not heat-stressed ones (nonsupplemented; retinol-, melatonin-, and zinc-supplemented). Among all the supplemented groups under heat stress, only retinol could bring a significant ($P < 0.05$) increase in oocyte maturation percentage compared to nonsupplemented OMM. There was also a significant ($P < 0.05$) difference with retinol supplementation between the nonstressed and heat-stressed groups. Supplementation with both melatonin and zinc during heat stress did not have any significant effect on the maturation percentage, but instead showed a significant ($P < 0.05$) difference in their competence as compared to nonstressed oocytes.

3.2. Experiment 2: effect of antioxidants on COC diameter

Table 2a shows the IVM of bovine oocytes in different supplemented mediums assessed after 24 h of maturation and the mean increase in diameter of grade A and B COCs. Analysis of variance (ANOVA) showing the effect of antioxidant supplementation on bovine COC maturation with and without heat stress is represented in Table 2b.

3.2.1. Without heat stress (38.5 °C)

The highest mean increase in grade A and B COC diameter during IVM was observed to be with retinol-supplemented

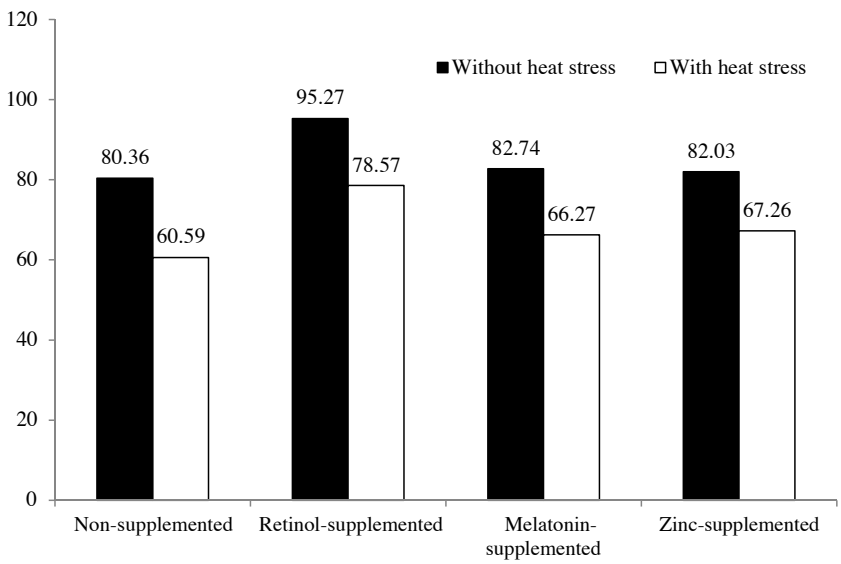

Figure 1. Percentages of matured bovine oocytes based on cumulus cell expansion in different antioxidant-supplemented oocyte maturation media with (41 °C) and without (38.5 °C) heat stress.

Table 1. Pairwise chi-square values of in vitro maturation of bovine oocytes based on cumulus cell expansion in different antioxidant-supplemented oocyte maturation medium (OMM), with and without heat stress.

			Without heat stress (38.5 °C)				With heat stress (41 °C)			
			OMM	OMM + retinol	OMM + melatonin	OMM + zinc	OMM	OMM + retinol	OMM + melatonin	OMM + zinc
Independent chi-square values	Without heat stress (38.5 °C)	OMM		8.74 **	0.16	0.08	7.97 **	0.94	4.28 *	3.73
		OMM + retinol			6.74 **	7.29 **	29.66 **	5.80 *	22.87 **	21.67 **
		OMM + melatonin				0.01	23.35 **	1.82	6.03 *	25.40 **
		OMM + zinc					9.54 **	1.51	5.47 *	4.84 *
	With heat stress (41 °C)	OMM						4.02 *	0.59	0.81
		OMM + retinol							1.56	1.24
		OMM + melatonin								0.02
		OMM + zinc								

*Significant at P ≤ 0.05; **significant at P ≤ 0.01.

medium. There was a significant (P < 0.05) increase in the grade A COC diameter in retinol-, melatonin-, and zinc-supplemented OMM compared to nonsupplemented ones. Although the significance was higher with retinol and melatonin supplementation than with zinc, there was no significant difference between the retinol- and melatonin-supplemented groups. Contrastingly, in grade B COCs, the COC diameter only increased significantly (P < 0.05) in retinol supplementation.

