



**Figure S1.** Data acquisition and selection of viable single human sperm cells in the BD Accuri C6 Plus flow cytometer. **(a)** Representative density plot comparing side (SSC-A) versus forward scatter area (FSC-A). Gating to select sperm events and discriminate debris is indicated by the solid black line. **(b)** Forward scatter height (FCS-H) versus FSC-A profile applied to the subpopulation selected in **a** to exclude aggregates and select single events. **(c)** Density histograms of Fluo3-AM (Fluo3) fluorescence emission for cell samples that were unstained (white), or Fluo3-stained and either untreated (dark green) or heat-killed (light green). The percentage of viable, non-viable or dead cells is indicated above the histograms when appropriate.