were microinjected into blastocele cavity of groups I and II. The hatching rate and the presence of GFP positive cells in inner cell mass (ICM) were evaluated at Day 5 of culturing.
**Results:** The results showed that the hatching rate was significantly higher in group 1 than control group (96.3±3.7 vs. 58.6±11.3) though, the presence of GFP positive ESCs was lower in group 1 than group 2 (57.2±9.2 vs. 86.3±5.9).
**Conclusion:** The increased hatching rate in group 1 compared to control is probably due to zona pellucida drilling by laser. The presence of greater numbers of GFP positive ESCs in group II might be related to the higher purity and homogeneity of cultured ESCs in feeder free culture system compared to the co-cultured group.
***Key words:*** *Embryonic stem cell, Blastocyst, Chimeric, Mouse, Microinjection.*

## P-104
## IVF outcome after using anti freeze protein in semen freezing of three Iranian ovine breeds

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**Introduction:** Despite the effects of antifreeze proteins (AFPs) on freezing point and their potential interaction with membranes, there are controversial reports on their effects on fertility in different species. In this study the effects of extender supplementation with AFP during semen freezing on embryo development were studied in three Iranian sheep breeds.
**Materials and Methods:** The collected semens from Shal, Afshari, and Moghani ram breeds, before freezing, were diluted in extender supplemented with (Shal-AFP, Afsh-AFP and Mogh-AFP, respectively) or without (Shal-Cnt, Afsh-Cnt and Mogh-Cnt groups, respectively)10µg/ml AFP III. The abattoir-derived COCs were separately fertilized with above frozen semen and the cleavage, blastocyst, and hatching rates were recorded.
**Results:** The highest and lowest blastocyst rates, on day 8, were recorded in shal-Cnt and Afsh-Cnt groups, respectively (26.61±8.58 and 16.61±4.94, respectively; $p \geq 0.05$). The highest and lowest hatching rates were observed in Afsh-Cnt and Mogh- Cnt groups, respectively (55.15±12.06 and 24.61±14.87, respectively; $p \geq 0.05$).
**Conclusion:** Neither the presence or absence of AFP III nor the breeds of rams had effect on the blastocyst formation and hatching rates in in vitro drived embryos.
***Key words:*** *AFP, Frozen, Semen, Breeds, Blastocyst, Hatching.*

## P-105
## Effect of α-tocopherol supplementation during ram semen cryopreservation on subsequent embryo development

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**Introduction:** This study was conducted to evaluate the effect of α-tocopherol (Toc) supplementation to semen extender during sperm freezing on developmental competence of derived embryos.
**Materials and Methods:** The pooled semen samples collected from three rams of Shal breed were diluted in extender with different concentrations (5 mM and 10 mM; Toc-5 and Toc-10 groups, respectively) or without Toc (control groups) before freezing. The abattoir-derived COCs were fertilized with above frozen semen (treatment and control group). The parameters of embryo development such as cleavage, blastocyst, and hatching rates were subsequently recorded. The data were analyzed using ANOVA test.
**Results:** The blastocyst rate on day 8 in Toc-5 group was greater than Toc-10 and control groups (48.2±11.1 vs. 36.4±7.8 and 39.3±8, respectively). However, the hatching in Toc-10 group was greater than Toc-5 and control groups (52.6±4 vs. 55.9±15.4 and 43.8±8.3, respectively).
**Conclusion:** The presence of different concentrations of α-tocopherol in semen extender may exert different effects on embryo developmental parameters using frozen-thawed semen.
***Key words:*** *Embryo, α-tocopherol, Sperm cryopreservation.*

## P-106
## In vitro proliferation and colonization of mouse spermatogonial stem cell using low intensity ultrasound

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**Introduction:** Spermatogonial stem cells (SSCs) are the foundation of spermatogenesis. Sound wave especially low intensity ultrasound (LIUS) can be effective on increasing the number of cells.
**Materials and Methods:** Isolated SSCs from neonate mice cultured in DMEM culture medium with 10% Fetal Bovine Serum. In the first phase, temperature controlled and in the second phase, SSCs stimulated by LIUS with 3 different Intensity dose (100, 200 and 300