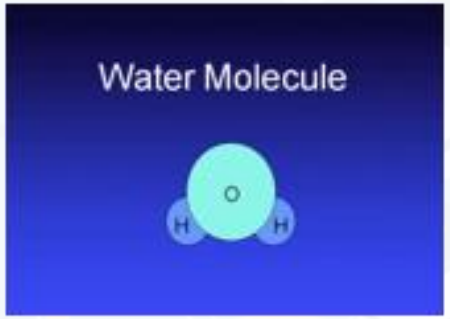
BME 174 – Cryopreservation and Muscle & Fat Differentiation

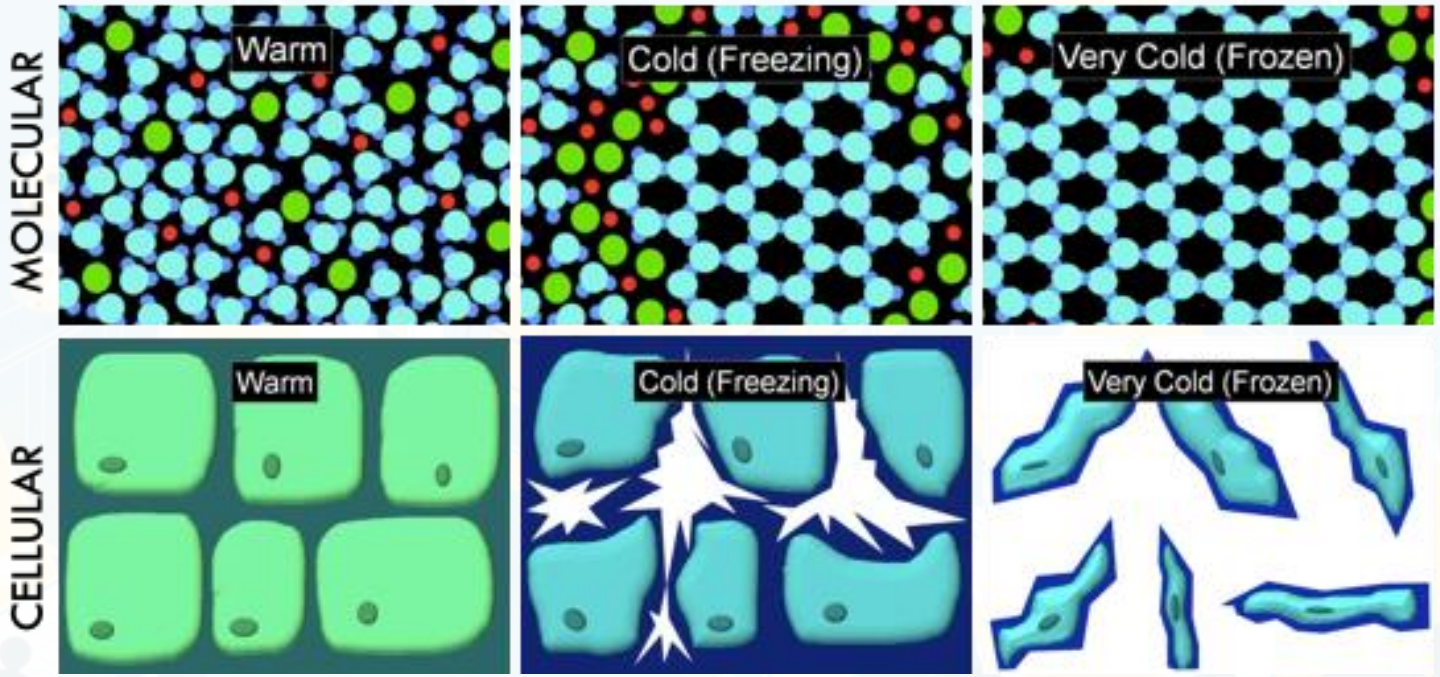
https://new-harvest.org/

Cryopreservation

- The use of very low temperatures to preserve structurally intact living cells and tissues
- Cryo-protective agents help minimize the formation of ice crystals/other cryodamage by reducing the crystallization process of water and increasing the viscosity of the solution
  - Examples: Dimethyl sulfoxide (DMSO), glycerol, ethylene glycol, propylene glycol

