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EFFECT OF CILOSTAZOL ON *IN VITRO* PRODUCTION OF SHEEP EMBRYOS: PRELIMINARY RESULTS

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INTRODUCTION

Cilostazol (CIL) is a cyclic adenosine monophosphate (cAMP) modulator that influences and improves nuclear and cytoplasmic *in vitro* maturation. Is a quinolinonic derivative, has an antiplatelet effect superior to acetylsalicylic acid and ticlopidine (KLASCO, 2004), in addition to being antithrombotic with vasodilating action (KAMBAYASHI *et al*., 2003; NAKAMURA; IKOMI; OHHASHI, 2006). Promote the increase of intracellular levels of cyclic adenosine monophosphate (cAMP), by producing potent and selective inhibition of PDE – 3 (OKUDA; KIMURA; YAMASHITA, 1993; CONE *et al*., 1999). However, its use on in vitro maturation media (IVM) of ovine oocytes has not yet been reported.

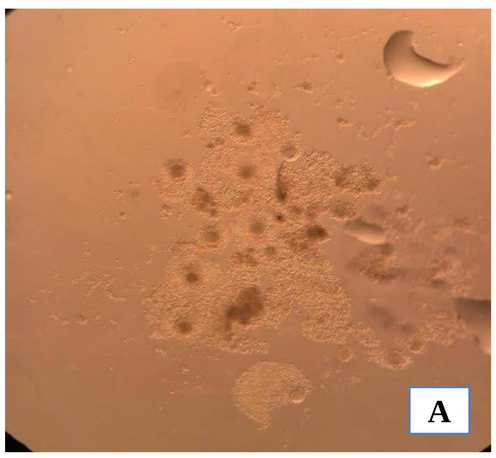
OBJECTIVE

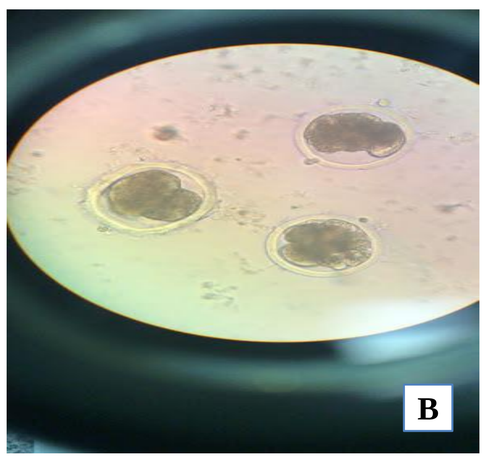
To evaluate the effect of cilostazol supplementation in the oocyte *in vitro* maturation medium on the *in vitro* production of sheep zygotes.

MATERIAL AND METHODS

Ovaries ovine were collected from local abattoir and oocytes were aspirated using a vacuum pump, then poured in petri dishes, analyzed under a stereomicroscope and taken to in vitro maturation (IVM), being divided into four groups: CIS group where cumulus-oocyte complexes (COCs) were immersed in a medium composed of TCM-199, supplemented with antibiotics and antimycotic, 0.2 mM sodium pyruvate, 10% (v/v) fetal bovine serum (SFB), 10 ng/ml EGF, 10 µg/ml FSH, 10 µg/ml LH and 10 µg/ml estradiol and 100 mM cysteamine; and CIL0.3; CIL1 and CIL10 groups, where COCs were immersed in the same medium as the CIS group, without adding cysteamine but including 0.3; 1 and 10 µM of cilostazol, respectively. COCs were placed in a petri dish with 15 COCs per drop of 75 µL of IVM medium, for 24 hours, in a CO 2 incubator, at 38.5°C in a humidified atmosphere with 5% CO_2. Matured oocytes were evaluated from cumulus cell expansion, being classified as high, moderate, and mild. After that, oocytes were proceeded to in vitro fertilization (IVF), together with selected semen for a period of 18 to 20 hours, in a CO_2 incubator, at 38.5°C, with a humidified atmosphere, containing 5% of CO_2. Presumptive zygotes were denuded by successive pipetting, evaluated for the presence of the 2nd polar body in the perivitelline space, using an inverted microscope. After evaluation, presumptive zygotes were followed for 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 Fisher's exact test (P < 0.05).

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





RESULTS

A total of 70 oocytes were submitted to IVM. There was no significant difference of expansion rate (P < 0.05) between oocytes treated with 0.3 µM, 1.0 µM and 10.0 µM of cilostazol when compared to control group with cysteamine (Figure 1A). Likewise, there was no difference (P < 0.05) also regarding the degrees of expansion, slight, moderate and high (Table 1).

Table 1 - Degrees of expansion (high, moderate and slight) within each group of *in vitro* maturation (CON, CIL0.3, CIL1, CIL10) of collected ovine oocytes by follicular puncture of a slaughterhouse ovary.

Treatments	N° CCOs I e II	Expansion rate% (n)	Degree of expansion % (n)		
			High	Moderate	Slight
CIS	12	100 (12)	2.00±1.41^{aA}	3.00±2.12^{aA}	1.00±0.70^{aA}
CIL 0,3	19	100 (19)	2.00±0.70^{aA}	4.00±1.41^{aA}	3.50±0.35^{aA}
CIL 1	17	100 (17)	3.00±0.70^{bA}	3.00±1.41^{abA}	2.50±1.06^{aA}
CIL 10	22	100 (22)	4.00±0.70^{aA}	4.00±1.41^{aA}	3.00±0.00^{aA}

a, b Lowercase letters indicate comparisons between columns (P < 0.05); A, B Uppercase letters indicate differences between lines (P<0,05).

Regarding fertilization, it was possible to verify that there was no difference (P < 0.05) in relation to the number of presumptive zygotes and also on the cleavage (Figure 1B) rates (Table 2).

Table 2. Number of presumptive zygotes and cleaved structures after *in vitro* fertilization of ovine oocytes

Treatments	N	Nº of presumptive zygotes	Nº of cleaved structures
CIS	11	2.50±1.76^{a}	3.50±2.47^{a}
CIL0,3	20	0.50±0.35^{a}	0.50±0.35^{a}
CIL 1	15	0.00±0.00^{a}	0.00±0.00^{a}
CIL 10	21	1.00±0.70^{a}	2.00±1.41^{a}

a, b Values with different lowercase letters between lines indicate a significant difference (P<0.05).

CONCLUSIONS

It is possible to concludes that, to date with preliminary results, cilostazol at different concentrations (0.3 µM, 1.0 µM, and 10 µM), can replace cysteamine in oocyte maturation medium of sheep, promoting quality oocyte maturation and *in vitro* production of sheep embryos.

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