3.2.2. With heat stress (41 °C)

The highest mean increase in grade A and B COC diameter during IVM was observed to be with retinol-supplemented medium. The diameter in heat-stressed COCs was correspondingly less than that of the unstressed ones. Except for retinol supplementation in the OMM of grade A COCs, none of the other supplements in either A or B grade COCs significantly increased the COC diameter.

When the mean grade A COC diameter in the nonsupplemented group without heat stress was compared to the corresponding nonsupplemented group with heat stress, there was approximately a 50% decrease in expansion of COC diameter.

When the mean grade B COC diameter in the nonsupplemented group without heat stress was compared to the corresponding nonsupplemented group with heat stress, there was approximately a 40% decrease in expansion of COC diameter, probably indicating that heat stress causes a decrease in COC diameter of grade A COCs slightly more so than grade B COCs during maturation.

3.3. Experiment 3: effect of antioxidant on nuclear maturation

A total of 651 oocytes were observed with various antioxidant supplementations in the maturation medium in order to observe the nuclear maturation stages metaphase I (MI) and metaphase II (MII), and the expression percentage of the stages observed with and without heat stress is presented in Figure 2.

Table 2a. Mean increase in diameter (μm) of grade A and B COCs during in vitro maturation in different antioxidant-supplemented oocyte maturation medium (OMM), with and without heat stress.

	Without heat stress (38.5 °C)		With heat stress (41 °C)	
	A grade	B grade	A grade	B grade
OMM	$157.42^{ad} \pm 12.44$	$124.49^{ae} \pm 18.43$	$79.99^{eh} \pm 18.25$	$75.47^{hj} \pm 5.72$
OMM + retinol	$380.19^{b} \pm 24.62$	$180.12^{df} \pm 18.65$	$130.97^{ai} \pm 19.87$	$104.43^{egij} \pm 16.22$
OMM + melatonin	$375.67^{b} \pm 22.10$	$151.24^{af} \pm 5.85$	$114.42^{aej} \pm 11.73$	$102.73^{egij} \pm 2.29$
OMM + zinc	$277.85^{c} \pm 22.72$	$136.87^{afg} \pm 16.25$	$93.37^{egij} \pm 10.29$	$76.97^{hj} \pm 15.28$

Means in a column or row bearing at least one common superscript do not differ significantly ($P \leq 0.05$).

Table 2b. Analysis of variance showing the effects of antioxidant supplementation on bovine COC maturation with and without heat stress, based on cumulus expansion.

Source of variation	Df	SS	MS	F-value
Temperature	1	568,685.6627	568,685.6627	237.48**
Antioxidant	3	178,096.4800	59,365.4933	24.79**
Grade of COC	1	243,214.1831	243,214.1831	101.57**
Temperature × antioxidant	3	55,058.0774	18,352.6925	7.66**
Temperature × COC grade	1	163,580.4766	163,580.4766	68.31**
Antioxidant × COC grade	3	56,658.3528	18,886.1176	7.89**
Temperature × antioxidant × COC grade	3	42,745.0484	14,248.3495	5.95**
Error	128	306,515.452	2394.652	

There was an approximately 75% decrease in the MII stage of nuclear maturation during heat stress when COCs in both nonsupplemented groups matured at different temperatures were compared. Moreover, the percentage of nuclear maturation stage MI was higher in heat-stressed oocytes, while the MII maturation percentage rate was higher in unstressed oocytes. Retinol supplementation showed the highest MII stage of nuclear maturation at both 38.5 °C and 41 °C. The percentage of oocytes in the MII stage was higher in all groups without heat stress than in corresponding groups exposed to higher temperature. For nuclear maturation stage MII, the pairwise chi-square values observed with different antioxidant-supplemented OMM with and without heat stress are presented in Table 3.

