Assessing endangered felid *Puma concolor* sperm fertility by *in vitro* fertilization with domestic cat oocytes.

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The *Puma concolor* population has been decreasing during the last 30 years. Semen cryopreservation of this specie has been accomplished successfully and offers the possibility of preserving endangered species. We previously showed that fertilizing capability of wild felid spermatozoa can be evaluated using ICSI with in vitro-matured domestic cat oocytes (Moro et al. 2014 Reprod Domest Animal 49, 693-700). Due to the lack of homologous oocytes, we evaluated the capability of the *Puma concolor* sperm to induce domestic cat oocyte fertilization and subsequent preimplantation embryo development. In the present study, cryopreserved sperm obtained by electroejaculation from five different males were used for *in vitro* fertilization (IVF) of *in vitro* matured domestic cat oocytes. Straws were thawed by exposing them to air for 10 s and then immersing in a 37°C water bath for 30 s. The contents of the straws were poured into a sterile 1.5-mL microtube pre-warmed to 37°C. The sperm suspension was diluted (1:3 v/v) by the slow (drop by drop) addition of a modified Tyrode's solution. For IVF, *in vitro* matured oocytes (n=370) were co-incubated with 0.5×10^5 motile spermatozoa mL^{-1} in an atmosphere of 21%O_2 in air at 38.5° C for 18–20 h. Presumptive zygotes were cultured *in vitro* in 50 ul drops of modified Tyrode's medium on 6.5%CO_2 in air at 38.5°C. Cleavage was determined 48h post fertilization, and 5% of FBS was added at day 5 of *in vitro* culture. Blastocyst stage was evaluated at Day 8. Results, mean (± s.e.m.), showed a high cleavage rate (179/370, 49.0 ± 4.0%), and a high development to morula stage (137/370, 34.4 ± 7.2%), and to blastocyst stage (94/370, 23.4 ± 4.7%) for all males. These results indicated that *Puma concolor* spermatozoa can induce domestic cat oocyte activation and development to blastocyst stage in similar rates to domestic cat homologous IVF: *in vitro* matured oocytes (n=291), cleavage rate (199/291, 67.1 ± 6.1%), development to morula stage (144/291, 47.8 ± 4.9%), and to blastocyst stage (86/291, 30.1 ± 1.6%). In conclusion, we demonstrated that domestic cat oocyte can be used to evaluated cryopreserve sperm samples from another felid specie.