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Supplementary Material

Successful sperm cryopreservation in Egyptian spiny mice *Acomys cahirinus*

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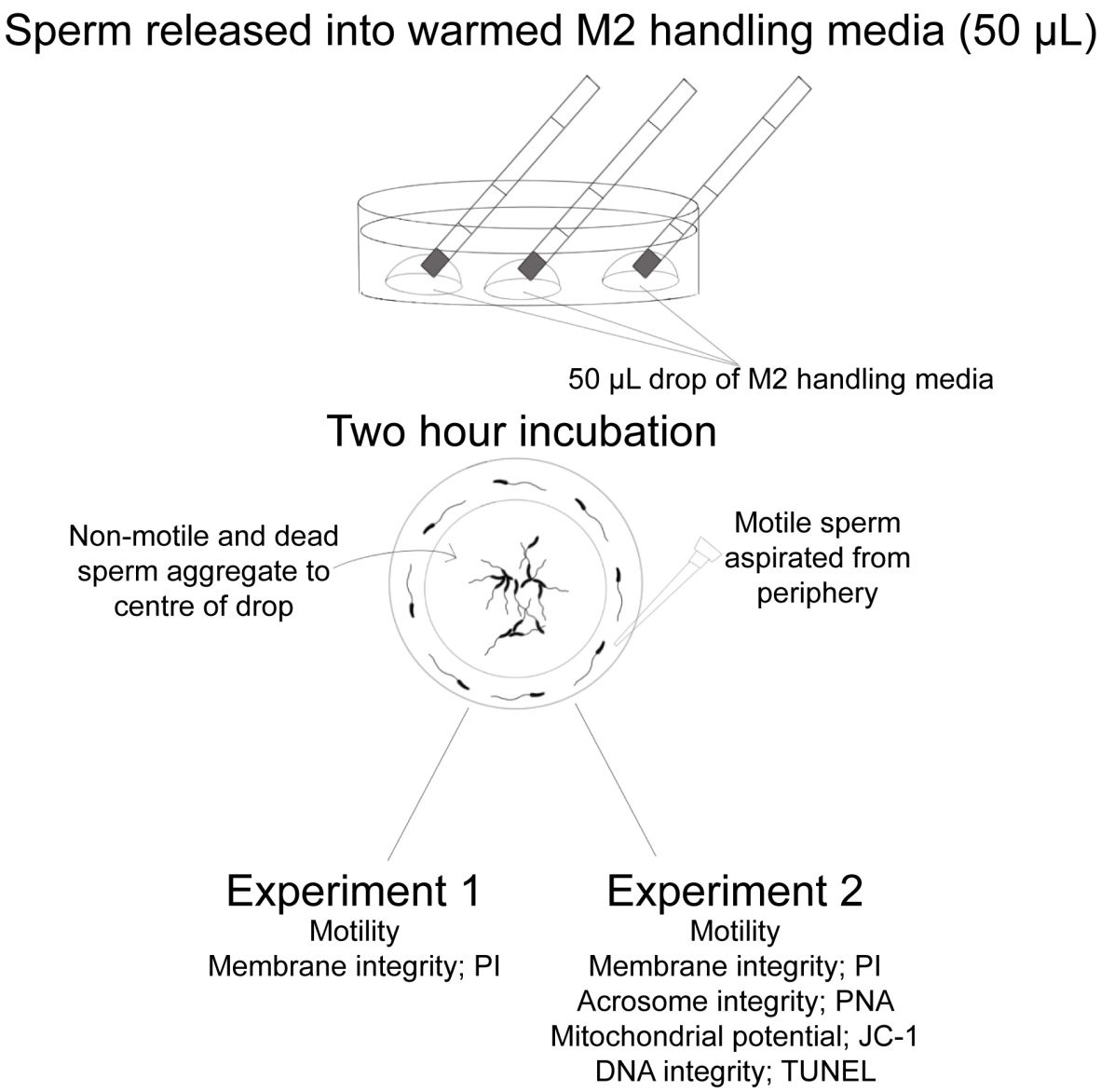
Fig. S1. Flowchart of sperm analysis procedure for both Experiment 1 and 2 adapted from Takeo and Nakagata (2010).

Fig. S2. Micrograph displaying sperm DNA fragmentation.

Table S1. Staining patterns of fluorescently labelled sperm.

Table S2. Comparative post-thaw motility and membrane integrity of spiny mouse spermatozoa when assessed immediately after thawing and following a two-hour incubation in warmed handling media.



