**Supplementary materials of the manuscript**

**“Prolonged oral administration of ethyl alcohol leads to histopathology of the epididymis and seminal vesicle and changes of metabolite composition in the tissue lumen”**

by

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**Supplementary Figures**

**Supplementary Figure S1.** Hematoxylin/eosin-stained sections of the cauda epididymis from EtOH rats revealed marked disorientation and collapse of the epithelium with no discernment of the basement membrane in 10% of the total epithelium. Tissue fragments were also observed in the epididymal lumen of this highly deformed epithelium. The scale bar in the inset is 20 μm.

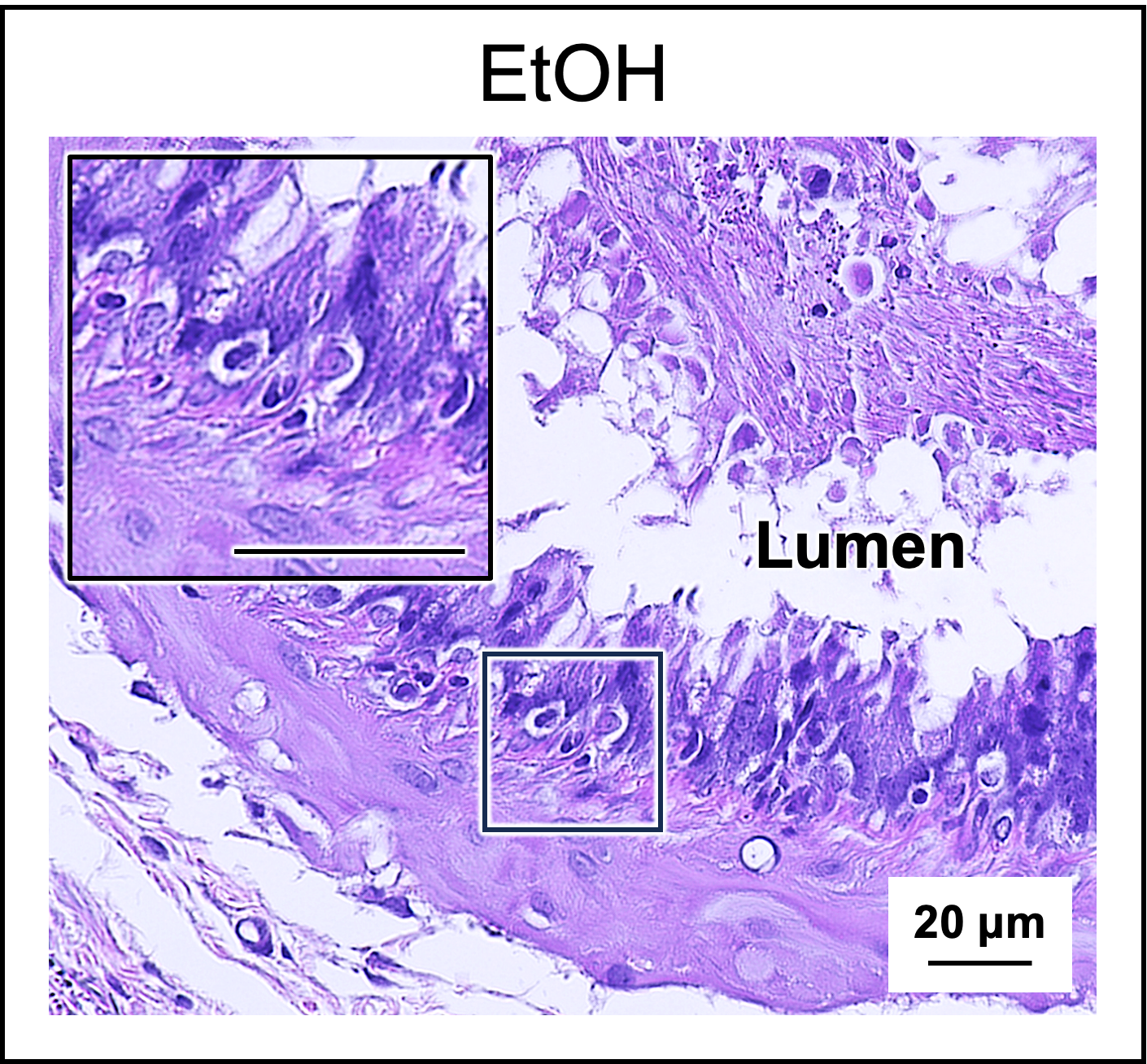
**Supplementary Figure S2.** Immunoblotting of the cauda epididymis and seminal vesicle with anti-caspase 9 antibody and anti-GAPDH antibody. The blot containing electrophoresed proteins from the cauda epididymis and seminal vesicle was cut between the MW of ~60,000 and 30,000, and this blot segment was used to probe with anti-caspase 9 and then reprobed with GADPH. This was to minimize the amount of antibodies needed for immunoblotting. The pro-caspase 9 band and cleaved-caspase 9 band as well as the GAPDH band in the first and second lanes were selected for presentation in Figure 5B. Similarly, for the presentation in Figure 6B, the pro-caspase 9 band and cleaved-caspase 9 band as well as the GAPDH band in lanes five and six were selected.