3.3.1. Without heat stress (38.5 °C)

Within the unstressed group, there was no significant difference except improvement with retinol supplementation ($P < 0.05$) when compared to the medium without any supplementation.

3.3.2. With heat stress (41 °C)

There was a dramatic and highly significant ($P < 0.01$) decrease of MII nuclear maturation stage of oocytes between the heat-stressed and nonstressed groups. All nonstressed oocytes had a highly significant ($P < 0.01$) increase in MII percentage compared to control and supplemented oocytes in the heat-stressed groups, except for retinol supplementation, which marginally gave a significant improvement of MII maturation stage ($P < 0.05$) in the heat-stressed group when compared to nonsupplemented groups as well as the groups supplemented with melatonin and zinc.

Extrusion of the first polar body of MII was observed after 24 h of maturation when visibly stained with either acetoorcein stain or DAPI. The nucleus could be easily seen with fluorescent stain. One parthenote was observed in heat-stressed nonsupplemented OMM stained with DAPI where only a female pronucleus and two polar bodies were present.

3.4 Experiment 4: effect of the best antioxidant on embryonic development

Since retinol consistently gave a better performance during in vitro maturation of COCs, it was further used during in vitro embryonic development at a working concentration of 7.5 μM supplemented in an IVC medium. The various stages of embryonic development using OMM and IVC medium supplemented with and without retinol

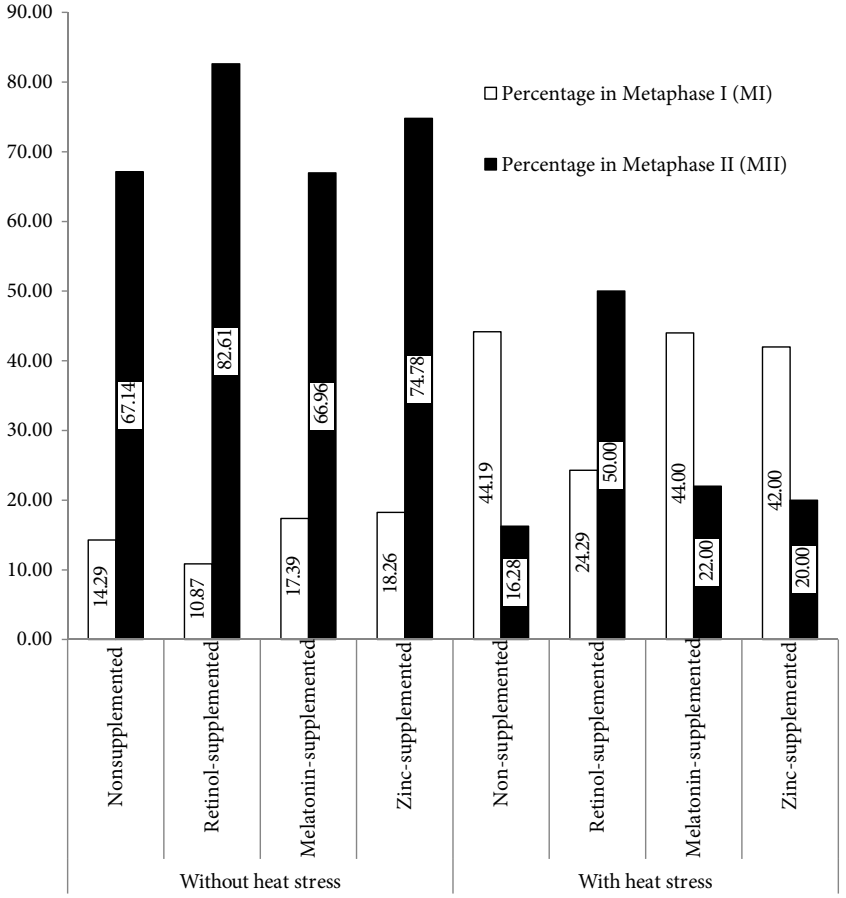

Figure 2. Expression percentages of nuclear maturation stages MI and MII observed during in vitro maturation of bovine oocytes in different antioxidant-supplemented oocyte maturation media (with and without heat stress).

were observed. The in vitro developmental competence is presented in Table 4.

