

**Supplementary Table S1** Survival of X and Y bearing sperm for 3 or 5 days under various culture conditions.

Culture period	Temperature	Medium pH	Sperm counted	% Sperm alive, mean (SD)		Sex chromosome ratio in live sperm	
				X bearing	Y bearing	Mean (SD)	
Initial		7.5	3331	65 (2.9)*	64 (5.0)*	0.97 (0.04)	
Day 3	4°C	6.5	2425	50 (12.9)	37 (5.0)	0.77 (0.11)	
		7.5	3089	49 (9.8)	41 (7.3) <sup>†</sup>	0.85 (0.04)	
		8.5	3130	41 (8.4)	39 (10.1)	0.93 (0.08)	
		6.5	3176	50 (3.8)	47 (7.2)	0.95 (0.06)	
	22°C	7.5	3257	56 (1.2)	51 (3.7)	0.92 (0.06)	
		8.5	2047	50 (0.4)	45 (0.8) <sup>‡</sup>	0.91 (0.01)	
		37°C	6.5	3168	33 (9.0)	29 (8.0) <sup>†</sup>	0.87 (0.02)
			7.5	3163	40 (7.2)	34 (8.9) <sup>‡</sup>	0.85 (0.09)
Day 5	4°C	8.5	3252	37 (7.6)	31 (7.3) <sup>†</sup>	0.84 (0.03)	
		6.5	3247	48 (12.5)	40 (11.1) <sup>†</sup>	0.84 (0.02)	
		7.5	3147	49 (18.5)	47 (19.5)	0.95 (0.06)	
		8.5	3107	32 (16.4)	27 (18.7)	0.80 (0.15)	
	22°C	6.5	3324	47 (11.0)	40 (8.8) <sup>‡</sup>	0.87 (0.02)	
		7.5	3139	49 (14.0)	47 (14.9)	0.95 (0.08)	
		8.5	3165	34 (10.7)	28 (9.1) <sup>†</sup>	0.86 (0.02)	
		37°C	6.5	3175	22 (11.0)	19 (9.5)	0.85 (0.19)
	7.5		3149	30 (17.9)	29 (18.3)	0.95 (0.10)	
	8.5		2998	33 (11.0)	28 (9.6) <sup>‡</sup>	0.82 (0.13)	

Sperm counts are presented as the total of three independent measurements.

Live X and Y sperm (%) and sex chromosome ratio are presented as mean  $\pm$  SD of three independent experiments.

\*Significant difference in viability in vertical column ( $P < 0.05$ ), <sup>†</sup>significant difference in viability between X and Y spermatozoa ( $P < 0.05$ ), <sup>‡</sup>significant difference in viability between X and Y spermatozoa ( $P < 0.01$ ).