FREEZING WITHOUT CRYO-PROTECTIVE AGENTS





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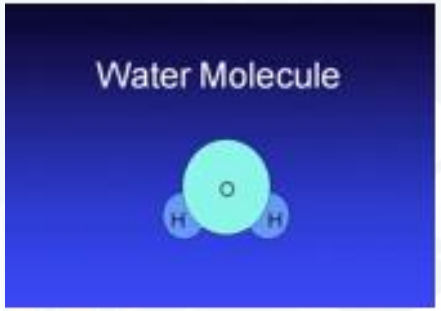
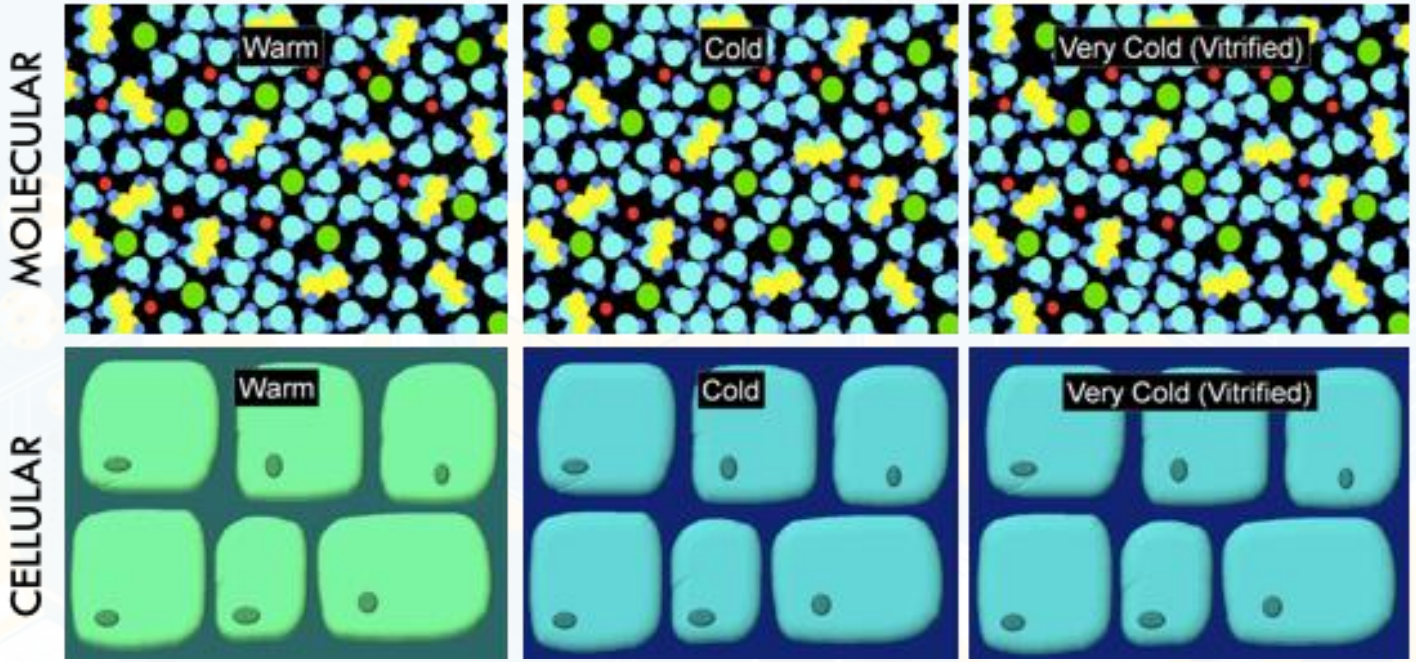




Cryopreservation

- Why would we want to freeze cells?
  - Give us a break from continuous culturing
  - Avoid total loss if contamination occurs
  - Minimize genetic change in continuous cell lines
  - Avoid aging and transformation in finite cell lines
- How do we freeze cells?
  - Medium with cryoprotective agent and protein
  - Storage in liquid nitrogen (-195°C)

