Cysteine, Lipid Peroxidation, Motility

P-138: The Effects of Omega-3, 6, 9 Fatty Acids on The Quality of Bovine Chilled Semen

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Background: Many studies are being conducted to improve the quality of bull sperm in storage conditions. It seems that increasing sperm membrane unsaturated fatty acids can enhance sperm quality. This study was aimed to investigate the effects of combination of omega- 3, 6, 9 fatty acids on characteristics of bovine chilled semen.

Materials and Methods: Different levels of oil containing omega- 3,6,9 fatty acids was added to semen extender. Five proven bulls were randomly selected and their ejaculates were collected by artificial vagina. Fresh semen was analyzed in terms of several parameters including volume, concentration, motility, viability and morphology. To emulsify the oil in semen extender, polyethylene glycol (PEG) was added as a more suitable solvent and solution was finally sonicated. Experimental groups included: control and treatments 1, 2, 3 and 4 as sham, levels 1, 2.5 and 5% of omega-3, 6, 9 fatty acids, respectively. In treatment 1, PEG was added alone to the diluents and in treatments 2, 3 and 4 different concentration of omega-3,6,9 fatty acids in combination with PEG were added to extender based Tris- citrate buffer, egg yolk and glycerol. After dilution, semen samples were packed into 0.5 ml straws and then cooling process was performed. Samples were evaluated in terms of motility, viability and morphology after 24 and 48 hours of storage in refrigerator (5°C). Motility and other dynamic parameters were analyzed by computer aided sperm analyzer (CASA) and viability and morphology were measured using phase contrast microscope after staining. The results were evaluated by repeated measure ANOVA using SPSS and $p<0.05$ was considered significant.

Results: Immobility increased and all other parameters (including motility, progressive sperm, viability, morphology…) significantly decreased in all groups including control compared with fresh samples. Moreover, some parameters including motility and progressive sperm were significant different between groups ($p<0.05$). Our result showed that PEG has significant detrimental effects on motility ($p<0.05$) while its effects was not significant on the viability and morphology ($p> 0.05$). Combination of omega-3, 6, 9 fatty acids could not attenuate harmful effects of PEG significantly. Moreover, they could not improve sperm motility, viability and morphology of bovine sperm in chilled storage condition.

Conclusion: Our result showed that this fatty acid combination does not appear to be a proper candidate for improving bovine sperm quality in cold conditions.

Keywords: Bovine, Sperm Quality, Cooling, Omega- 3,6,9 Fatty Acids

P-139: Compaison of Swim Up and Histoprep® Continuous Gradient for Isolation of Motile Epididymal Sperm in Ram

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Background: Isolation of motile sperm is essential for routine IVF programs. One of most important source of sperm for IVF is isolation of epididymal sperm (ESP). The problem regarding use of ESP is somatic cells which are recovered through sperm collection. The swim up and the swim down procedures are use to isolate motile sperm. The aim of the present study was to compare swim up and Histoprep® continuous gradient (HP) for isolation of ram ESP.

Materials and Methods: Ram testes epididymes were collected from local slaughterhouse and transported to the Lab. Basic medium for separation of sperms from epididymis was caffeine treated BO solution. Histoprep was provided from the company. After incubating of cauda epididymis in the basic medium for 15 minutes, primary sperm parameters including sperm motility and concentration were recorded. Two equal volumes of sperm solution were use in swim up solution and on Histoprep gradient. We followed the routine swim up procedure by incubation of 800 µl of BO-caff in CO_2 incubator for 30-45 minutes. For Histoprep, we followed the company protocol for isolation of human lymphocytes. After sperm recovery in two procedures, the secondary sperm parameters were recorded. To find the effects of the procedure on sperm parameters, proportion of secondary to primary sperm parameters, for each method, was calculated.

Results: The results showed that proportion of motile sperms were 1.094 ± 0.04 and 1.19 ± 0.06 in Histoprep and swim up respectively ($p>0.05$). The proportion of secondary to primary sperm count for two procedure was similar (0.27 ± 0.05 and 0.25 ± 0.07 for Histoprep and swim up, respectively).

Conclusion: The results showed that Histoprep and swim up have similar effects on motile sperm isolation for ram ESP.

Keywords: Epididymal Sperm, Swim Up, Histoprep, Ram

P-140: Effects of The Method of Motile Epididymal Sperm Isolation on Embryo Production in Ewe

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Background: Motile sperm is essential for successful embryo production. The aim of the present study was to compare the *in vitro* embryo development following