Evaluation of pre-freeze and post-thaw sperm quality of epididymal canine sperm cooled *in situ* or extended overnight

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- *Introduction and objectives*

Sperm harvesting from the epididymal cauda represents the last chance to obtain progeny in case of an unforeseen decease or castration. However, not all small animal clinics perform sperm cryopreservation and epididymides need to be shipped to specialized facilities. Unfortunately, the lack of technical knowledge regarding its optimal storage and shipping conditions prevents the preservation of canine epididymal sperm. Hence, our study aimed to define the best cooling method for canine epididymal sperm prior cryopreservation at 4 ˚C: within the epididymis or extended.

- *Materials and methods*

Testicles were collected from 11 healthy dogs of different breeds immediately after castration. One epididymis was immediately stored at 4 ˚C for 24 h (Time 0). The contralateral epididymis was flushed with CaniPlus Chill®, extended to reach 150 × 10^6 sperm/ml and preserved at 4 ˚C. After 24 h of cooling, the stored epididymis was flushed and processed as the former. Samples were centrifuged, and the obtained pellet was resuspended at 150 × 10^6 sperm/ml in CaniPlus Freeze® medium, plus 20% egg yolk (v/v). Sperm were packed in 0.5 ml straws, cooled at 4 ˚C for 1 h, exposed to liquid nitrogen vapor for 20 minutes and plunged into liquid nitrogen. Sperm quality was assessed in fresh and frozen-thawed samples at all the time points: 0 and 24 h (fresh samples) and after thawing (37 ˚C for 1 min). Motility was evaluated using a CASA system; viability, mitochondrial membrane potential (MMP) and DNA integrity were assessed by flow cytometry using SYBR-14/PI, JC-1 and sperm chromatin structure respectively. Results are expressed as the mean ± SEM in % comparing epididymal cooling *vs*. extended sperm. ANOVA and student *t*-test were used to compare normally distributed data; Kruskal-Wallis test and Mood's median test for non-normally distributed data; $p < 0.05$ was considered as significant.

- *Results*

  Canine epididymal sperm, cooled for 24 h at 4 ˚C within the epididymis, showed significantly differences compared to the cooled-extended samples respectively for viability (69.9 ± 1.8 *vs.* 59.6 ± 3.4, mean % ± SEM; $p < 0.05$), higher MMP (64.9 ± 3.7 *vs.* 51.1 ± 2.7, mean % ± SEM; $p < 0.05$), and beat cross frequency (BCF) (7.5 ± 0.5 *vs.* 5.6 ± 0.6, mean % ± SEM; $p < 0.05$). After cryopreservation, significant differences were also found in MMP (50.9 ± 2.2 *vs.* 39.1 ± 4.9, mean % ± SEM; $p < 0.05$) and in viability (48.5 ± 2.7 *vs.* 39.5 ± 4.2, mean % ± SEM; $p < 0.05$) in samples cooled in the epididymis *vs*. extended 24 h prior cryopreservation, respectively. DNA fragmentation remained unaffected in all the treatments ($p > 0.05$).

- *Conclusions*

  The study demonstrates that epididymal sperm better maintains its quality when cooled within the epididymis prior freezing. This finding opens a new horizon in the small animal clinics procedures to store and/or ship epididymal sperm in dogs prior freezing. Funding: RYC-2017-21545 (AEI/FEDER/UE, Spain); TA18008 (Junta de Extremadura, Spain).