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In vitro oocyte maturation with eugenol antioxidant improves the quality of bovine embryos

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INTRODUCTION

In vitro production of bovine embryos is used worldwide, although only 30 to 40% of selected oocytes reach the blastocyst stage. It occurs due to the excessive formation of reactive oxygen species (ROS), especially in the *in vitro* maturation (IVM) stage. Therefore, new potent antioxidants such as eugenol (EU; 4-allyl-2-methoxyphenol) represent a promising strategy to reduce ROS, improve oocyte maturation status and blastocyst formation. EU is a natural phenolic compound present in essential oils of some aromatic plants, such as cloves. It has several pharmacological properties, including antioxidants. Due to its great antioxidant importance, the EU has been used in the culture of different cell types. However, it has not yet been tested in the IVM medium. Thus, this work aimed to investigate the impact of EU supplementation to the IVM medium during *in vitro* production of bovine embryos.

METHODOLOGY

Therefore, a total of (n = 516) cumulus-oocyte complexes were *in vitro* matured for 22-24h in TCM-199^{+} alone (control treatment) or supplemented with EU at concentrations of 10 (EU-10), 20 (EU- 20) or 40 µM (EU-40; **Fig. 1**). After IVM, the oocytes were submitted to *in vitro* fertilization (IVF) for 18-22h and further culture of embryos for 3, 7 and 10 days. The maturation (22-24h), cleavage (day 3), blastocysts (days 7 and 10) and hatching rates (day 10), as well as the total number of cells/expanded blastocyst (day 7), was observed. The experiment was repeated three times. The variables were analysed by one-way ANOVA followed by chi-square, Fisher's exact, post hoc or Pearson's correlation tests. Statistical significance was defined as $P < 0.05$.

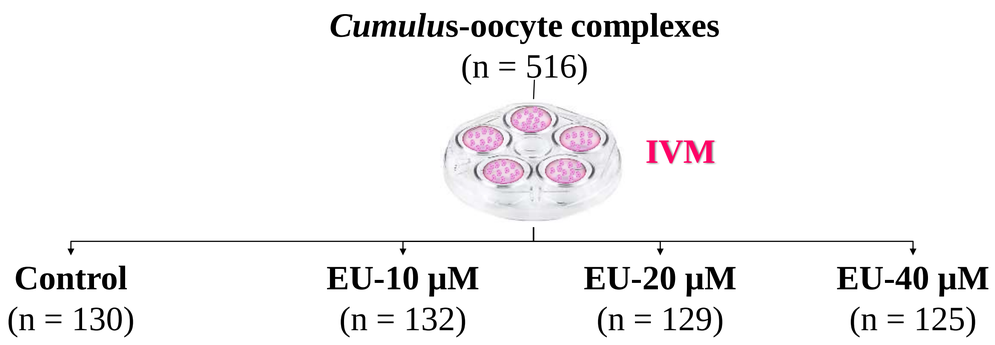

Fig 1. Schematic and representative design of the experimental phase.

RESULTS AND DISCUSSION

Resulting no significant ($P < 0.05$) difference was observed between treatments for the maturation **(Fig. 2A** and **B)**, blastocyst and hatching rates (**Table 1).** However, the addition of 40 µM EU to the IVM medium improved ($P < 0.05$) the cleavage rate than the control treatment. Furthermore, a positive correlation ($r = 0.61$, $P < 0.03$; **Fig. 3**) was observed between cleavage rate and EU concentration. The EU-10 and EU-20 treatments had higher ($P < 0.05$) total number (mean) of cells/expanded blastocyst than the control and EU-40 treatments.

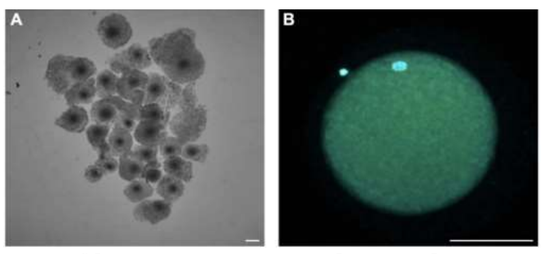

Fig 2. (**A**) Representative image of bovine oocytes with cumulus expanded after 22-24 h of IVM in the EU-10 treatment. (**B**) Metaphase II oocyte, with the extrusion of the first polar body. Bar scale = 100 µm (**A** and **B**).