Retinol-supplemented COCs demonstrated highly significant ($P < 0.01$) maturation rates compared to controls and a significantly higher ($P < 0.05$) fertilization rate than nonsupplemented controls. Although cleavage performance with retinol supplementation was higher at all embryonic stages (2–4 cells, 8–16 cells, morula, and blastocyst), a significantly ($P < 0.05$) higher performance was also observed in the 2–4 cell stage compared to the nonsupplemented control group.

4. Discussion

The present results demonstrate that incorporation of antioxidant supplements in the maturation medium could bring significant improvements in maturation competence at a controlled temperature (38.5 °C). All antioxidants were effective in enhancing cumulus diameter in controlled conditions in both grade A and B COCs, while only retinol seemed effective in grade B ones. However, except for retinol, none could significantly aid in progression to a nuclear maturation event. In this study, retinol (7.5 µM) was found to cause an expansion of cumulus cells and an increase in MII nuclear maturation, similar to earlier studies (17) in water buffaloes with 5 µM retinoic acid and bovine oocytes (18) using 0.1, 0.25, 0.5, and 1 µM retinoic acid. Likewise, the results of cumulus expansion and increase in MII nuclear maturation using 1 nM melatonin were comparable to earlier reports in bovine oocytes and mice COCs (14,19). Further, nuclear maturation achieved with the incorporation of zinc (approximately 11 mM) in the maturation medium was comparable with that reported earlier in pig COCs (20). Understandably, subtle variations from earlier reports in maturation percentage, expansion of cumulus cells, and nuclear maturation values in different supplemented and nonsupplemented media could arise due to the variations in the working concentrations of supplementations. Furthermore, variations may also be attributable to the initial quality of oocytes, genotype, physiology, and reproductive status of the animals from which the ovaries were collected.

Table 3. Pairwise chi-square values of nuclear maturation stage MII observed after in vitro maturation of bovine oocytes in different antioxidant-supplemented oocyte maturation medium (OMM) with and without heat stress.

			Without heat stress (38.5 °C)				With heat stress (41 °C)			
			OMM	OMM + retinol	OMM + melatonin	OMM + zinc	OMM	OMM + retinol	OMM + melatonin	OMM + zinc
Independent chi-square values	Without heat stress (38.5 °C)	OMM	-	4.21 *	0.00	0.78	10.51 **	2.12	9.92 **	10.83 **
		OMM + retinol		-	3.78	1.05	15.46 **	8.18 **	16.09 **	17.04 **
		OMM + melatonin			-	0.85	8.79 **	1.98	8.58 **	9.34 **
		OMM + zinc				-	12.04 **	4.47 *	12.14 **	13.03 **
	With heat stress (41 °C)	OMM					-	4.94 *	0.26	0.12
		OMM + retinol						-	4.03 *	4.67 *
		OMM + melatonin							-	0.03
		OMM + zinc								-

*Significant at $P \leq 0.05$; **significant at $P \leq 0.01$.

Exposure of COCs during maturation to elevated temperatures (41 °C) during the first 12 h of maturation disrupts oocyte function as determined by a reduction of cumulus expansion and nuclear maturation. Supplementation of antioxidants in the maturation medium could bring partial or significant improvement in maturation competence in heat-stressed COCs, pointing to an underlying mechanism of oxidative stress.

Of particular interest is the protective role of retinol during elevated temperature whereby it brought about the most competent cumulus expansion compared with the other antioxidant supplements used, though this seemingly was limited to grade A COCs only; however, specific execution mechanisms were not determined. Similarly, only retinol could marginally ameliorate the effects of heat stress on MII nuclear progression, while both melatonin and zinc showed no effect. The benefits of retinol to improve the oocyte MII progression and decrease the exocytosis pattern have been documented (21), where heat stress dramatically affected bovine oocyte maturation at both nuclear and cytoplasmic levels. Reportedly, there is a strong indication of retinol's influence to be transcriptionally oriented and mediated by specific receptors in COC cells (22,23), resulting in an effectual oocyte maturation and therefore its overall competence. This, and further evidence of the retinoid inducible MAP kinase phosphatase gene (24) and the gene expression of midkine (25), is thus a plausible explanation for retinol-induced maturation competence.

