The effect of hydroalcoholic extract of *Thymbra spicata* on in- vitro maturation of ovine oocyte

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Abstract

In vitro maturation (IVM) of oocytes and subsequently, in vitro fertilization (IVF) for the generation of embryos in the laboratory have important values. Considering that antioxidants are known as effective free radicals scavenger, it is possible to improve the in vitro oocyte maturation and the fetal quality. Therefore, the aim of this study is to evaluate the effect of Thymbra spicata hydroalcoholic extract as a source of antioxidant on in-vitro sheep oocyte maturation. Cumulus oocyte complexes (COCs) were collected from ewe ovaries and were cultured for 24 hours in maturation medium in TCM supplemented with FSH, LH, FBS, cysteamine, pyruvate sodium and antibiotics (control group) and in maturation medium without cysteamine (as an antioxidant) supplemented with different doses of Thymbra spicata hydroalcoholic extract (1mg/ml: group 1, 10 mg/ml: group 2, 50 mg/ml: group 3) as an antioxidant. In-vitro maturation stages and resumption of meiotic was assessed by determination of cumulus cells mass expansion and number of oocytes in metaphase II stage of meiotic division in all groups. Cumulus cells mass expansion was similar between control, 2 and 3 groups. However, in group 1 was lower than control group. Nuclear maturation was similar between control and group 3 and both of them were different with groups 1 and 2. The results of this study showed that the Thymbra spicata hydro alcoholic extract, has a positive effect on oocyte maturation that is doses dependent. So with increasing concentration of Thymbra spicata hydroalcoholic extract, the rate of maturation immature oocytes is increased. Generally, we conclude that addition of appropriate amounts of natural extracts such as Thymbra spicata to maturation medium improves oocytes maturation.

Key words: In vitro maturation (IVM), Oocyte, Sheep, Thymbra spicata

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Refrences

- Abedi, A., Rouhi, L., & Ghasemi Pirbalouti, A. (2014). Effect of *GundeliaTournefortii*leaves extract on in immature mouse oocytes. 2: 84-89.
- Akin, M., Oguz, D., & Saracocoglu, H. (2010). Antibacterial activity of essential oil from thymbra spiata var. spicata L. and teucrimpolium (stapf Brig). *Int J Pharm Appl Sci*, 1(1), 56-58
- Alaee, S., Rezaee, S., & Ziaei, GH. (2016). Evaluation of the Effects of MenthaSpicata Extract on In-Vitro Maturation of Mouse Oocytes. *Journal of Advanced Medical Sciences and Applied Technologies (JAMSAT)*, 2(2), 200-203.
- Alpinar, K., Ozyurek, M., Kolak, U., Guclu, K., Aras, C., Altun, M., et al. (2009). Antioxidant capacities of some food plants wildly grown in Ayvalik of Turkey. *Food SciTechnol Res*, 5, 59–64.
- Banihosseini, SZ. GhaffariNovin, M., Nazarian, H., Piryaei, A., Parvardeh, S., & Eini, F. (2018). Quercetin improves developmental competenceof mouse oocytes by reducing oxidative stress duringin vitro maturationAnn. Anim. Sci, 18,1 87–198 DOI: 10.1515/aoas-2017-0029.
- Barakat, IAH. Al-Himaidi, AR., & Rad, AM. (2014) .Antioxidant Effect of Green Tea Leaves Extract on in vitro Production Sheep Embryos.Pakistan J. Zool, 46(1), 167-175.
- Compoti, M. (1989). Three models of free radical induced cell injury. Chemico Biological Intractions, 72, 1-56.
- Eimani, H., Hassani, F., Haeri Rohani, S., Nasr Esfahani, M.H., Rezazadeh, M., & Dalman, A. (2005). Effect of cysteamine on in vitro maturation, resumption of miosis and embryo development of immature mouse oocytes. *Yakhteh*, 7(1), 1-6.
- Eimani, H., Hassani, F., Nasresfahani, M.H., Dalman, A., Shahverdi, A.H., & Eftekhari Yazdi, P. (2006). Effect of B-Mercaptoethanol with and without Bso (Dl-Buthionine Sulfoximine) on resumption of meiosis, iin vitro maturation and embryo development of immature mouse oocytes. *Yachted*, 7(4), 236-241.
- Faleiro, L., Miguel, G., Gomes, S., et al. (2005). Antibacterial and antioxidant activities of essential oils isolated from thymbra capitata L (cav.) and origanum vulgare L. *J Agric Food Chem*, 53(21),8162-8.
- Ghasemipirbalouti, A., Bahmani, M., & Avijgan, M. (2009). Anti –candida activity of some of the Iranian medicinal plants. *Electronic J Biol*, 5(4), 85-88.
- Golkar-Narenji, A., Eimani, H., Samadi, F., Hasani, S., Shahverdi, A., & Eftekhari-Yazdi, P. (2010). Effect of Papaver rhoeas extract on in vitro maturation and developmental competence of immature mouse oocytes. *Reproductive Medicine and Biology*, 9(4), 211-215.
- Gordon, I. (2003). Laboratory production of cattle embryos. 2nd edition. CAB International publishing, Wallingford.
- Guerin, P., El Mouatassim, S., & Menezo, Y. (2001). Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. *Human Reproduction. Update*, 7, 175–189.
- Hajian, S. & Haidari Nasrabadi, M. (2017). Effect of Fennel essence on nuclear maturation of bovine oocyte. *NCMBJ*, 7(25), 27-32.
- Hreinsson, JR., Friden, B., & Levkov, L. (2003). RecombinantLH is equally effective as recombinant hCGinpromoting oocyte maturation in clinical invitromaturationprogramme: a randomized study. *Hum.Repro*, 18, 2131-2136.
- Hashimoto, S., Saeki, K., Nagao, Y., Minami, N., Yamada, M., & Utsumi, K. (1998). Effects of cumulus cell density during in vitro maturation on the developmental competence of bovine oocytes. *Theriogenology*, 49, 1451-1463.
- Jurema, M.W., & Ogueira, N. (2006). In vitro maturation of human oocytes for assisted reproduction. *Fertility* and sterility, 86, 1277-1291.
- Khodabandeh, M. Roohi, L. & Ghasemi Pirbalooti, A. (2016). Dose dependent effects of *Gundeliatournefortii* root extract on meiosis restart and in vitro maturation of mouse immature oocytes. *Herbal drugs*, 6(3), 161-166.

