

Effect of three different extenders and two thawing temperatures on frozen-thawed canine sperm.

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Frozen canine semen does not have the same viability as fresh semen and its survival in the female reproductive tract is reduced. Canine semen cryopreservation is important to canine breeders and to standardize protocols to freeze semen from endangered canine species. The aim of this study was verify the effect of three different extenders and two thawing temperatures on frozen-thawed canine sperm characteristics.

One ejaculate from each of nine different dogs was collected and processed (n=9). Each ejaculate was divided into 3 equal samples and centrifuged in 1:1 skim milk plus glucose extender for 5 min. at 800g. The pellets were re-suspended using each of the 3 extenders for each dog separately. **GEY extender** (0.6g glucose, 0.6g fructose, 1g sodium citrate, 0.47g glycine, 0.03g Na-benzylpenicillin, 0.1g streptomycin solubilized in 80ml of distilled water plus 20ml egg yolk and 0.4ml of Orvus est Paste) without glycerol. After 5 minutes the same amount of GEY extender with 12.8% glycerol was added and filled in 0.5ml straws. **TRIS extender** (2.4g TRIS; 1.4g citric acid; 0.8 g glucose, 0.06 g Na-benzylpenicillin, 0.1 g streptomycin, solubilized in 80ml of distilled water plus 20 ml egg yolk and 1 ml of Orvus est Paste) with 3% glycerol. After 5 min. the same amount of TRIS extender with 7% glycerol were added and filled in 0.5ml straws. **M9 extender** (33% of Merck-egg yolk extender; 33% Kenney's extender; 33% DME and 5% glycerol at the final solution) and filled in 0.5ml straws. A total of 54 straws (6 from each dog, being 2 with each extender) with a mean of 33.73×10^6 sperm/straw were prepared. The sperm motility, vigour and plasma membrane integrity from a sample from each protocol were immediately evaluated and the straws were put in a refrigerator to reach temperature of 5°C in 20 minutes and then were maintained in the refrigerator for an additional 40 minutes. After that, a sample from each protocol was warmed up in a water bath 37°C for 5 min. and sperm motility, vigour and plasma membrane integrity were evaluated. The straws were frozen by suspending 4 cm above liquid nitrogen in a Styrofoam box. One straw from each protocol was thawed at 37°C for 30 sec and another at 72°C for 8 sec and the sperm motility; vigour and plasmatic membrane integrity were evaluated. At this time, acrossomal status was verified using FITC-PNA stain. Plasma membrane, sperm motility and vigour were evaluated after a 1-hour incubation at 37°C. Thawing at 72°C for 8 sec had a positive effect ($p < 0,05$) on frozen-thawed canine sperm characteristics. Canine semen frozen in GEY extender and thawed 72°C for 8 sec had the highest ($p < 0.05$) sperm motility, vigour and when associated had a high membrane integrity and low acrossome reaction.

Keywords: Semen, canine, cryopreservation, extender, thawing temperature.