Protocol

Isolation of Progenitor and Stem Cells from Equine Adipose Tissue

⚠ All procedures must be performed in biosafety hoods.
⚠ This procedure is intended for processing equine subcutaneous adipose tissue only.
⚠ Surgically remove the subcutaneous fat pad using sterile techniques.
⚠ Using fresh adipose tissue will yield the best results.

❶ Pre-warm the processing solution (sterile PBS or sterile Lactated Ringer's) to 37˚C. Measure the tissue weight in a pre-zeroed sterile container; this will be used to calculate the processing solution volume and Celase® GMP volume.

❷ Wash the tissue with processing solution if bloody.

❸ Use surgical scissors to mince the tissue to a 3-5 mm^3 size or until no obvious large pieces are observed. Add a small amount of processing solution if the tissue is too dry.

❹ Reconstitute the Celase GMP vial with 5 mL of the processing solution. Invert the vial several times to fully reconstitute the enzyme into a clear solution (reference Celase GMP IFU).

❺ Place the washed and minced tissue into a sterile container, usually 6 times the size of the original tissue volume. For a given amount of tissue, add the appropriate volumes of processing solution and Celase GMP to the container; tightly close the container.

Adipose Tissue	Processing Solution	Celase GMP
5 mL	20 mL	0.7 mL
10 mL	40 mL	1.4 mL
15 mL	60 mL	2.1 mL
20 mL	80 mL	2.8 mL
25 mL	100 mL	3.5 mL
30 mL	120 mL	4.2 mL
35 mL	140 mL	4.9 mL
40 mL	160 mL	5.6 mL
45 mL	180 mL	6.3 mL
50 mL	200 mL	7.0 mL

❻ Place the container in a 37˚C shaking water bath for 60 ± 15 minutes until the tissue becomes visibly broken down. If a shaking water bath is not available, gently swirl the container contents by hand continuously or in 5 minute increments for 60 ± 15 minutes.

❼ Collect the non-buoyant solution into multiple 50 mL centrifuge tubes. Place the tubes in a centrifuge and concentrate the cells at 600 g for 5 minutes at room temperature with a low-medium brake speed.

❽ Carefully aspirate the top lipid, floating layer and the solution that contains Celase GMP.
⚠ Do not disturb the cell pellet and leave 5-10 mL of the solution in the tube.

❾ Resuspend the cell pellet in each 50 mL tube with the remaining solution. Combine the cell pellets from each tube and then wash with the processing solution 2 times to reduce residual enzyme. Resuspend the combined cell pellet with 25-30 mL of the processing solution and pass through a 100 µm strainer followed by a 40 µm strainer.

❿ Centrifuge 1 more time and resuspend with the remaining processing solution into the final desired volume for cell counting and further *in vitro* and *in vivo* studies.