

EFFECT OF TRANS-FERULIC ACID ON *IN VITRO* PRODUCTION OF SHEEP ZYGOTES: PRELIMINARY RESULTS

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INTRODUCTION

PIV is a biotechnology that consists of assisted production of embryos in laboratories until the desired embryonic stage, which makes it possible to accelerate genetic gain and increase the reproductive potential of sheep (LIMAVERDE; RONDINA; FREITAS, 2003). Currently, in the *in vitro* maturation stage, several antioxidants have been added to the media. An example of them is trans ferulic acid. Ferulic acid has attracted attention for its antioxidant capacity and benefits to human health (KADOMA; FUJISAWA, 2008). Studies show that ferulic acid, in addition to being an antioxidant, has diverse therapeutic activity, including anti-inflammatory, antithrombotic, anticancer, neuroprotective, cardioprotective effects and, when applied to the skin, photoprotective action (MERLIN *et al.*, 2012).

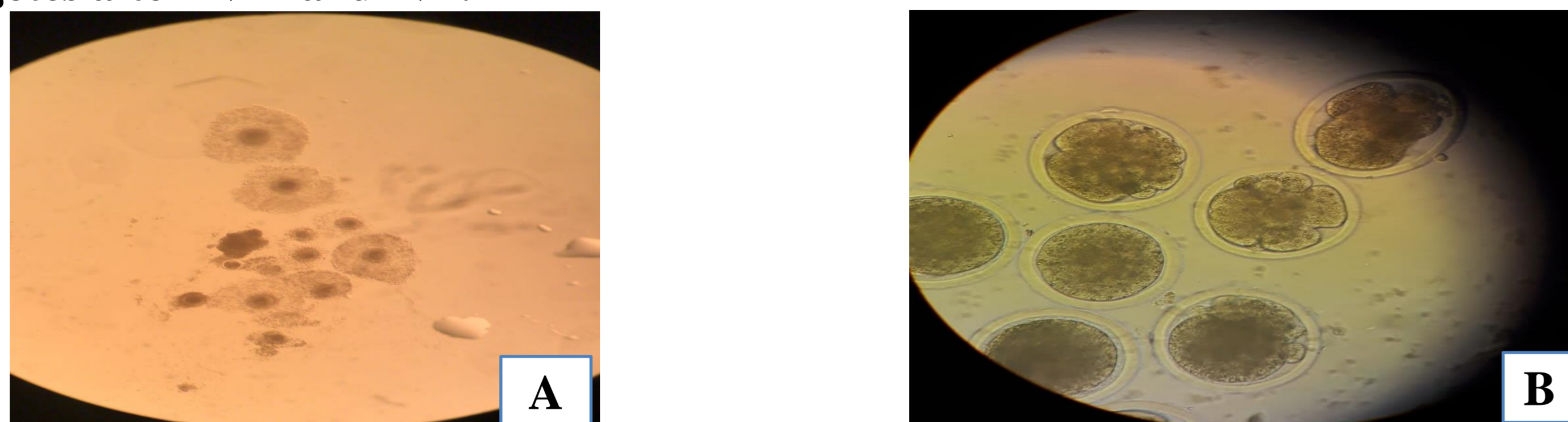
OBJECTIVE

To evaluate the effect of including trans-ferulic acid in the *in vitro* oocyte maturation medium on the *in vitro* production of ovine zygotes.

MATERIAL AND METHODS

Oocytes were collected from ovaries obtained from a local slaughterhouse. A vacuum pump was used to collect oocytes, which, soon after, were classified and taken to the IVM, where they were divided into four groups: CIS group, with medium containing TCM-199, supplemented with 0.2 mM of pyruvate sodium, 10% fetal bovine serum, 1% antibiotic-antimycotic solution, 20 mg/mL FSH/LH and 100 mM cysteamine; in ATF10, ATF50 and ATF100 groups, oocytes were matured in the presence of a medium with the same composition as the CIS group, but without the addition of cysteamine and including 10 µM, 50 µM and 100 µM of transferulic acid, respectively. The oocytes with the medium were incubated for 24 h at 38.5 °C, with 5% CO₂. Then, *in vitro* fertilization (IVF) was performed, where IVF medium was used, together with selected spermatozoa for a period of 18 to 20 hours, under the same conditions as the MIV. The presumptive zygotes were evaluated for the presence of the 2nd polar body in the perivitelline space, using an inverted microscope. After evaluation, the presumptive zygotes went to CIV, with SOF medium supplemented with 3 mg/mL of BSA and, from that, were evaluated for the occurrence of D1 and D2 cleavage. ANOVA and Tukey's test were used to compare parameters between the studied groups. 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).

