6. *IN VITRO* ASSESSMENT OF FUNCTION OF SEX-SORTED FROZEN–THAWED RAM SPERM

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Lambs of predetermined sex have been produced by laparoscopic AI with low numbers of frozen–thawed sex-sorted sperm. However, sperm that have undergone both sorting and freezing may have lower fertility than those that have only been frozen (1). In the present study, we attempt to evaluate *in vitro* the altered function of sex-sorted sperm in the female reproductive tract (FRT). Semen (3 rams, 3 ejaculates per ram) was collected and either a) used directly (FRESH), b) frozen by a commercial method (FT Control; 1), or c) Hoechst 33342 stained and sex-sorted using a modified high-speed cell sorter (2) then frozen (FT Sort; 1). Assessments of sperm (*i*) binding to ovine oviduct epithelial cell (OEC) monolayers (3), (*ii*) migration through artificial cervical mucus (HA; 4), (*iii*) acrosomal integrity using FITC-PNA, and (*iv*) motility were made over 4 h. FRESH sperm bound to the OEC during incubation (61.6, 74.6 and 75.9% bound at 0.5, 2 and 4 h, SEM = 5.3%) whereas FT Control and FT Sort sperm were released from the OEC with incubation (52.1, 45.6, 29.9% and 45.6, 29.0 and 18.1% bound respectively at 0.5, 2 and 4 h). More FT Sort sperm were released at 2 h than FT Control sperm ($p<0.05$). More FRESH sperm (241.6 ± 11.9 sperm; $p<0.05$) migrated 0.5 cm into the HA than either the FT Control (76.4 ± 11.9) or FT Sort (73.9 ± 11.9) samples. There was no difference between treatments in the distance migrated by the vanguard sperm. Overall, more FT Sort sperm (88.6 ± 1.5%, $p<0.05$) were acrosome-intact than FRESH (84.0 ± 1.5%) and FT Control sperm (81.8 ± 1.6%). Motility of FT Sort (51.1, 39.4 and 23.3% motile, SEM = 2.6%) decreased more rapidly during incubation ($p<0.05$) than that of both FRESH (89.4, 85.6 and 78.9% motile) and FT Control sperm (67.2, 58.3 and 35.6% motile at 0, 2 and 4 h, respectively). The rapid release of FT Sort sperm from OEC and their decreased longevity may indicate that FT Sort sperm have a shorter period of time within the FRT to encounter the oocyte than FT Control sperm.

(1) Hollinshead *et al*. (2002) *Reprod. Fertil. Dev.* **14**:505. (2) MoFlo®, DakoCytomation, Fort Collins, CO, USA. (3) Gillan *et al*. (2000) *Reprod. Fertil. Dev.* **12**:237. (4) Hollinshead *et al*. (2002) *Reprod. Fert. Dev.* Suppl. 14:53.

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