Melatonin at the concentration presently used (1 nM) could not produce an improvement in nuclear development competence. Takada et al. (26) similarly opined that although melatonin (10^{-9} M) during IVM can decrease the DNA damage in cumulus cells of cattle, it could not significantly affect development rates. On the other hand, there are also suggestions that melatonin's regulation of oocyte maturation capacity is dose-dependent (27) and induces nuclear maturation through the maturation-

Table 4. In vitro developmental competence of bovine COCs in retinol-supplemented OMM and IVC medium.

	Maturation performance			Fertilization performance		Cleavage performance			
	No. of COCs	No. of COCs matured	Maturation %	No. fertilized	Fertilization %	2–4 cells (%)	8–16 cells (%)	Morula (%)	Blastocyst (%)
Nonsupplemented	281	225	80.07	145	64.44	145 (64.44)	90 (62.07)	44 (30.34)	44 (30.34)
OMM/IVC + retinol	241	229	95.02	168	73.36	168 (73.36)	120 (71.42)	63 (37.50)	63 (37.50)
Chi-square value		25.59 **		4.22 *		4.22 *	3.08	1.77	1.77

*Significant at $P \leq 0.05$; **significant at $P \leq 0.01$.

promoting factor and germinal vesicle break-down of oocytes (28). The toxic effects of melatonin have also been highlighted, in that 10^{-4} M melatonin alleviated bovine oocytes from the harmful effects of heat stress, but it was toxic for bovine oocytes at 10^{-3} M concentrations (29). Contrarily, melatonin was found to lower maturation in mouse oocytes (27). It is therefore suggested that a higher nontoxigenic concentration could probably have a better performance in alleviating the harmful effects of heat stress.

Zinc used at a concentration of 1.5 µg/mL failed to show any significant increase in cumulus cell expansion, but it increased the diameter of grade A COCs in controlled conditions. It was found to be the least effective antioxidant in ameliorating the harmful effects of heat stress.

It is likely that the parthenote observed in the present study was due to heat stress, as reported earlier (30) in murine oocytes exposed to extreme elevations in temperature (44 to 45 °C). This could probably occur by a mechanism allowing progress through MII-meiotic arrest, induced through intracellular calcium oscillations akin to fertilization by sperm.

Retinol (7.5 µM) was found to show consistently better results in maturation events during both controlled and elevated temperatures and hence was selected for embryonic development studies. Likewise, it demonstrated better maturation, fertilization, and cleavage performance compared to nonsupplemented control COCs. Similar findings have been reported using 5 µM retinoic acid on water buffalo embryos (17). Of particular interest is the significant increase in the 2–4 cell stage of cleavage with retinol supplementation in the present study. Reportedly, development of bovine blastocysts below 20% was dramatically increased with 5 µM retinol (7). Several transcripts of retinoid receptors have been identified in bovine oocytes and embryos from the 2-cell to the hatched blastocyst stage (23), thought to be targets of the transcriptional influence of retinol.

In the present study, exposure to a working concentration of 7.5 µM retinol during IVM and culture was found to be effective in bringing a desired increase in oocyte and embryonic performance. Although the free radical or transcript events were not monitored, it is presumed that the favorable effects induced by 7.5 µM retinol supplementation during embryonic development may have been realized via one or more of the pathways of retinol influence.

In conclusion, supplementations of antioxidants in optimum concentrations have been demonstrated to have a positive effect on oocyte maturation and embryo development events. In comparison, 7.5 µM retinol supplementation not only showed better maturation results, but also was a better antioxidant in overcoming the deleterious effects of elevated temperature. Since this study was conducted using a single antioxidant concentration, it is advocated that an optimum nontoxic concentration may be worked out for bringing effective enhancement in oocyte maturation and embryonic development competence.

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