



Supplementary Figure S1 Experimental design. Semen from donors, IVF patients and those patients having previous failed/low fertilization at IVF (recall) was prepared using density gradient centrifugation and a wash step. The primary assay was the Ca^{2+} influx induced by progesterone. Depending on cell availability, the samples were then subjected to one or a combination of the progesterone-induced penetration into viscous media; 4-aminopyridine (4-AP)-induced hyperactivation (HA) assay. Spermatozoa from men with a defective Ca^{2+} response were asked to provide additional samples. In men where there was a consistent and robust failure of the Ca^{2+} influx induced by progesterone, i.e. repeatable in more than one semen sample, a direct assessment of CatSper function was performed using electrophysiology (patch clamping).