ABSTRACTS

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Kittens born following transfer of frozen-thawed embryos produced by intracytoplasmic sperm injection using sperm recovered from cryopreserved testicular tissue

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OBJECTIVE AND METHODS: Cryopreservation of gametes and embryos associated with assisted reproduction can be highly beneficial for genetic conservation of several endangered species (1). Testicular tissue cryopreservation is a good approach to expand the option of preserving the fertility of an individual male, while embryo cryopreservation is a potential technique for infinite storage of the whole genetic complement of both parents. However, in vitro development of frozen-thawed embryos produced via intracytoplasmic sperm injection (ICSI) using sperm recovered from cryopreserved testicular tissues has yet to be examined prior to apply this technology to endangered species. Although early embryos (4-8 cell stage) are very sensitive to cryopreservation (2), our preliminary study indicated that Forskolin, a lipolytic agent, could enhance cryosurvival of treated embryos and live offsprings were delivered after transfer (3). The objective of this study was to assess the effect of cryopreservation on developmental competence of early embryos derived from ICSI using sperm from cryopreserved testicular tissues.

Testicular tissues from adult male cats, were cryopreserved by conventional slow freezing technique using 5% (v/v) glycerol as a cryoprotectant. Testicular spermatozoa were extracted from the thawed tissue and were injected into in vitro matured cat oocytes. At 24 h post injection, presumptive zygotes/cleaved embryos were treated with 10 µM forskolin for delipidation. At 48 h post injection, part of the embryos (4 to 8 cell-stage; n = 163; in 5 replicates) were cryopreserved with 10% (v/v) ethylene glycol-based medium by a controlled rate slow freezing. The straws were slowly cooled from 24°C to -6°C at 2°C/min. After 5 min, each straw was seeded manually and held at -6°C for an additional 5 min. Cooling was continued at 0.3°C/min to -33°C, where the straws were held for 10 min before being plunged into liquid nitrogen. After thawing, the embryos were cultured for 6 days for assessment of morula and blastocyst formation. Fresh ICSI embryos (n = 93; 5 replicates) served as control and were cultured in parallel. To assess the developmental potential in vivo, an additional group of 209 early embryos (from 8 replicates) were froze, thawed and transferred into the oviducts of 7 eCG/GnRH treated cat recipients (approximately 15-50 embryos per queen). Radiography was performed on day 49 after transfer to detect the fetal skeletons.

RESULTS: After ICSI with cryopreserved testicular sperm, the percentage of cleavage embryo (related to injected oocytes) was approximately 30%. However, the morula and blastocyst rates of frozen-thawed embryos were lower (P < 0.05) than fresh control (22.6 vs 45.2 % and 21.3 vs 37.6%, respectively). After transfer, three out of the seven recipients were pregnant. One queen aborted a fetus at 49 days of gestation, while a kitten from another queen died pregnant. One queen delivered 2 healthy kittens at 64th day of gestation (both male), while a kitten from another queen died in utero (65 days of gestation). Thus, our study confirmed that cryopreserved testicular sperm remained their ability to fertilize oocyte after ICSI resulted in embryo development and pregnancy (4). Frozen-thawed ICSI embryos have potential to develop in vivo, although spontaneous abortion and fetal death following transfer were observed. These unwanted results could be due to the embryo damage during the freezing and thawing processes (5).

CONCLUSION: We reported the first time live kittens derived from ICSI and cryopreservation program using cryopreserved testicular tissue as a source of sperm but improvement of these techniques is prerequisite for preserving the genetic potentials of endangered species.


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