137 Assessment of fertilizing ability of Merino ram semen cold stored up to 48 h by heterologous IVF of bovine oocytes

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Abstract

The use of cold-stored ram semen has been applied in sheep AI programs, because it preserves its fertilizing ability similar to fresh. Besides, the heterologous IVF has been successfully employed to assess semen fertilizing ability in several species. Hence, we aimed to evaluate the fertilizing ability of ram semen cold stored up to 48 h at 5°C by assessing heterologous IVF using bovine oocytes. Fifteen pools of 3 normospermic Merino ram (2-7 years) ejaculates were collected using artificial vagina, diluted to 200×10^6 spermatozoa mL^{-1} with ultra-heat-treatment-based extender (skim milk-6% egg yolk) and cold stored up to 48 h. *In vitro* matured zona-intact bovine oocytes were subjected to heterologous IVF using fresh semen (FS, $n = 707$), semen cold stored to 24 h (CS24, $n = 832$) or semen cold stored to 48 h (CS48, $n = 611$). In parallel, homologous IVF (control, $n = 1356$) and parthenogenesis (parth control non-fertilized oocytes, $n = 334$) were performed. Ram non-selected and selected (BoviPure, Nidacon International, Mölndal, Sweden) semen parameters were evaluated by computer-assisted semen analysis. Sperm-oocyte interaction was assessed at 2.5 h post-insemination (hpi) by evaluating the number of bound spermatozoa, whereas penetration and polyspermy were evaluated after 12 hpi. Presumptive zygotes were fixed and stained with Hoechst 33342 at 18, 20, 22, 24 and 26 hpi to assess pronuclear formation using phase contrast and confocal microscopy. Cleavage rate was evaluated in all groups at 48 hpi. Data obtained from 5 replicates were analysed using one-way ANOVA. Data was expressed as mean ± standard error of the mean. In terms of sperm storage time, non-selected semen showed a significant decrease ($P < 0.05$) for CS24 and CS48 compared with FS on progressive motility [SPM (%): 52.30 ± 4.1 and 36.9 ± 5.5 *v.* 71.3 ± 1.6] and straight-line velocity (mm s^{-1}: 132.2 ± 6.1 and 109.7 ± 6.3 *v.* 176.7 ± 4.3), respectively. However, selected semen showed a decrease ($P < 0.05$) only for CS48 when compared with CS24 or FS on SPM (35.6 ± 3.9 *v.* 56.1 ± 6.91 and 59.3 ± 2.6) and straight-line velocity (83.5 ± 4.4 *v.* 105.3 ± 6.5 and 110 ± 2.0), respectively. No differences were observed between heterologous IVF groups in all parameters evaluated. Homologous IVF showed a higher percentage of penetration only when compared with heterologous FS group (44.4 ± 6.8 *v.*12.5 ± 4.5%; $P < 0.01$). The polyspermy was higher in heterologous CS24 group when compared with homologous IVF (11.4 ± 3.4 *v.* 3.8 ± 2.2; $P < 0.05$). The homologous IVF group, as expected, showed the higher percentage of pronuclear formation at 18 hpi compared with heterologous IVF with FS (67.3 ± 5.8 *v.* 35.2 ± 5.6%), CS24 (72.1 ± 4.5 *v.* 37.2 ± 5.7%) and CS48 (63.0 ± 6.0 *v.* 27.0 ± 5.6%), respectively ($P < 0.001$). Likewise, cleavage rate was higher in homologous group compared with heterologous IVF and parthenogenetic groups for FS (78.3 ± 2.6.8 *v.* 46.3 ± 3.2 and 7.0 ± 2.3%), CS24 (78.4 ± 2.6 *v.* 48.3 ± 3.2 and 4.9 ± 2.0%), and CS48 (78.4 ± 3.3 *v.* 43.3 ± 3.5 and 4.3 ± 1.2%), respectively ($P < 0.001$). In conclusion, Merino ram semen cold stored up to 48 h maintains its fertilization ability to the same extend as fresh and can be used for sheep crossbreeding programs.

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