**Supplementary Figure S3.** Immunoblotting of the cauda epididymis and seminal vesicle with anti-caspase 3 antibody and anti-GAPDH antibody. The blot containing electrophoresed proteins from the cauda epididymis and seminal vesicle was cut between the MW of ~10,000 and ~35,000, and between the MW of ~35,000 and ~40,000. The first blot segment was used to probe with anti-caspase 3, whereas the second one was probed with GADPH. This was to minimize the amount of antibodies needed for immunoblotting. The pro-caspase 3 band and cleaved-caspase 3 band as well as the GAPDH band in the first and second lanes were selected for presentation in Figure 5C. Similarly, for the presentation in Figure 6C, the pro-caspase 3 band and cleaved-caspase 3 band as well as the GAPDH band in lanes five and six were selected.

**Supplementary Tables**

**Supplementary Table S1.** Identification of metabolites in the caudal epididymal fluid (CEF) based on their chemical shift values (ppm) from the website, https://hmdb.ca.

**Supplementary Table S2.** Identification of metabolites in the seminal vesicle fluid (SVF) based on their chemical shift values (ppm) from the website, https://hmdb.ca.

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**Supplementary Table S1.** Identification of metabolites in the caudal epididymal fluid (CEF) based on their chemical shift values (ppm) from the website, https://hmdb.ca.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **No.** | **Chemical shift**  **(ppm)** | **Multiplicity** | **STOCSY** | **Metabolite** |
| 1 | 1.32374 | d\* | 1.32374 (d), 4.09023 (q) | Lactate |
| 2 | 1.47547 | d | 1.47547 (d), 3.73798 (q) | Alanine |
| 3 | 1.92124 | s\* | 1.92124 (s) | Acetate |
| 4 | 2.07701 | s | 2.07701 (s), 2.52312 (dd), 2.62405 (dd), 3.20709 (s), 3.60610 (d), 3.86852 (dd) | Acetylcarnitine |
| 5 | 2.14699 | s | 2.14699 (s), 2.63414 (t), 3.86852 (t) | Methionine |
| 6 | 2.42993 | dd\* | 2.42993 (dd), 3.23333 (s), 3.42779 (m) | Carnitine |
| 7 | 3.04156 | s | 3.04156 (s), 3.93412 (s) | Creatine |
| 8 | 3.20709 | s | 3.20709 (s), 3.47960 (m), 4.06129 (m) | Choline |
| 9 | 3.23333 | s | 3.23333 (s), 3.59634 (m), 3.86852 (m), 4.30621 (m) | Glycerophosphocholine |
| 10 | 3.23333 | s | 3.23333 (s), 3.90586 (s) | Betaine |
| 11 | 3.26126 | s | 3.26126 (s) | Trimethylamine N-oxide |
| 12 | 3.28413 | t\* | 3.28413 (t), 3.52199 (dd), 3.60610 (t), 4.06129 (t) | Myo-inositol |
| 13 | 3.56337 | d | 3.56337 (d), 3.58692 (d), 3.65387 (m), 3.99367 (m) | Fructose |
| 14 | 3.65387 | q\* | 3.65387 (q), 3.90586 (m), 4.05120 (s) | Fructose 2,6-bisphosphate |
| 15 | 8.19706 | s | 8.19706 (s), 8.21623 (s) | Adenine |
| 16 | 8.46082 | s | 8.46082 (s) | Formate |

s: singlet; d: doublet; t: triplet; q: quartet; dd: doublet of doublet.

**Supplementary Table S2.** Identification of metabolites in the seminal vesicle fluid (SVF) based on their chemical shift values (ppm) from the website, https://hmdb.ca.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **No.** | **Chemical shift**  **(ppm)** | **Multiplicity** | **STOCSY** | **Metabolite** |
| 1 | 0.89176 | m\* | 0.89176 (m), 1.62586 (m), 3.70905 (t) | Leucine |
| 2 | 0.92540 | t\* | 0.92540 (t), 0.97318 (d), 1.21675 (m), 1.36613 (m), 2.18198 (m), 3.67271 (d) | Isoleucine |
| 3 | 1.32374 | d\* | 1.32374 (d), 4.08316 (q) | Lactate |
| 4 | 2.09753 | s\* | 2.09753 (s), 2.94366 (dd), 4.37283 (m) | Acetylcysteine |
| 5 | 2.52279 | d | 2.52279 (d), 2.68130 (d) | Citrate |
| 6 | 3.04123 | s | 3.04123 (s), 3.93412 (s) | Creatine |
| 7 | 3.23333 | s | 3.23333 (s), 3.59601 (m), 3.85573 (m), 4.30554 (m) | Glycerophosphocholine |
| 8 | 3.23333 | s | 3.23333 (s), 3.90586 (s) | Betaine |
| 9 | 3.26092 | t | 3.26092 (t), 3.52266 (dd), 3.60610 (t), 4.06096 (t) | Myo-inositol |
| 10 | 3.59601 | d | 3.59601 (d), 3.64008 (d), 3.80829 (d), 4.02294 (m), 4.06386 (m) | Fructose |
| 11 | 3.70905 | dd\* | 3.70905 (dd), 3.80829 (dd) | Glycerate |
| 12 | 7.83538 | s | 7.83538 (s) | Xanthine |

s: singlet; d: doublet; t: triplet; m: multiplet; dd: doublet of doublet.