

# Effect of Concentration of Sexed Bovine Sperm Sorted at 40 and 50 psi on Developmental Capacity of In-Vitro Produced Embryos

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Frozen sex sorted sperm undergo a series of procedures that compromise fertility with IVF. The objective of this experiment was to determine whether there was a difference in fertilization and blastocyst rates with sperm sorted at either 40 or 50 psi. To test this at critical sperm numbers, a dose response of sperm concentration in the fertilization medium was done with X and Y-sorted sperm in each subclass. Thus, a factorial experiment was designed with 2 sorting pressures, 3 sperm concentrations (1, 0.33 and 0.11 x 10<sup>6</sup> sperm/ml), 2 sexes and replicated with sperm of 6 bulls. Oocytes (n=2160) were aspirated from 2-8mm follicles from slaughterhouse ovaries and matured in M-CDM supplemented with 0.5% fatty acid free-BSA, 15ng/ml NIDDK-oFSH-20, 1 g/ml USDA-LH-B-5, 1 g/ml E2, 50ng/ l EGF and 0.1mM cysteamine for 23 hours at 38.8 C and 5% CO<sub>2</sub> in air. Sorted sperm frozen with 2 x 10<sup>6</sup> cells per straw were thawed and centrifuged at 400 g through 2ml 45% and 2ml 90% Percoll gradient for 20 minutes. Then, the supernatant was discarded and 2ml of FCDM supplemented with 0.5% FAF-BSA, 2mM caffeine and 2 g/ml heparin was added to the sperm pellet and centrifuged at 400 g for 5 minutes. The supernatant was discarded leaving approximately 50 l of sperm suspension. Matured oocytes were washed once in FCDM and transferred in groups of 15 in 5 l into 25 l drops of FCDM under mineral oil. Fertilization took place by adding 10 l of sperm suspension per drop for 18 hours at 38.8 C, 5% CO<sub>2</sub> in air. Presumptive zygotes were cultured in CDM1 for 2 days and CDM2 for 4.5 days at 38.5 C, 5 % O<sub>2</sub>, 5% CO<sub>2</sub> and 90% N<sub>2</sub>. On day 7.5, blastocyst development was evaluated: Quality 1-4 (1=excellent and 4=poor) and stage of development, 6-8 (6=full, 6.5=expanding, 7=expanded, 7.5=hatching and 8=hatched blastocyst). Data (Table 1) were analyzed by ANOVA and Tukey's hsd after arc sin transformation.

Bull:	H023	H026	H025	H026	H027	H028	Average	
Sperm Concentration (10 <sup>6</sup> )	C/B	C/B	C/B	C/B	C/B	C/B	C	B
0.11	18/1	6/1	42/11	36/15	20/7	54/15	30 <sup>a</sup>	8 <sup>a</sup>
0.33	44/4	7/2	72/31	62/21	29/11	68/18	47 <sup>b</sup>	14 <sup>b</sup>
1.0	56/18	35/14	85/43	83/34	72/27	85/29	69 <sup>c</sup>	28 <sup>c</sup>

<sup>a,b,c</sup> Values without common superscripts within groups differ, P<0.01