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Supplementary Material

Reduced cytochrome oxidase activity and increased protein tyrosine phosphorylation of mitochondria-rich fractions of buffalo (*Bubalus bubalis*) spermatozoa after a cycle of freezing and thawing

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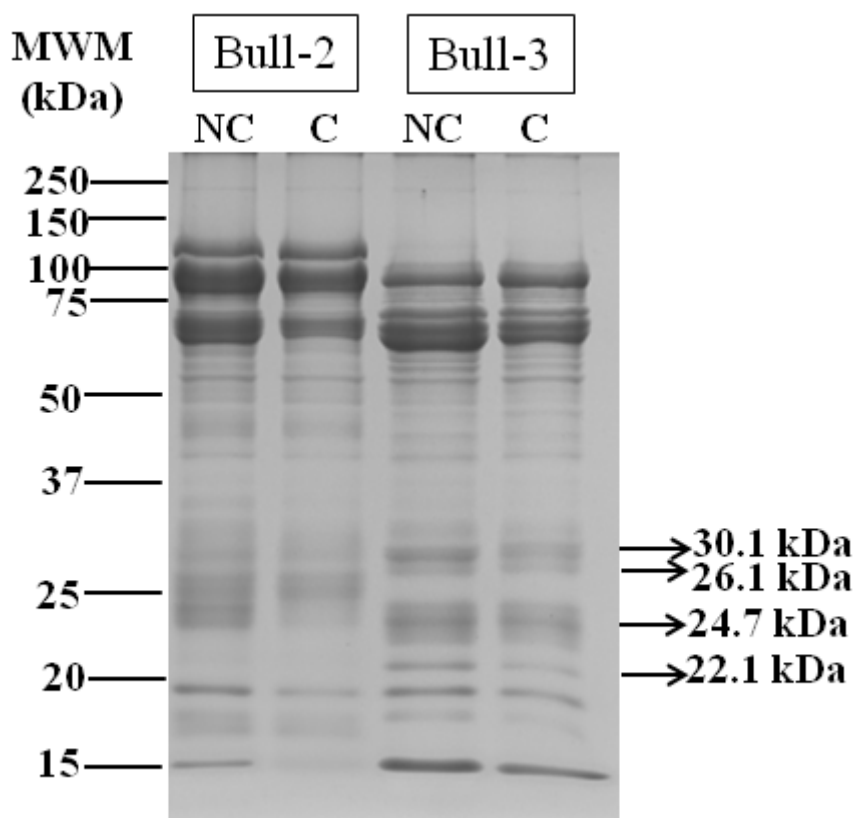


Fig. S1. Reducing SDS-PAGE analysis of proteins of band-3 fractions from non-cryopreserved (NC) and cryopreserved (C) sperm of two additional buffalo bulls. Coomassie Brilliant Blue R-250 stained 10% uniform gel. MWM, standard molecular weight markers. Approximately 50 μ g of protein was loaded in each well. Arrows on the right-hand side of the gel indicate molecular weights (in kDa) of the major mitochondrial proteins the intensities of which reduced after cryopreservation procedure.

