O-1 Comparison of Preimplantation Developments in Simple and Complex Medium Posterior to Vitrification of Cumulus-free Mouse Oocytes

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Objective: To evaluate preimplantaion development of the embryos derived from frozen/thawed mouse mature oocytes in simple medium compared with complex one.

Methods: Oocytes were collected from 5 to 6 weeks old ICR female mice, and denuded from the cumulus cells by 0.1% hyaluronidase. Recovered mature oocytes in study group were cryopreserved by vitrification method using EM gred for 5~7 days. In brief, oocytes were exposed in dPBS containing 1.5 M EG and 5.5 M EG + 1 M sucrose for 2.5 minutes and 20 seconds each, and then executed vitrifcation by plunging in LN2 after loading on EM grid. Thawing treated by exposure of 1, 0.5, 0.25 and 0.125 M sucrose solution for 2.5 minutes each in order and used for experiments. Spermatozoa were aspirated form the epididymis of 12 weeks old ICR male mice and capacitated for 1.5~2 hours before insemination. Simple (T6), complex (αMEM) and high phosphate (0.90 mM) simple medium containing 0.3~0.4% BSA used for fertilization and further development.

Results: In simple medium, fertilization rate after thawing in cryopreservation group (70.8%) was lower ($p<0.05$) than that of control group (86.1%). The embryo developmental rates from 2-cell to morula were not significantly different, however, the blastocysts and cell number of blastocysts in study group (69.9%, 58.9±9.2) were lower compared with those of control group (58.7%, 63.5±8.9). In complex medium, fertilization rate after thawing in cryopreservation group (57.4%) was lower ($p<0.05$) than that of control group (67.2%). And also, the embryo developmental rates from 4-cell to morula in study group were lower ($p<0.001$) compared with those of control group. In high phosphate simple medium, fertilization rate after thawing in cryopreservation group (58.0%) was lower ($p<0.05$) than that of control group (88.5%). And the embryo developmental rates morula and blastocyst in study group were lower ($p<0.05$) compared with those of control group.

Conclusion: By these experimentations, vitrification is very effective freezing method for mouse mature oocyte. And simple media may be better for preimplantion development of embryos derived from frozen-thawed mouse oocytes than complex or simple medium containing high concentration of phosphate.