BOR-Papers in Press. Published as DOI: 10.1095/biolreprod.111.091546

**ODET ET AL., SUPPLEMENTAL DATA:**
**EXPERIMENTAL PROCEDURE/MATERIALS AND METHODS**

***Digestion and Mass Spectrometry***

Immunoprecipitates were separated by SDS-PAGE and each gel lane was excised manually as 24 separate samples and digested with trypsin (Promega) for 8 hours in an automated fashion with a Progest robotic digester from Genomic Solutions. Resulting peptides were lyophilized and then resuspended in 40 µl of 0.1% formic acid. NanoLC-ESI-MS and MS/MS analyses were performed using an Agilent 1100 nanoLC system on-line with an Agilent 6340 ion trap mass spectrometer with the Chip Cube Interface. Briefly, 20 µl of digest were loaded onto an Agilent C18 chip (75 µm x 43 mm) followed by a linear gradient from 5% acetonitrile, 0.1% formic acid to 50% acetonitrile, 0.1% formic acid over 45 min. The mass spectrometer was used in the positive ion, standard enhanced mode and included settings of a mass range from 200 to 2200 m/z, an ionization potential of 2.1 kV, an ICC smart target of 100000 or 200 milliseconds of accumulation, and a 1.0 volt fragmentation amplitude. MS/MS data were acquired using a data dependent acquisition format, with the six most abundant ions from each MS scan with a threshold of 5000 counts further interrogated by MS/MS.

***Automated Database Searching***

Peak lists were generated from the data obtained from each nanoLC-ESI-MS/MS analysis using the Data Extractor feature of the SpectrumMill software from Agilent. The resulting extracted data were then searched against the NCBI non-redundant database using the MS/MS Search function in the SpectrumMill software. Search settings included tryptic specificity with up to two missed cleavages allowed, a precursor ion mass tolerance of 1.5 Da, a product ion mass tolerance of 1.0 Da, variable methionine oxidation and a minimum matched spectral intensity of 70%. Sequence assignments of MS/MS spectra were manually validated.

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## ODET ET AL., SUPPLEMENTAL FIGURE LEGENDS

**Supplemental Figure S1.** [$^{13}$C] NMR spectrum of WT (A) and KO (B) sperm after 30 min and 20 h incubation. Signals of α and β anomers of [1-$^{13}$C]-glucose and [3-$^{13}$C]-lactate are indicated. In some case, a few peaks were detected but were identified as background produced by the HEPES buffer.

**Supplemental Figure S2:** Effect of a methylene blue treatment on NAD, NADH, ATP levels, and sperm motility. WT, HET and KO sperm were incubated 2h at 37°C in 5% CO2 in humidified air in TYH 5.5 mM glucose without (control) or with 5μM methylene blue. Values are the mean ± SEM, n=3. Letters (a, b) above the means denote no significant difference if identical or significant difference if not identical.

**Supplemental Figure S3.** (A) Inhibition of total LDH activity by the inhibitor sodium oxamate in protein preparations from WT and KO sperm. (B) Consequence of an increase of sodium oxamate on ATP level (B. 1), motility (B. 2), progressive motility (B. 3), and hyperactivity (B. 4) of WT sperm after 2 h incubation at 37°C in 5% CO2 in humidified air in TYH containing 5.5 mM glucose. Values are the mean ± SEM, n=3; *$p<0.05$ compared to the control.

**Supplemental Figure S4**. Co-immunoprecipitation followed by detection by western blot. Protein extracts from testis (T) or from sperm (S) were used. (A) First two lanes contain sperm extracts from WT and KO mice stained with antibody to ANT4. Immunoprecipitations with an anti-LDHC ($IP_{LDHC}$) were performed with testis (T) or sperm (S) protein extracts from KO mice (negative control) or WT mice and ANT4 was detected by western blot. (B) First two lanes contain sperm extracts from WT and KO mice stained with antibody to LDHC. Samples were immunoprecipitated from testis (T) or sperm (S) extracts from wild type (+) mice with ANT4 antibody ($IP_{ANT4}$) and stained with antibody to LDHC. Addition of normal goat IgG (-) was used as a negative control for ANT4 immunoprecipitation. (C) and (D) Validation using the same antibody for immunoprecipitation and western blot assays ((C) ANT4; (D) LDHC; data shown are from experiments performed with sperm extract. The migration of size markers is shown at the left. The example shown is representative of three experiments.

