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Effect of myo-inositol on *in vitro* production of sheep zygotes: preliminary results

E.K.O. Aureliano[1]*, L. K. P. Oliveira[1], D.R.P. Santos[1], W.M. Souza[1], J.M. Silva[1], P.R.V. Silva[1], M. F. Cordeiro[1], E. S. Lopes Júnior[1]

[1]Laboratório de Fisiologia e Biotecnologia da Reprodução Animal – UNIVASF, Petrolina, PE, Brasil. [2]Instituto Federal de Educação, Ciência e Tecnologia do Sertão Pernambucano – IF SERTÃO-PE, Petrolina, PE, Brasil.

INTRODUCTION

The success of IVP comes mainly from the quality of oocytes and the peculiarities of the methods of recovery of cumulus-oocyte complexes (CCOs) (RODRÍGUEZ *et al*., 2006), requiring the use of antioxidants such as myo-inositol (MI). MI acts in the maintenance of cell osmolarity by presenting an antioxidant action and being considered an osmotic regulator (CONDORELLI *et al*., 2012). In addition, it showed an important role in oocyte maturation and sperm capacitation, through its role in the regulation of intracytoplasmic calcium (LOWTHER *et al*., 2009).

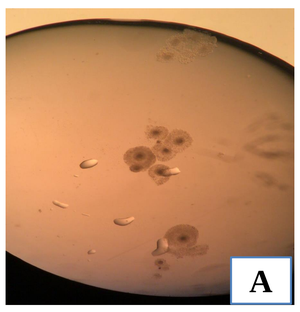
OBJECTIVE

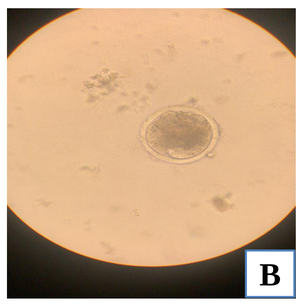
To evaluate the effect of including three concentrations of myo-inositol (20.0, 30.0 and 40.0 mM) in the oocyte *in vitro* maturation medium on the oocyte maturation rate, oocyte maturation quality and the *in vitro* production of ovine embryos.

MATERIAL AND METHODS

Oocyte collection was performed by follicular aspiration, using a vacuum pump and from ovaries obtained from a local slaughterhouse. The oocytes were poured into a petri dish, analyzed under a stereomicroscope and forwarded to in vitro maturation (IVM), being divided into four groups: CON group, oocytes were matured in medium containing TCM-199, sodium pyruvate, fetal bovine serum, antibiotic-antimycotic solution, FSH / LH and 100 mM cysteamine; groups MIO20, MIO30 and MIO40, the oocytes were matured in the medium of the CON group, supplemented with 20.0, 30.0 and 40.0 mM myo-inositol, respectively. The drops of IVM were covered with mineral oil and incubated for 24 hours, at 38.5°C, with 5% of CO_2 . After that, the oocytes were evaluated for the degree of expansion of the cumulus cells, being classified as: High, Moderate and Slight and then destined for in vitro fertilization (IVF), where they were placed in drops with IVF medium, together with selected spermatozoa and capacitated in SOF medium supplemented with 10 µg/mL of sodium heparin and 10%(v/v) of FBS. After evaluating the presumptive zygotes (PZ), they were switched to in vitro culture medium (IVC) composed of SOF medium supplemented with bovine serum albumin and cleavages at D1 and D2 were evaluated. The conditions of the IVC were the same as for IVM and IVF. Data were submitted to Analysis of Variance, followed by Tukey's Test and Fisher's Exact Test, with a significance level of 5%.($P < 0.05$).

Figura 1 – (A) Oocytes after *in vitro* maturation with addition of MI; (B) cleaved structure after IVM, IVF and initial IVC.





RESULTS

As for the degree of expansion of cumulus cells (Figure 1A), there was no difference between them. When you can see an overall expansion rate of 88.88%, when you take into account the MIO20 group, note an overall expansion rate of 94.44% (Table 1).

Table 1. Degree of expansion of cumulus cells (High, Moderate and Slight) in the *in vitro* maturation of ovine oocytes.

Treatments	N° CCOs I e II	Expansion rate% (n)	Degree of expansion % (n)		
			High	Moderate	Slight
CON	27	88.00 (24)	2.00±0.76^{aA}	3.33±1.28^{aA}	2.66±1.02^{aA}
MIO 20	36	94.44 (34)	2.00±0.76^{aA}	5.00±0.00^{aA}	4.33±0.51^{aA}
MIO 30	36	91.66 (33)	3.66±0.51^{aA}	4.00±0.76^{aA}	3.33±1.28^{aA}
MIO 40	36	86.11 (31)	2.00±1.15^{aA}	4.00±1.53^{aA}	4.33±1.28^{aA}

a, b Lowercase letters indicate differences between columns (P<0.05); $^{A, B}$ Uppercase letters indicate differences between lines (P<0.05).

Regarding the comparison within the treatment groups, there was no significant difference (P>0.05) regarding the relationship between the presence and absence of cumulus cells for the CON group (Table 2).

Table 2. With expansion and without expansion of cumulus cells in *in vitro* matured oocyte oocytes

Treatments	N	With expansion of cumulus cells	No cumulus cell expansion
CON	27	8.00±3.07^{aA}	1.00±0.76^{aA}
MIO 20	36	11.33±1.28^{aA}	0.66±0.51^{bA}
MIO 30	36	11.00±1.15^{aA}	1.00±0.76^{bA}
MIO 40	36	10.33±2.05^{aA}	1.66±0.64^{bA}

a, b Lowercase letters indicate differences between columns (P<0.05); $^{A, B}$ Uppercase letters indicate differences between lines (P<0.05).

Taking into account the number of presumptive zygostes and the number of cleaved structures (Figure 1B), the effect of myo-inositol after *in vitro* fertilization of ovine oocytes was evaluated, noting that there was no significant difference. (Table 3)

Table 3. Number of presumptive zygotes and cleaved structures after *in vitro* fertilization of ovine oocytes.

Treatments	N	Nº of presumptive zygotes	Nº of cleaved structures
CON	27	3.00±2.12^{a}	0.00±0.00^{a}
MIO 20	36	5.50±0.35^{a}	3.00±1.41^{a}
MIO 30	36	4.50±1.06^{a}	3.50±0.76^{a}
MIO 40	36	4.50±1.76^{a}	2.00±0.00^{a}

a, b Lowercase letters indicate differences between columns (P<0.05).

CONCLUSIONS

Based on this information, it can be concluded that, to date and in possession of preliminary results, myo-inositol showed an effective antioxidant action, in addition to acting in a relevant way in oocyte maturation, maintaining and improving the quality of oocyte maturation. Furthermore, it can also be used in the *in vitro* production of sheep zygotes. Later sessions will likely elucidate some questions that have not yet been answered.

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