

(0.5 mL plastic straws and 1.0 mL cryotube vials) on post-thaw motility, viability and changes in morphology of domestic cat epididymal spermatozoa. Epididymides were harvested from testes of 40 male feral cats after routine castration. Sperm samples were collected by macerating the cauda epididymides in 1.0 mL of Kenney's type semen extender (EZ, Animal Reproduction Systems) with a scalpel blade to release spermatozoa. Immediately after collection, sperm samples were evaluated for motility, viability and changes in morphology (eosin-nigrosin) and divided into four aliquots. Each aliquot was centrifuged ($700 \times g$ for 8 min), supernatant was removed and the resulting pellet was randomly assigned to one of the four treatment combinations arranged in a 2×2 factorial design (two cryoprotective extenders and two packaging types). After dilution and equilibration, both straws and vials were cooled by forced LN₂ vapor in programmable freezer (Micro Digitcool ZH 300 & UE 300 cycle freezer with 900 HP programmer 3T software CE (IMV Technologies, L'Aigle, France) until frozen and then transferred to liquid nitrogen storage dewar for at least 1 week before being subjected to post-thaw semen analyses. A two-way ANOVA was used to evaluate the effects of the two factors and the interaction on sperm motility, survival and morphology. Mean ($\pm$ S.E.M.) percentages of motile and viable spermatozoa, immediately after collection, were $62.8 \pm 12.7\%$ and $84.1 \pm 5.1\%$, respectively. The mean ($\pm$ S.E.M.) percentage of morphologically normal spermatozoa after collection was $61.2 \pm 10.4\%$. After thawing, findings supported that cryopreservation of domestic cat epididymal spermatozoa frozen using the LE cryoprotective media in cryotube vials resulted in a more vigorous post-thaw motility ($27.5 \pm 2.5\%$, $P < 0.05$), a higher level of viability ($77.9 \pm 1.1\%$, $P < 0.05$) and the greatest percentage of morphologically normal spermatozoa ($42.5 \pm 1.9\%$, $P < 0.0001$). There was, however, a marked decrease in motility, viability and appearance of normal morphology after freezing and thawing. Therefore, a more in-depth study addressing each stage of cryopreservation is needed to gain further understanding of where and when damage occurs.

Keywords: Feline; Epididymal spermatozoa; Cryopreservation; Cryoprotective media; Semen packaging

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EVALUATION OF THREE DIFFERENT TECHNIQUES TO RECOVER CANINE EMBRYOS USING A POST-MORTEM MODEL

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Reproductive technologies such as embryo transfer in canids may become a very important tool in conservation efforts. The dog is a good model for implementing such studies and could lead to important advances in these technologies that could be applied to wild animals such as foxes and other endangered wild canids. The present study compared three different recovery techniques, using simulation models in which denuded oocytes were introduced in recently recovered uteri. Ten denuded oocytes were deposited in the ovarian tip of each uterine horn using a tomcat catheter. Twenty replicates were made for each protocol. Technique one (T1) consisted of a puncture made close to the ovarian tip of the uterine horn with a 20-gauge needle connected to a 20 mL syringe loaded with 20 mL of PBS (flush medium); in the base of each horn a 2.5 cm incision was made to place a tomcat catheter, from which the fluid was recovered into a Petri dish. For technique two (T2), a puncture in the ovarian tip was made (as in T1), but the oocytes were recovered with an 8-French catheter that was passed through the cervix and the fluid recovered in a Petri dish. In technique three (T3), an 8-French catheter was inserted through the cervix and was used for infusing and recovering collection media. Each horn was flushed independently (20 mL of PBS was used for each horn). The recovery per horn was 7.3 ± 2.4 (73%), 8.5 ± 1.9 (85%) and 5.8 ± 2.2 (58%) for T1, T2 and T3, respectively. The first two techniques were not different ($P > 0.5$) and were selected to conduct an in vivo trial with six bitches whose embryos were collected 5 days after a diestrous smear. The average of embryos flushed using either technique was 6 ± 2 .

The findings of the study suggested that canine embryos could be retrieved using a less invasive technique by passing an 8-French catheter through the cervix, thereby reducing the damage to the uterus and the possible generation of adhesions.

Keywords: Embryos; Oocytes; Embryo transfer; Recovery; Canine

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