Cryopreservation Materials

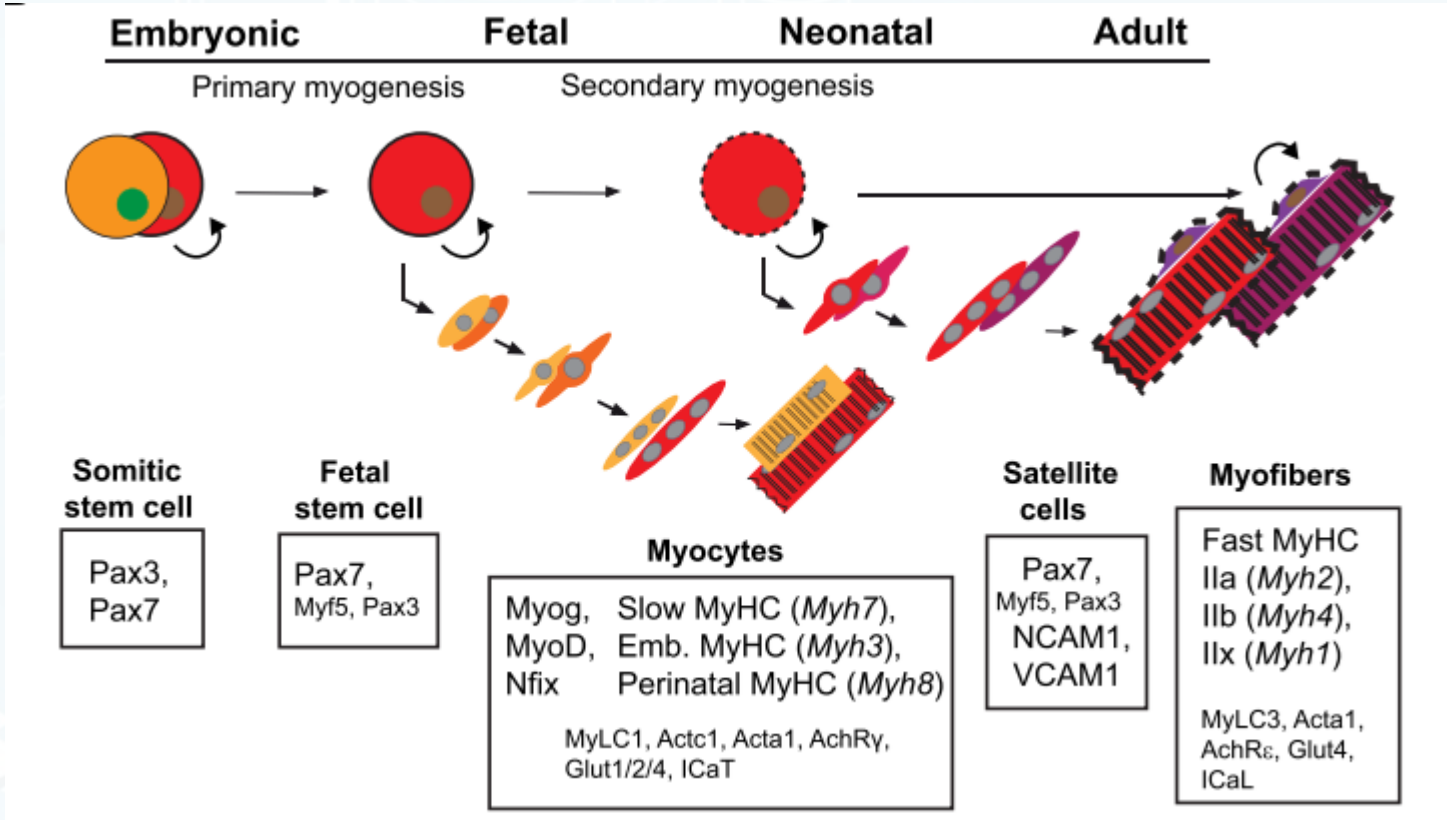
- Cells
- BSC-GM (contains protein for cells)
- DMSO as cryoprotectant
- Mr. Frosty Freezing Container
  - Filled with isopropanol to control freezing rate
- Cyrovials
  - Tested for storage in liquid nitrogen