Figura 1 – (A) oocytes after *in vitro* maturation with addition of ATF; (B) presumptive zygotes after IVM and IVF.



RESULTS

A total of 97 oocytes were used for *in vitro* maturation (IVM), so it was observed that there was no significant difference between the groups with transferulic acid and the CIS group, in relation to the expansion rate 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 cumulus cells	No cumulus cell expansion
CIS	25	10.50±1.06 ^{AA}	2.00±1.41 ^{AA}
ATF 10	24	10.00±1.41 ^{AA}	2.00±1.41 ^{AA}
ATF 50	24	10.00±1.41 ^{AA}	2.00±1.41 ^{AA}
ATF 100	24	10.50±1.06 ^{AA}	1.50±1.06 ^{AA}

a, b Letras minúsculas indicam comparações entre colunas (P<0,05); A, B Letras maiúsculas indicam diferenças entre linhas (P<0,05).

Likewise, no significant difference (P>0.05) was observed within each moderate and mild expansion treatment group (Figure 1A). However, a significant smaller number of oocytes with high expansion was observed in the group of oocytes matured in the presence of 50 µM transferulic acid (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	25	84.00 (25)	4.00±0.00 ^{AA}	2.00±0.70 ^{AA}	4.50±0.35 ^{AA}
ATF 10	24	83.33 (24)	2.50±1.06 ^{AA}	3.00±1.41 ^{AA}	4.50±1.76 ^{AA}
ATF 50	24	83.33 (24)	1.00±0.70 ^{BA}	3.00±0.70 ^{abA}	6.00±0.00 ^{AA}
ATF 100	24	87.50 (24)	1.50±1.06 ^{AA}	5.50±.06 ^{AA}	3.50±1.06 ^{AA}

a, b Letras minúsculas indicam comparações entre colunas (P<0,05); A, B Letras maiúsculas indicam diferenças entre linhas (P<0,05).

As for the success of fertilization, there was no significant difference between the treatment groups in relation to the presumptive zygotes (Figure 1B). Likewise, no significant difference was observed between the treatment groups regarding the number of cleaved structures (Table.3)

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

Treatments	N	N° of presumptive zygotes	N° of cleaved structures
CIS	25	4.50±0.35 ^a	1.50±1.06 ^a
ATF 10	24	5.00±0.70 ^a	3.50±1.06 ^a
ATF 50	24	5.00±0.70 ^a	1.50±0.35 ^a
ATF 100	24	5.50±0.35 ^a	5.00±0.00 ^a

CONCLUSIONS

Based on the preliminary results, it is possible to conclude that the addition of transferulic acid does not interfere in the *in vitro* maturation of ovine oocytes when compared to the use of cysteamine. Further studies are needed to see the real effect of transferulic acid on *in vitro* maturation of sheep embryos, which may prove to be an effective alternative to cysteamine.

REFERENCES

- KADOMA, Y.; FUJISAWA, S., A comparative study of the radical-scavenging activity of the phenolcarboxylic acids caffeic acid, p-coumaric acid, chlorogenic acid and ferulic acid, with or without 2-mercaptoethanol, a thiol, using the induction period method. *Molecules*, v. 13, p. 2488-2499, 2008.
- LIMAVERDE, J. B.; RONDINA, D.; FREITAS, V. J. F. Produção *in vitro* de embriões ovinos. *Ciência Animal*, v. 13, p. 79-87, 2003.
- MERLIN, J. P. J.; PRASAD, N. R.; SHIBLI, S. M. A.; SEBEELA, M. Ferulic acid loaded Poly-d,l-lactide-co-glycolide nanoparticles: Systematic study of particle size, drug encapsulation efficiency and anticancer effect in non-small cell lung carcinoma cell line *in vitro*. *Biomedicine & Preventive Nutrition*, v. 2, p. 69-76, 2012.