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EFFECT OF ALL-TRANS RETINAL (RAL) ON *IN VITRO* MATURATION OF SHEEP OOCYTES AND *IN VITRO* EMBRYO PRODUCTION: PRELIMINARY RESULTS

B.C. Freitas [1] , L. K. P. Oliveira [1] *, A.M.C. Lima [1] , E.K.O. Aureliano [1] , D.R.P. Santos [1] , T. T. S. Souza [2] , 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

All trans retinal acid (RAL) is a vitamin A precursor that can be a potent antioxidant, increasing resistance to oxidative stress and helping to prevent tissue aging and block oxidative effects (RIBET; NOBILE; ROSSI, 2019). In mice, RAL improved photoreceptor dysfunction in diabetic mice, regardless of the visual cycle, through an antioxidant mechanism (BERKOWITZ *et al*., 2015), however there are no reports of its use in ovine oocyte IVM yet.

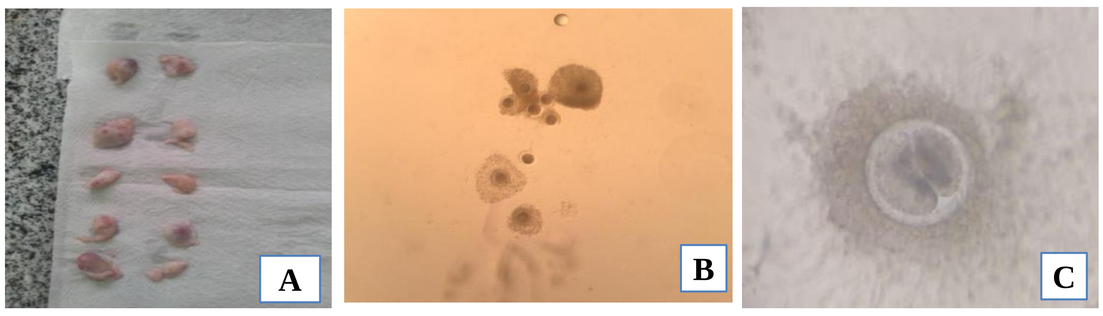
OBJECTIVE

This study aimed to evaluate the effect of including RAL as an antioxidant on *in vitro* production of sheep embryos.

MATERIAL AND METHODS

For this purpose, oocytes were aspirated from abattoir ovaries (Figure 1A) of sheep by using a vacuum pump. After oocyte classification, the best quality cumulus-oocyte complexes (COCs) were kept in the follicular fluid until maturation. The total of selected COCs was equally divided into four maturation groups: CIS group, composed of oocytes matured in a medium composed of TCM-199, supplemented with antibiotics and antimycotic, 0.2 mM sodium pyruvate, 10% (v/ v) fetal bovine serum (FBS), 10 ng/ml EGF, 10 µg/ml FSH, 10 µg/ml LH and 10 µg/ml estradiol and 100 mM cysteamine; and, in RAL1.5, RAL3 and RAL6 groups, with the same medium as CIS group, but without adding cysteamine and including 1.5 µM, 3.0 µM and 6.0 µM of RAL, respectively. COCs were placed in MIV medium, under mineral oil, for 24 hours in a CO_2 incubator, at 38.5ºC, in a humidified atmosphere with 5% CO_2 and saturated humidity. Mature oocytes were evaluated from cumulus cell expansion; after that, the oocytes went to *in vitro* fertilization (IVF), together with selected and trained semen for a period of 18 to 20 hours, in a CO_2 incubator, at 38.5ºC, with a humidified atmosphere, containing 5% of CO_2. Presumptive zygotes (PZ) were evaluated for the presence of the 2nd polar body in the perivitelline space, using an inverted microscope. After evaluation, the presumptive zygotes proceeded to *in vitro* embryo culture (IVC) with SOF medium supplemented with 3 mg/mL of BSA, and cleavages were evaluated at D1 and D2. The conditions of the IVC were the same used in the IVM and IVF. One-way ANOVA was used to compare the parameters between groups, followed by the Tukey test. Percentage data were submitted to the Fisher's exact test ($P < 0.05$).

Figure 1 – (A) Ovine ovaries used for follicular aspiration (B); oocytes after *in vitro* maturation with addition of RAL; (C) cleaved structure, after IVM and IVF.



RESULTS

A total of 91 oocytes were submitted to IVM, there was no significant difference between the treatment groups when compared to CIS group regarding the rate of expansion of cumulus cells (Table 1).

Table 1. With expansion and without expansion of cumulus cells in *in vitro* matured oocyte oocytes

Treatments	N	With expansion of cummulus cells	No expansion of cummulus cells
CIS	20	7.50±1.76^{aA}	2.50±1.76^{aA}
RAL 1,5 µM	19	9.00±0.70^{aA}	0.50±0.35^{aB}
RAL 3,0 µM	27	13.50±2.47^{aA}	0.00±0.00^{aA}
RAL 6,0 µM	25	11.50±1.06^{aA}	1.00±0.70^{aB}

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

Likewise, there was no significant difference ($P>0.05$) between treatment groups for any degree of cumulus cell expansion (Figure 1B). However, comparing the degrees of expansion of cumulus cells within each treatment group, a significantly higher number of oocytes with high expansion was observed in the group of oocytes matured in the presence of 1.5 µM retinal, with no significant difference observed for the other treatment groups (Table 2)

Table 2. Degree of expansion of cumulus cells (High, Moderate and Light) in the *in vitro* maturation of ovine oocytes.

Treatments	N° CCOs I e II	Expansion rate % (n)	Degree of expansion% (n)		
			High	Moderate	Mild
CIS	20	75.00 (20)	2.50±0.35^{aA}	1.50±0.35^{aA}	3.50±1.76^{aA}
RAL 1,5 µM	19	94.74 (19)	5.50±0.35^{aA}	2.50±0.35^{bA}	1.00±0.00^{bA}
RAL 3,0 µM	27	100.00 (27)	8.50±3.18^{aA}	3.50±0.35^{aA}	1.50±0.35^{aA}
RAL 6,0 µM	25	92.00 (25)	6.00±2.12^{aA}	2.00±0.00^{aA}	3.50±1.06^{aA}

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

As for the successful fertilization, the RAL6.0 group (RAL6.0: 5.50±1.06) had a significantly higher number of presumptive zygotes when compared to the RAL1.5 group, no difference between the other groups were observed. However, there was no difference between the treatment groups regarding the number of cleaved structures (Figure 1C) (Table 3).

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

Treatments	N	Nº of presumptives zygotes	Nº of cleaved structures
CIS	20	2.50±0.35^{ab}	0.50±0.35^{a}
RAL 1,5 µM	19	1.50±0.35^{b}	0.50±0.35^{a}
RAL 3,0 µM	27	4.00±0.00^{ab}	0.00±0.00^{a}
RAL 6,0 µM	25	5.50±1.06^{a}	0.50±0.35^{a}

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

CONCLUSIONS

Based on the above, it is concluded that, to date and with preliminary results, the addition of all-trans-retinal acid, in none of the concentrations tested, interferes with the *in vitro* maturation of ovine oocytes compared to the use of cysteamine; however, the use of RAL at a concentration of 6 µM showed superior performance in the number of presumptive zygotes after *in vitro* fertilization. Further repetitions and studies are needed to elucidate the possible mechanisms of using RAL as an effective alternative to cysteamine.

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