Successful ICSI with vitrified epididymal cat spermatozoa

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Introduction and objectives. Albeit more common for oocytes and embryos, vitrification has more recently been applied to mammalian spermatozoa, but it is far from standardization. In the domestic cat, model of choice for the development of gamete preservation protocols for endangered felids, few conflicting reports were published. The aims of this study were to evaluate the morphofunctional integrity of cat epididymal spermatozoa vitrified in pellets or straws with two different extenders and to assess their fertilizing ability by intracytoplasmic sperm injection (ICSI).
Material and methods. Epididymal spermatozoa (10 cats) were analyzed as fresh (FS) or vitrified (VS). For vitrification they were diluted 1:1 with Extender 1 (E1: TRIS + 20% egg yolk + 0.25M sucrose) or Extender 2 (E2: Ham's F-10 + 1% BSA + 0.4M sucrose) and, after 5 min equilibration, 10 µl were dropped into liquid nitrogen or loaded in straws and then immersed into liquid nitrogen. Warming was performed at 37°C in TRIS (E1) or HF-10 (E2). In all groups [FS; VS at warming (T0) and after 6 hours (T6)], motility, morphology (Bengal Rose/Victoria Blue B), membrane (hypo-osmotic swelling test) and acrosome integrity (fluorescein isothiocyanate/propidium iodide staining) were assessed. The best extender (E1/E2) and packaging (pellet/straw) were chosen for ICSI. In vitro matured oocytes were microinjected with VS (n=20), or frozen spermatozoa (positive control, n=26), or just pierced without sperm injection (negative control, SHAM-ICSI, n=21). Presumptive embryos were cultured for up to 7 days and embryo development was recorded. The number of nuclei was confirmed by Hoechst staining. Data were analyzed by Kruskal-Wallis and Dwass-Steel-Critchlow-Fligner test (morphofunctional parameters) or Fisher's exact test (ICSI) with significance set at $p \leq 0.05$.
Results. FS had better motility (mean % ± SD: 61±15.2), normal morphology (41.4±24.7), membrane (75.3±13.9) and acrosome (78.2±9.66) integrity than VS at warming (T0), irrespective of the extender used (E1/E2). In VS no differences were found for morphology, membrane and acrosome integrity, regardless of extender (E1/E2), packaging (pellet/straw) or time (T0/T6), although these parameters tended to worsen along time. Motility was similar between extenders and packagings at T0 (E1 pellet 21.7±7.4; E2 pellet 12.7±8; E1 straw 17.7±10.2; E2 straw 11.4±8.3) and decreased at T6 (p=0.05; E1 pellet 3.6±2.9; E2 pellet 0±0; E1 straw 2.7±3; E2 straw 0.1±0.3). E1 pellet was chosen for ICSI since its motility remained higher over time (p=0.03 vs E2 at T6). These VS were able to support embryo formation, with 25% cleavage and 5% morulae rate, which were similar to those obtained with frozen spermatozoa (46.2% cleavage, 11.5% morulae, p=0.21). No cleavage was observed with SHAM-ICSI (p=0.02 vs sperm-injected groups).
Conclusions. Feline epididymal spermatozoa maintained better morphofunctional parameters after vitrification with E1. Such VS were able to successfully fertilize mature cat oocytes by ICSI and produce embryos until advanced stages of in vitro development. This study demonstrates that the ultimate goal of having a fast and easy technique for cryopreservation of male germplasm under field conditions is becoming more than achievable.
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