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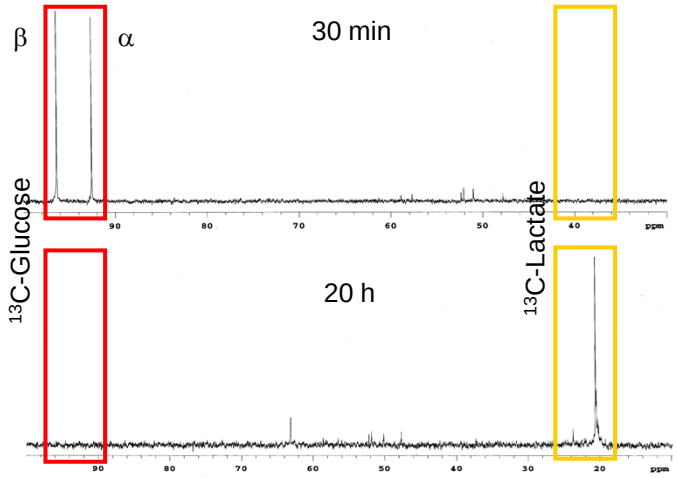
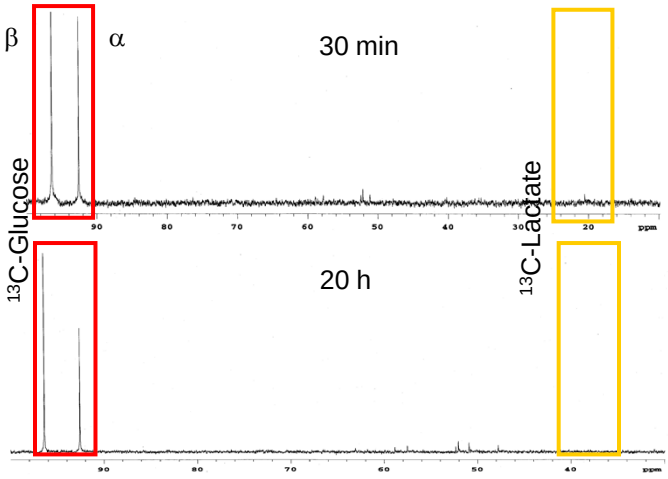
# Odet et al., Supplemental Data Figure S1