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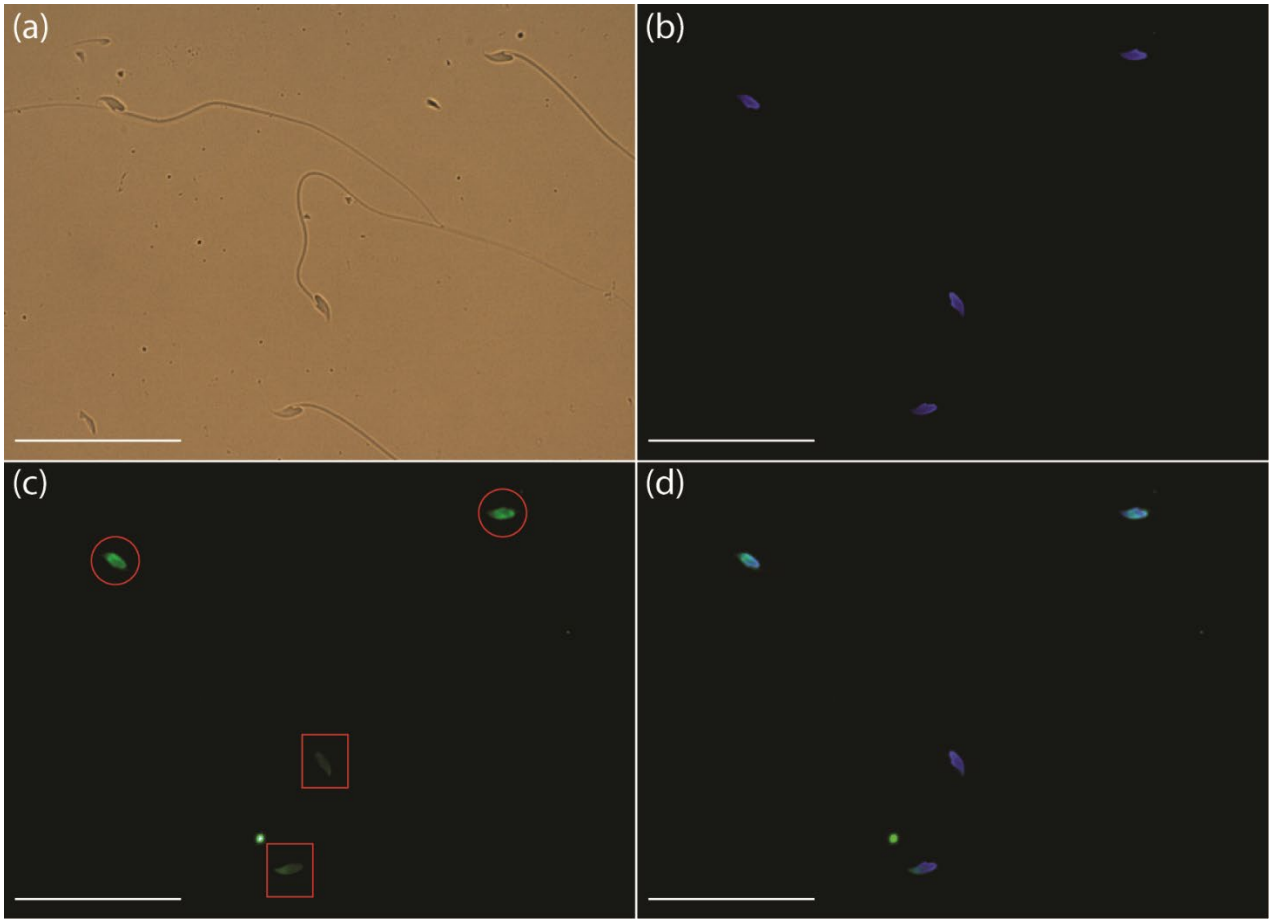

Fig. S2. Micrograph displaying sperm DNA fragmentation. (*a*) Bright field image of *A. cahirinus* spermatozoa; (*b*) H33342 positive sperm; (*c*) TUNEL positive (circles) and negative (squares) spermatozoa; (*d*) Merged image showing TUNEL and H33342 positive (circles) and negative (rectangles) spermatozoa. Scale bars indicate 50 µm.

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Table S1. Staining patterns of fluorescently labelled sperm

+, fluorescent positive; -, fluorescent negative

Staining pattern			Sperm classification		
PI + (purple) - (blue)	PNA + (green) - (absent)	JC-1 + (orange/yellow) - (green)	Membrane integrity	Acrosome integrity	Mitochondrial potential
-	-	+	Intact	Intact	High
		-			Low
-	+	+	Intact	Damaged	High
		-			Low
+	-	+	Damaged	Intact	High
		-			Low
+	+	+	Damaged	Damaged	High
					Low

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Table S2. Comparative post-thaw motility and membrane integrity of spiny mouse spermatozoa when assessed immediately after thawing and following a two-hour incubation in warmed handling media

Data are mean of six animals and two slides per animal. Motility quality is described as + non-progressive, ++ slow-progressive and +++ rapid-progressive motility

	Immediate assessment				Two-hour incubation			
Cryopreservation solution	18R3SM	15R3SM	15R5SM	10R5SM	18R3SM	15R3SM	15R5SM	10R5SM
Average motility (%)	14	17	17	19	34	35	35	19
Motility quality	++	+	+	+	++	++	++	++
Average membrane integrity (%)	24	22	24	27	36	39	42	25