Induced immotility using the CLONE chilled semen kit during long-term storage at +5°C does not prolong survival of dog spermatozoa

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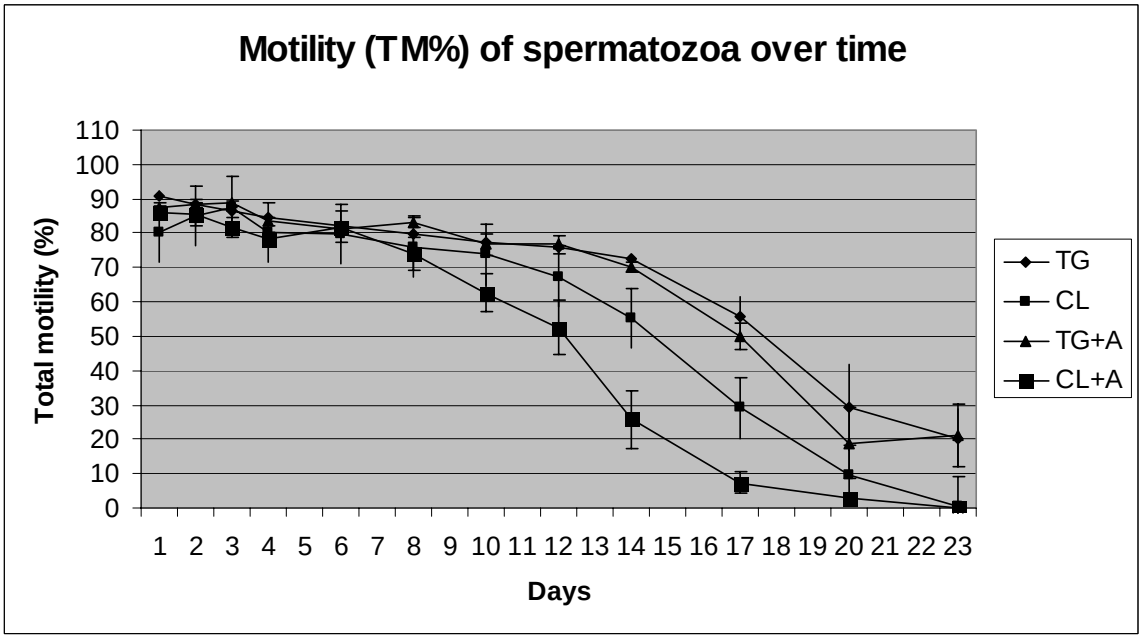
Abstract

This study investigated whether the immotility induced by the CLONE chilled semen extender prolongs the lifespan of dog spermatozoa stored at 5°C, compared with a Tris-egg yolk-glucose (TG) extender, which maintains motility. Pooled semen was split in four aliquots, centrifuged, and the four sperm pellets mixed with TG extender; with the CLONE chilled semen (CL) extender; with TG extender mixed with an activator ($TG+A^{TG}$); or with the CLONE extender mixed with the CLONE activator ($CL+A^{CL}$). Samples were stored at 5ºC for 23 days and examined twelve times for sperm motility, plasma membrane and acrosome integrity, glucose consumption, and DNA fragmentation index (DFI). The experiment was performed in triplicate. Glucose consumption was not significantly different between extenders until in the period 15-23 days, when it was higher in CL and $CL+A^{CL}$ than in TG (P=0.0055) and $TG+A^{TG}$ (P=0.0010). No breakdown of DNA chromatin (P>0.05) occurred until day 14. **Spermatozoa preserved in TG or $TG+A^{TG}$ showed better values for all the different parameters throughout the experiment compared with sperm subjected to CL or $CL+A^{CL}$.** In conclusion, the immotility induced by the CLONE chilled semen extender during long-term cold storage at 5°C did not prolong the lifespan of spermatozoa compared with the lifespan following storage in Tris-egg yolk-glucose. In addition, **our results indicate that good quality dog semen may possibly be stored for up to 14 days in TG extender at 5°C, with retained fertilizing capacity**. *In vivo* studies should, however, be performed to further support this conclusion.



Total motility percentages (TM%) of spermatozoa over time (means ± S.E.M).

Published in: Theriogenology 2007;68:920-933.