A. WT




t20h:
G = 5.1 ± 7.2 %
L = 94.9 ± 7.2 %

B. KO




t20h:
G = 95.4 ± 2.3 %
L = 4.6 ± 2.3 %

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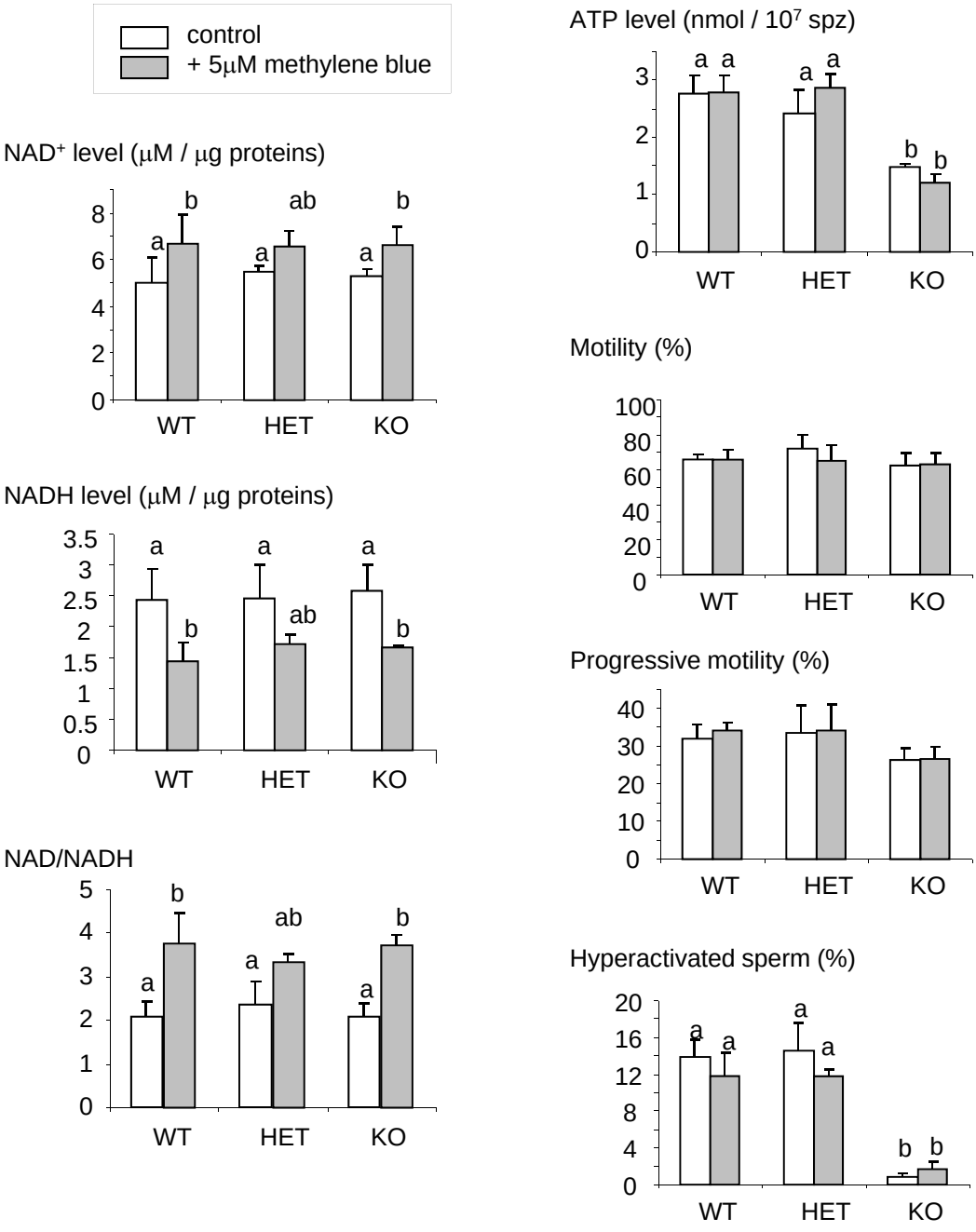
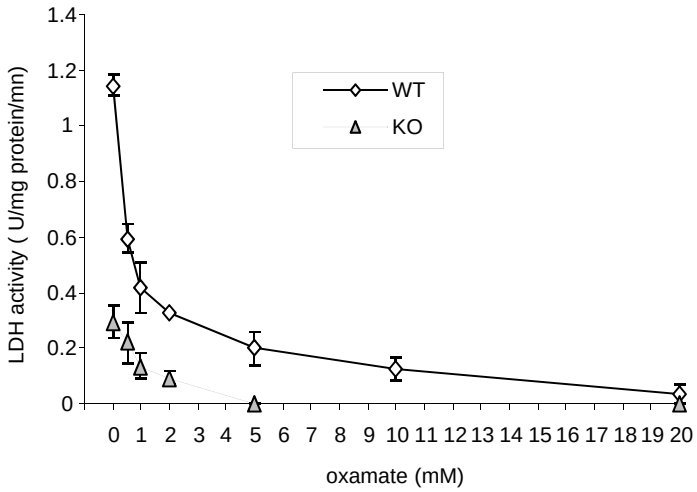
# Odet et al., Supplemental Data Figure S2

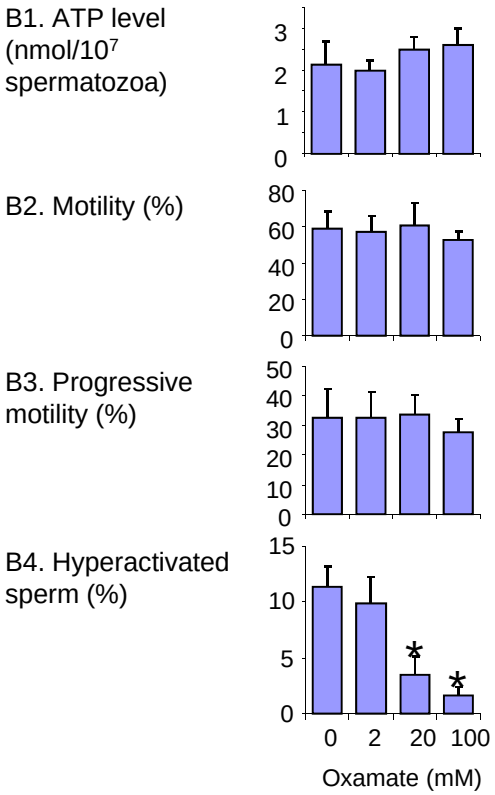


# Odet et al., Supplemental Data Figure S3

A. LDH activity inhibition by sodium oxamate on WT and KO sperm extract.




B. Consequences of an increase of sodium oxamate concentration on WT sperm.



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Odet et al., Supplemental Data Figure S4