# IMPROVING THE CONSERVATION OF FRESH RAM SEMEN

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Sheep production is under development in Canada, however, artificial insemination (AI) is limited by the short storage time required for fresh semen (8 h). Three hypotheses have been tested to improve fresh semen conservation. (1) The removal of glycerol from egg yolk-based extender improves sperm conservation at 5°C. (2) Semen pre-incubation for 4 h at room temperature between collection and dilution increases sperm resistance to cooling and storage. (3) Inclusion of seminal plasma to the extender benefits conservation. Ram ejaculates were aliquoted, diluted according to hypothesis (± 7% glycerol; ± 10 or 25% seminal plasma) and placed in straws. For the second experiment, one aliquot was held undiluted at room temperature for 4 h before extension. Straws were cooled and stored to 5oC in a transport box. At different times until 24 h post-collection, straws were diluted into a physiological medium, synthetic oviductal fluid (SOF; 39°C, 5% CO2) to mimic the genital tract of the ewe. Samples from SOF were then assessed for sperm quality based on computer-assisted motility parameters, viability by eosin-nigrosin staining and capacitation status using the chlortetracycline (CTC) assay.

In the experiment testing the effects of glycerol (n = 6), no differences were observed; sperm quality was unaffected during conservation at 5°C in glycerol-free egg yolk extender at all times (0, 8, 16, 24 h; $P<0.28$ for motility data, viability, CTC pattern distribution) and incubation for 0, 6 or 24 h in SOF did not discriminate between treatments ($P<0.59$ for all parameters). A 4 h pre-incubation prior to dilution reduced the % motile sperm and proportions of normal and capacitated sperm according to CTC patterns F and B, respectively during conservation for 8 or 24 h ($P<0.01$; n = 4). Pre-incubated sperm tended to be less motile in SOF for up to 6 h than controls ($P=0.07$) although survival tended to be improved ($P=0.08$). Inclusion of seminal plasma to the egg yolk-glycerol extender did not modify semen quality during conservation at 5°C for 8 or 24 h ($P<0.33$ for all parameters; n = 4); no differences were observed during incubation in SOF for 0, 4 or 8 h

($P<0.29$). Studies are underway to test other extenders of fresh ram semen.

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