7- Summary

The objective of the present study was to compare cryoprotective solutions such as Ethylene Glaycol (EG),Dimethyle-Sulfoxide (DMSO) and Ficoll 70 with different combination ratios for vitrification of mature bovine oocytes and embryos produced in vitro. In addition to the demonstration of the effect of the straw diameter on post thawing viability of the thawed matured oocytes and embryos.

1- Vitrification of mature bovine oocytes:

For this purpose, a total of 728 oocytes were collected from 175 ovaries by slicing technique. The recovered COCs were classified according to their morphological criteria into four grades; I, II, III and IV. The good quality recovered oocytes grade I, II (n=551) were selected to undergo in vitro maturation. The maturation medium was supplemented with gentamycin sulfate, FCS in addition to hormonal combination of FSH, hCG and E_2. Following maturation, Excellent and good mature oocytes were vitrified. In another experiment the excellent and good mature oocytes were used for fertilization then the zygotes were placed in culture medium for further development to morula and blastocysts. In this respect, IVM, IVF and IVC were done in CO_2 incubator adjusted at 39 ^{0}C, 5% CO_2 and 95% relative humidity.

The mature bovine oocytes were subjected to Vitrification in DMSO group by equilibration of cells in Vitrification solution (VS_1) containing 10% EG + 10% DMSO in TCM-199 as a base medium for 2 min. at 22 ^{0}C followed by their transfer into VS_2 containing 20% EG + 20% DMSO + 0.3 Trehalose in BM for 30 seconds then the cells were loaded in 0.5 ml and 0.25 ml straw that were sealed before their plunged into LN. While in Ficoll group , the mature bovine oocytes were divided into three group as 40% EG + 18% Ficoll 70 (volume ratio of 2:1 ml), 40% EG + 18% Ficoll 70 (volume ratio of 3:1 ml) and 40% EG + 18% Ficoll 70 (volume ratio of 3:2 ml). Then loaded in the straws as mentioned. The rate of post-thawing morphologically normal oocytes and as well as in vitro developed rates were recorded in order to compare between the different treatments and recorded the best one that can used for Vitrification.

Analysis of data reveals that:

Matured oocytes frozen in solutions containing 20% EG+ 20% DMSO + 0.3 M trehalose had mean survival rate of (44.43±4.98%). While mature Oocytes frozen in solutions containing 40% EG and 18% Ficoll 70 by a ratio of 1 : 1, 2 : 1 and 3 : 2 in volume had a mean survival rate of 49.22±1.66, 54.33±3.11 and 62.00±3.71%, respectively. Also using of mini straw for the cryopreservation of mature bovine oocytes had post-thawing viability significantly higher (< 10%) than using midi straw

2- Vitrification of bovine embryos:

For this purpose a grade I and II immature oocytes (n= 639) were collected from 208 ovaries and maturation occurred as mentioned

After fertilization the cleavage rate, morula rate and blastocyst rate % were 45.39±2.11, 28.00±2.81 and 15.29±2.79 % respectively.

For Vitrification of embryos, cryoprotectant solutions were prepared as in case of Vitrification of mature oocytes.

The present study revealed that blastocysts cryopreserved in media containing EG + Ficoll (3:2) had a significantly (P<0.01) higher recovery rate (79.28±13.08), compared to 45.00±16.24 blastocyst in embryos cryopreserved in DMSO, respectively. Moreover, recovery rates of blastocysts cryopreserved in media containing EG + Ficoll (٢:1) and in media containing EG + Ficoll (٣:1) were numerically higher than those cryopreserved in DMSO group (50.00±3.74 and 63.49±6.83, respectively).

It is worth to mention that evaluation of post-thawing mature oocytes and embryos can be confirmed by Trypan blue (0.05% in PBS) for 2 min.

So, combination of 40% EG + 18% Ficoll 70 by the ratio of 3:2 act as a good cryoprotectant combination for Vitrification of mature bovine oocytes and embryos.

Also using midi-straw for cryopreservation of cells resulted in high post-thawing viability than midi-straw.