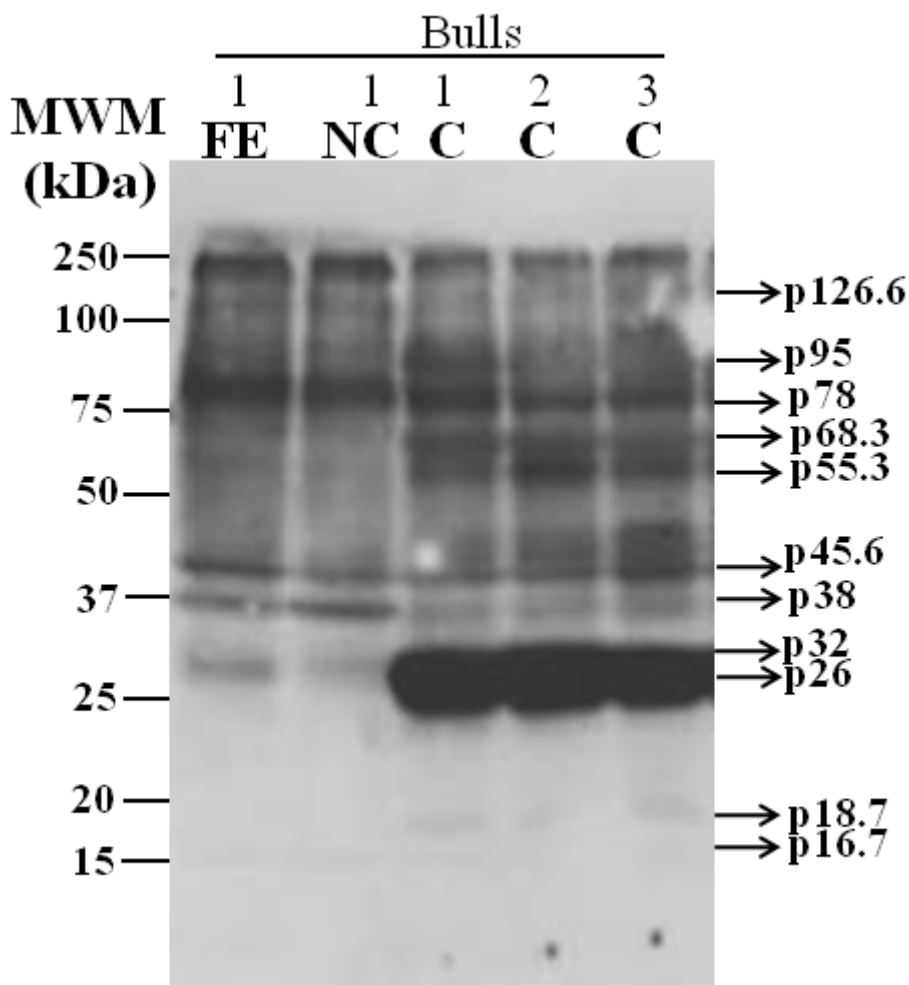


Fig. S2. Western blot for detection of tyrosine phosphorylated proteins in freshly ejaculated (FE), non-cryopreserved (NC) and cryopreserved (C, $n = 3$) whole sperm lysates. Approximately 50 μ g of protein in each lane was resolved by 10% uniform reducing SDS-PAGE. MWM, standard molecular weight markers. Tyrosine phosphorylated proteins were detected using mouse monoclonal anti-phosphotyrosine antibody. Arrows on the right side of the gel indicate different tyrosine phosphorylated proteins detected in the three groups (molecular weight in kDa prefixed with p for protein). A protein of 26.1 kDa was tyrosine phosphorylated intensely in cryopreserved (C) groups.

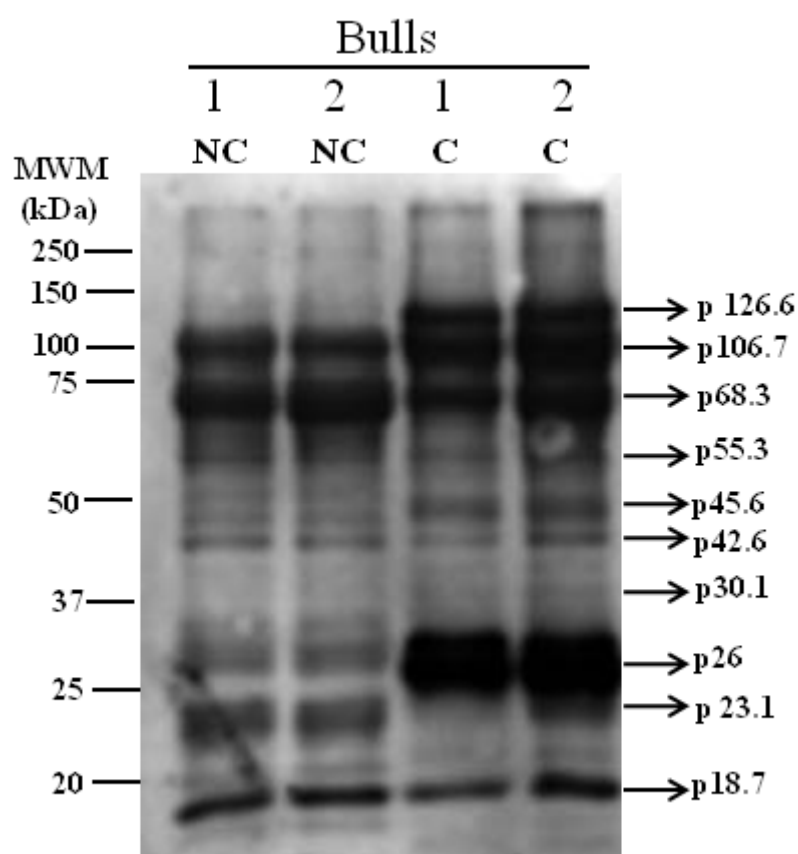


Fig. S3. Western blot for detection of tyrosine phosphorylated proteins in non-cryopreserved (NC) and cryopreserved (C) mitochondrial proteins of band-3 fractions of two additional buffalo bulls. Approximately 50 μ g of protein in each lane was resolved by 10% uniform reducing SDS-PAGE. MWM, standard molecular weight markers. Tyrosine phosphorylated proteins were detected using mouse monoclonal anti-phosphotyrosine antibody. Arrows on the right side of the gel indicate different tyrosine phosphorylated proteins detected in both the groups (molecular weight in kDa prefixed with p for protein).