



Use of a Plant Polysaccharide Gradient to Wash Bull Sperm Improves Fertilization & Embryonic Development

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Experiment 1

A continuous gradient of 22% plant polysaccharides (arabinogalactan) in a buffered salt solution (Sperm Concept-INGfertility, Spokane, WA) was compared to a standard Percoll density gradient of 45 % and 90% using frozen-thawed bull sperm (Select Sires).

Studies were designed to evaluate sperm recovery and performance in a routine IVF system as determined by fertilization rates and subsequent embryonic development. Frozen sperm from 4 bulls (2 replicates each) were washed through 4 ml of Sperm Concept (SC) or gradient Percoll (P) for 30 min at 300 x g.

The pellet of sperm from P was washed again in a TALP medium prior to use. No extra wash step was used in the hemocytometer. Sperm motility was also evaluated both subjectively and objectively with an HTM Analyzer.

All data are expressed as the mean SEM for SC or P treated sperm, respectively. Statistical analyses were conducted using ANOVA.

Conclusions

Recovery of sperm was significantly higher ($p=0.02$) after SC washing than with P (72.6% vs 53.5%). SC also tended ($p=0.08$) to recover more of the motile sperm than did P (88.9% vs 73.6%). Overall percent motilities were high for both groups and did not differ ($p=0.18$; 95.03% vs 90.3%).

Experiment 2

Pooled frozen bull sperm was also washed through either SC or P as above, treated routinely with 10 IU heparin/ml and placed with total of 1100 IVM oocytes over three replicates.

Oocytes were obtained from slaughtered beef heifers. Cleavage rates were determined visually on Day 3 of culture in CZB medium. Embryo quality was recorded on Day 9 of coculture on BRL cells, followed by embryo staining



(Hoechst 33258) and cell counts. Embryo evaluators were blind to the sperm washing treatment utilized prior to IVF.

Conclusion

Fertilization rates for the oocytes were improved after washing sperm through SC versus P ($p=0.001$; 73.2% versus 53.3%). The percentage of fertile oocytes able to develop to blastocysts by Day 9 of culture was higher after washing sperm through SC versus P ($p=0.009$; 59.4% vs 26.5%).

Overall production of blastocysts as a percentage of total oocytes introduced into the IVF system was greater after washing sperm through SC versus P ($p=0.004$; 43.4% vs 18.3%). Total cell counts for blastocysts formed after fertilization with either SC or P treated sperm did not differ ($p=0.25$; 85.2 vs 88.2).

Overall Conclusion

Washing frozen thawed bull sperm through SC appears to offer several advantages over P gradients. A pellet of highly motile sperm cells can be obtained in one centrifugation step. Sperm exposed to the arabinogalactan-containing SC wash show improved ability to fertilize oocytes and contributed to better embryonic development to the blastocyst stage.