



RNA isolation protocol from human ejaculates

After liquefaction, 1 ml of ejaculate was washed with 50 ml 1 x PBS. Cells were pelleted by centrifugation (500 x g, 15 min, 4°C) and frozen at -80°C. Total RNA was isolated by the RNeasy Plus Micro Kit (Qiagen, Hilden, Germany) according to the instructions of the manufacturer. Briefly, frozen cell pellets were immediately suspended in RLT Plus Lysis buffer in the presence of 10 mM β -mercaptoethanol (1.2 ml/2x10⁷ spermatozoa). Homogenization was conducted using an Ultra-Turrax™ for 30 sec. The lysate was divided into aliquots corresponding to maximally 2x10⁷ spermatozoa. Two aliquots were directly used for RNA isolation, using one set of columns for each aliquot and the remaining aliquots were frozen at -80°C for later RNA isolation. Genomic DNA was eliminated from the RNA lysate by DNA binding spin columns (gDNA columns), and the flowthrough bound to RNA Micro extraction spin columns that efficiently bind even minor amounts of RNA. After on-column washing steps, the RNA was eluted with 20 μ l RNase free water. The two RNA aliquots from the same sample were combined and purified from residual contamination with genomic DNA by (a) a second round with the above procedure or by (b) digestion with DNase I (Sigma-Aldrich, Hamburg, Germany) according to the supplier's protocol for 15 minutes at ambient temperature. Purified RNA was stored at -80 °C.