Standard operating procedure (EPA)

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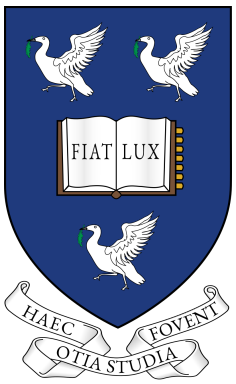

Equine Protein Atlas

Adipose Tissue

Background

Adipose tissue, or fat, plays a crucial role in energy storage, insulation, and hormone regulation in animals, including horses. It consists primarily of adipocytes (fat cells), which store lipids that can be mobilized for energy when needed. There are two main types of adipose tissue:

Adipose tissue is distributed throughout the body, with the most visible deposits found in the subcutaneous layer (beneath the skin), especially around the neck, withers, and flanks. The distribution of fat in horses is influenced by genetics, diet, and health status.

Fat behind the eye, known as retro-orbital fat, is part of the connective tissue surrounding the eye socket. This tissue helps cushion and protect the eyeball, contributing to the structural stability of the eye within the orbit. In horses, retro-orbital fat can vary in size depending on body condition and overall fat storage. While it plays a protective role, excess fat around the eye area can indicate an overall increase in body fat, which may be associated with metabolic conditions such as equine metabolic syndrome.

The yellow colour is due to the carotenoids (such as beta-carotene) present in the diet, particularly if the horse is fed a diet rich in grass or hay that is high in these compounds.

To isolate adipose tissue we collect samples from behind the eye.

Equipment needed

1. Scalpel
2. Rounded Scissors
3. Scissors
4. 10% Formalin
5. Liquid nitrogen

Methods

1. Collect equine heads from abattoir or following informed consent and ethical approval from horses donated for veterinary research . Take details of age, breed and sex from passport. Avoid leaving head in fridge for more than 24h.
2. If this is not possible use equine dentition to determine age and sex Click here
3. To gain access to the eyeball, first use a scalpel to cut around the eye lid (Left side)
4. Then use rounded scissors to cut any connective tissue
5. When the eye can be gently lifted cut the optic nerve
6. Gently remove the eyeball to reveal adipose tissue behind it
7. Cut the adipose tissue with clean scissors (300mg)
8. Split the tissue into two

   A. One part into 10% formalin in an appropriate container for histology and one for protein which will be snap frozen.

   B. Place into an appropriate sized and LN proof tube. Ensure tubes are suitable for liquid nitrogen
9. Snap freeze at least 200mg in liquid nitrogen
10. Annotate sample with age, type of tissue and date collected
11. Transfer to labelled box store at -80°C (Age, Type of Tissue, Date of collection)

12. Remove more adipose tissue for histological processing
13. Store in 10% Formalin