

Effect of vitrification of ovine immature oocytes on subsequent oocyte and blastocyst HSP70 gene expression

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Abstract

Vitrification removed many of the problems related to cryopreservation of oocytes and embryos. On the hand, some problems related to in vitro produced embryos may indicate any alteration in embryonic genome transcription. The present study aimed to investigate whether oocyte vitrification may alter expression of a gene that can change following environmental stress, i. e., HSP70 or not. Total of 120 immature germinal vesicle stage ovine cumulus oocyte complexes (COC) were retrieved from abattoir collected ovine ovaries. The COCs were subjected to vitrification in HTCM based (with 20% FBS) media as V1: DMSO (10 %), Ethylene glycol (10 %) for 30 min, V2: DMSO (10 %), Ethylene glycol (10 %) and 0.5 M sucrose immediately left on the cryotop device and immersed within liquid nitrogen. At least after 48 hrs of vitrification, the oocytes were warmed in warming solutions as W1: basic medium with 1 M sucrose, W2: basic medium and 0.5 M sucrose and W3 basic medium and 0.25 M sucrose, each of them for 5 min. The vitrified-warmed COCs (n=60) and fresh COCs (n=60) were subjected to routine IVM, IVF and IVC procedures of the laboratory with SOF based media. The developmental stages of oocytes were compared and the expression rate of HSP70 to an average of β -actin and B2m genes expressions were compared between blastocysts and oocytes before and after vitrification. The results of the study showed the impact of vitrification of germinal vesicle stage oocytes on the next developmental competence of the respected embryos in all stages. The expression of HSP70 was significantly different between oocytes and blastocysts; however the vitrification of immature ovine oocytes did not affect the expression rate of HSP70 in oocytes the respective blastocysts. In conclusion, vitrification does not affect the vitrified ovine immature oocytes and the next developed blastocysts.

Keywords: Ovine COCs, Vitrification, HSP70, Blastocysts, Oocyte

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