

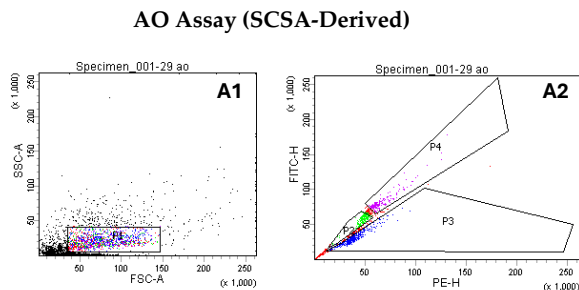
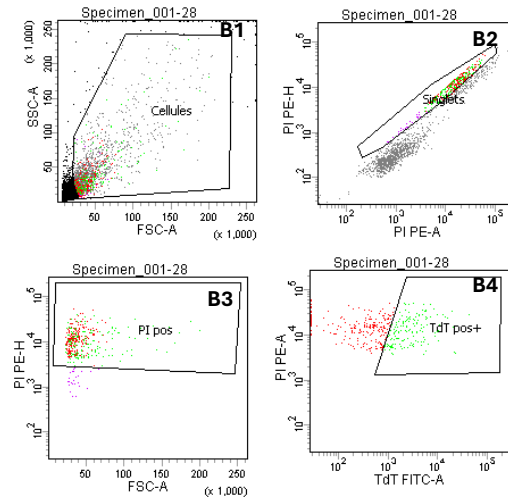
A**B****TUNEL Assay**

Figure S1. Flow cytometry acquisition and gating strategy for the Acridine Orange (A) and TUNEL (B) assays.

(A) Acridine Orange (AO) assay.

(A1) *FSC-A vs. SSC-A plot*: the main sperm population was identified, and debris were excluded.

(A2) *AO metachromatic fluorescence plot*: green fluorescence (FL1) corresponds to intact double-stranded DNA, whereas red fluorescence (FL3) indicates denatured or single-stranded DNA. The red-fluorescent population represents the metachromatic fluorescence distribution (MFD) and is used to calculate SDF (%).

(B) TUNEL assay.

(B1) *FSC-A vs. SSC-A*: gating of the sperm population after removal of debris.

(B2) *PI-A vs. PI-H plot*: selection of singlets to exclude doublets and aggregates.

(B3) *TUNEL fluorescence plot (FITC vs. PI)*: FITC-positive spermatozoa represent fragmented DNA following incorporation of fluorescein-dUTP.

(B4) *Positive control*: the TdT-positive sample shows the expected shift toward the FITC-positive region and was used to define the threshold for TUNEL-positive events.