E. & Maziero, RRD. (2014). Control of oocyte maturation. Anim. Reprod,. 11(3), 150-158.

- Lee, SE. Kim, EY. Choi, HY. Moon, JJ. Park, MJ. Lee, JB., et al. (2014). Rapamycin Rescues the Poor Developmental Capacity of Aged Porcine Oocytes. Asian-Australas J Anim Sci, 27(5), 635–647.
- Menne, LI., Sapinho, D., & De bree, A. (2004). Consumption of foods rich in flavonoids is related to a decreased cardiovascular risk in apparently Healthy French women. *J Nutr*, 134 (4), 923-926.
- Nadi, S., Ravindranatha, BM., Gupta, PSP. & Sarma, PV. (2002). Timing of sequential changes in cumulus cellsand first polar body extrusion during in vitromaturation of buffalo oocytes." *Theriogenology*, 57, 1151–1159.
- Nasr-Esfahani, MH. (1991). The origin of reactive oxygen species in mouse embryos cultured in vitro. *Development*, 113,551-560.
- Sabzali, S., Rostam zad, A., Panahi, J., Havasian, M., Haghani, K., & Bkhtiary, S. (2014). Investigation on the Inhibitory effects of hydro-alcoholic extract of Thymbra spicata on the growth of lung cancer cell line SK-Mes-1. Sjimu, 22(4), 153-158.
- Salimi, M., Salehi, M., Masteri Farahani, R., Dehghani, M., Abadi, M., Novin, MG. Nourozian, M., & Hosseini, A., 2014. The Effect of Melatonin on Maturation, Glutathione Level and Expression of H MGB1 Gene in Brilliant Cresyl Blue (BCB) Stained Immature Oocyte. *Cell J*, 15(4), 294-301.
- Son, YJ. Lee, SE. Hyun, H., Shin, MY. Park, YG. et al. (2017). Fibroblast growth factor 10 markedly improves in vitro maturation of porcine cumulus-oocyte complexes. *Mol. Reprod. De*, 84, 67–75.
- Sovernigo, Adona, PR., Monzani, PS., Guemra, S., Barros, FDA., Lopes, FG., & Leal, CVL. (2017). Effects of supplementation of medium with different antioxidants during in vitro maturation of bovine oocytes on subsequent embryo production. *Reprod Dom Anim*, 52,561–569.
- Tavana, S., Eimani, H., Azarnia, M., Shahverdi, A., & EftekhariYazdi, P. (2012). Effects of Saffron (*Crocus sativus L.*) Aqueous Extract on *In vitro* Maturation, Fertilization and Embryo Development of Mouse Oocytes. *Cell Journal (Yakhteh)*, 13(4), 259-264.
- Uday, B., Dipak, D., & Ranajit, K. (1999). Reactiv oxygen species: oxidative damage and pathogenesis. *Current Sien*, 77, 658-666.
- Wang, X., Falcone, T., Attaran, M., Goldberg, JM., Agarwal, A., & Sharma, RK. 2002. Vitamin C and vitamin E supplementation reduce oxidative stress-induced embryo toxicity and improve the blastocyst development rate. *Fertil Steril*, 78(6), 1272-7.
- Wani, N., Wani, G., & Al-Saigh, MN. (1999). Effect of different factors on the recovery rate of oocytes for in vitro naturation and in vitro fertilization procedures in sheep. *Small Rumin Res*, 34, 71-76.
- Wojdyto, A. (2007). Antioxidant activity and phenolic compound in 32 selected herbs. *J food Chem*, 105, 940-949.
- Zabihi, A., Shabankareh, HK. Hajarian, H., & Foroutanifar, S. (2019). Resveratrol addition to in vitro maturation and in vitro culture media enhances developmental competence of sheep embryos. *Domest Anim Endocrinol*, 68, 25-31.
- Zhu, G., Guo, B., Pan, D., Mu, Y., & Feng, S. (2008). Expression of bone morphogenetic proteins and receptors in porcine cumulus oocyte complexes during in vitro maturation. *Anim reprod sci*, 104, 275-283.