Optimal Handling of Adipose Tissue from Obese Mice

For accurate measurement of individual fat cell size

Care must be taken to not apply too much mechanical pressure while handling the tissues. Try to time it such that samples are harvested on Tuesday and fixed overnight. Samples then get processed on Wednesday and are ready to embed on Thursday. If sectioning is done on a Friday, the slides will dry over the weekend and can then be stained on Monday.

Procedure

1. After deep anesthesia till moribund, proceed with perfusion fixation of the mouse: flush with PBS then perfuse with fresh formalin, using a perfusion pump to maintain a constant flow rate.
2. Carefully dissect out fat tissue, making sure to ***not crush*** the fat pads with forceps.
3. Fix in pre-labelled 50-mL conical tubes filled with at least 10 volumes of fresh 10% neutral buffered formalin overnight at room temperature.
4. Transfer fixed tissue into labelled cassettes (may require jumbo cassettes to prevent smashing lid against larger fat pads).
5. Process samples using the "long" cycle programmed in the processor for fat because it takes longer for solvents to infiltrate fatty tissue. It may be necessary to do three changes of paraffin for 1hr with vacuum instead of two for the really large epididymal fat pads.
6. Embed gently: use deeper molds and don't press tissue to the bottom of the cassettes.
7. Section at 10 µm macrophages if the goal is to get good morphological evaluation on adipose cell size on H&E slides (*may need to cut deeper into block to get a full cross-section*). Thinner sections may be better for F480 stains.
8. Dry slides at least overnight then bake in vacuum oven before deparaffinizing and staining.