Table 1. Embryo development and quality after *in vitro* maturation of bovine oocytes in the different treatments.

	Embryo development and quality			
	% Cleavage	% Blastocysts		Quality of blastocysts
Treatments†	**Day 3**	**Day 7**	**Day 10**	**TCN/Expanded blastocysts (Day 7)**
Control (n = 130)	64.6 (84)[a]	44.6 (58)[a]	46.1 (60)[a]	137.6 ± 4.6 (33)[a]
EU-10 (n = 132)	69.7 (92)[ab]	38.6 (51)[a]	40.1 (53)[a]	164.4 ± 4.4 (29)[b]
EU-20 (n = 129)	72.8 (94)[ab]	40.3 (52)[a]	42.6 (55)[a]	156.1 ± 3.7 (34)[b]
EU-40 (n = 125)	79.2 (99)[b]	41.6 (52)[a]	44.8 (56)[a]	136.1 ± 5.1 (29)[a]

[a,b] Different letters differ within the same column. TCN, total cell number.
† TCM-199^{+} alone (control treatment) or supplemented with eugenol at the concentrations of 10 µM (EU-10), 20 µM (EU-20) or 40 µM (EU-40).

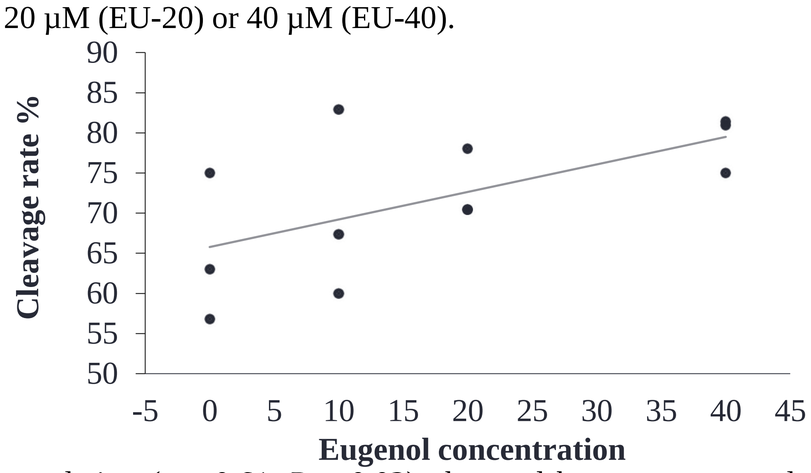

Fig 3. Positive correlation ($r = 0.61$, $P < 0.03$) observed between oocyte cleavage rate and eugenol concentration. Each dot represents a replicate within a treatment.

A previous study showed that lower EU concentrations (10-12.5 µg/mL) increased cell viability and the expression of genes related to cell survival and proliferation after murine mesenchymal stem cell culture. By considering the antioxidant effect of EU reported previously during *in vitro* culture of isolated cells i.e., human mononuclear and murine macrophages. We suggest that EU increased cleavage rate and embryo quality may be due to maintaining the redox balance in the IVM medium. In the present study, although EU at the highest concentration (40 µM) increased the cleavage rate without improving embryo quality. Previous studies have shown that the EU is a competitive inhibitor of α and β 17-β-estradiol receptors. Therefore, it may be due to the higher EU concentration during IVM may have reduced the efficiency of estradiol in improving oocyte maturation rate and embryo quality.

FINAL CONSIDERATIONS

In conclusion, the addition of EU to the IVM medium did not affect the oocyte maturation rate and blastocyst formation, but improved cleavage rate and bovine embryo quality in a concentration-dependent manner. Suggesting, the EU can be used as a potential supplement to improve *in vitro* embryo production in other species, a matter of research.

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