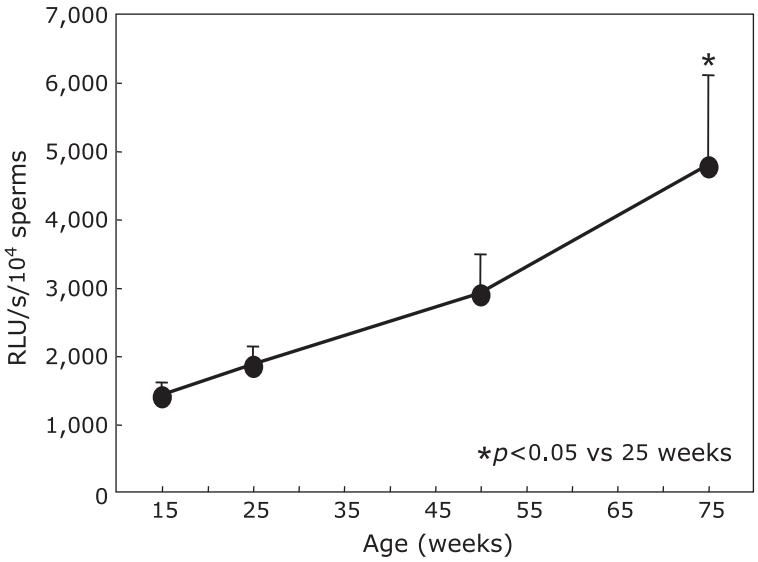

Supplemental Fig. 1. Effects of aging on ROS production in sperm. ROS generation in the sperm solutions was determined using L-012 (a luminol derivative that is highly sensitive for detecting ROS such as $O_2^{\bullet-}$, H_2O_2, and $OH^{\bullet}$). L-012 itself does not cause redox cycling. Sperm suspensions (1 ml of serum-free DMEM) were recorded at 37°C with a chemiluminescence reader (AccuFlex Lumi 400; Aloka, Tokyo). After 1 min of incubation, 50 μl of 2 mM L-012 solution was added through a reagent-dispensing unit and continuously recorded for 3 min. When SOD (50 U/ml) was added to the reaction mixture, ROS generation was almost completely abolished. Superoxide release from the spermatozoa was expressed as relative chemiluminescence calculated as the difference between the peak level observed and the level after the addition of SOD (50 U/ml). Chemiluminescence assays revealed that the ROS levels increased in the sperm cells in an age-dependent manner.