Performance of CaniPlus Freeze Extender for freezing canine semen

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1) Introduction

Freezing dog semen in semen straws has recently become more relevant worldwide, although the method has been established since the early sixties. TRIS-based extenders have been developed and proved in numerous publications that they ensure high sperm motility rates after thawing. CaniPlus Freeze is a commercially available freezing extender for canine semen based on TRIS (Tris(hydroxymethyl)aminomethane).

Other ingredients used in CaniPlus Freeze are glycerol as a cryoprotective agent, gentamicin (antibiotic), fructose, citric acid, antioxidants and specific stabilising agents.

The production of CaniPlus Freeze is performed in Minitüb GMP-certified facilities which are dedicated to the production of media for semen and embryos of various species. Minitüb is DIN ISO-certified (ISO 9001:2008), regularly audited, and complies with international quality management standards.

2) Materials and Methods

In 5 trials, a total of 13 ejaculates from 5 dogs were tested. The sperm rich fraction of each ejaculate was split, diluted with either CaniPlus Freeze or with a standard control extender and submitted to a standard freezing protocol. For each trial (1 to 5), the semen samples were collected from the dogs, processed and analysed on the same day in a reference research lab.

Motility (phase contrast microscope) was assessed for all 5 trials. Percentages of progressively motile, local and immotile sperm were determined before and after freezing. In 3 of the 5 trials, the membrane integrity of frozen-thawed semen was analysed with a flowcytometer and acrosome integrity was assessed using a specific stain and phase contrast microscopy.

The following freezing protocol was used:
The diluted semen was filled in 0.5 ml straws at ambient temperature. The straws were placed on a rack suspended 4 cm above liquid nitrogen (LN) and allowed to freeze in the LN vapour for 10 minutes. The straws were then plunged into the liquid nitrogen. The straws were thawed in a 38°C water bath for 30 seconds immediately prior to motility testing.
3) Results

The main parameter for predicting semen fertility is the percentage of progressive motile sperm. In these trials, CaniPlus Freeze semen had more than a 16% higher post thaw progressive motility when compared to semen frozen in the control extender: mean value 65.62% compared to 48.80%. The progressive motility before freezing was similar in both extenders (mean values 89.78% and 89.46%).

![Progressive Motility before freezing](image1)

**Figure 1:** Progressive Motility of 13 ejaculates before freezing.

![Post Thaw Motility](image2)

**Figure 2:** Post Thaw Motility of the 13 ejaculates.
There were more membrane intact sperm cells after thawing when frozen in CaniPlus Freeze (mean value 48.1% compared to 41.7%)

Figure 3: Membrane intact sperm cells post thaw

CaniPlus Freeze had also a positive effect on acrosome integrity: 64.3% intact sperm cells compared to 47.4%.

Figure 4: Acrosome intact sperm cells post thaw