Cryopreservation Overview

- Passage but do NOT seed cells
- Count and **THEN** pellet cells
- Resuspend in freezing medium (10% DMSO)
- Move to cryovial
- Place in a cell freezing container and move to -80°C freezer
  - Controls the rate of freezing to -1°C / min
- Move to liquid nitrogen once fully cooled (usually the next day)

Muscle Differentiation

- Muscle differentiation towards myotubes
- Protocols for bovine satellite cells are well-defined
  - Serum starvation: Cessation of feeding
  - Serum reduction: Lower FBS to 2%



Chal, J., & Pourquié, O. (2017). Making muscle: Skeletal myogenesis in vivo and in vitro. *Development (Cambridge)*, *144*(12), 2104–2122. https://doi.org/10.1242/dev.151035

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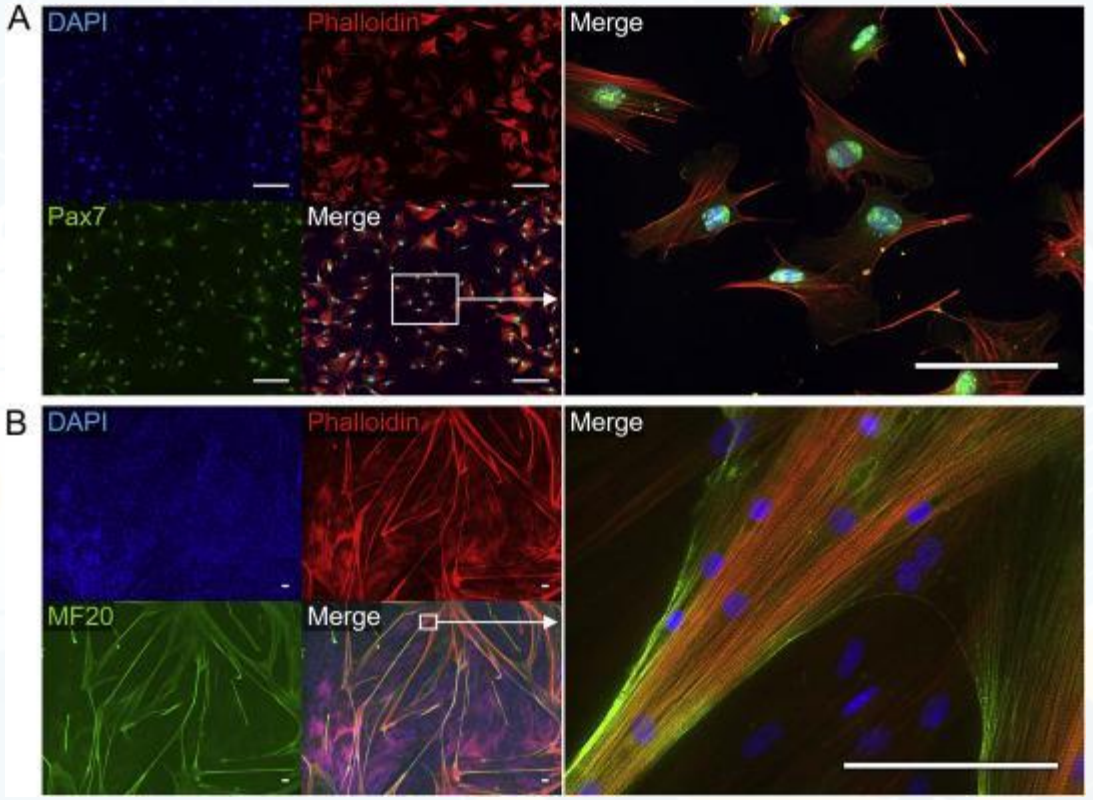
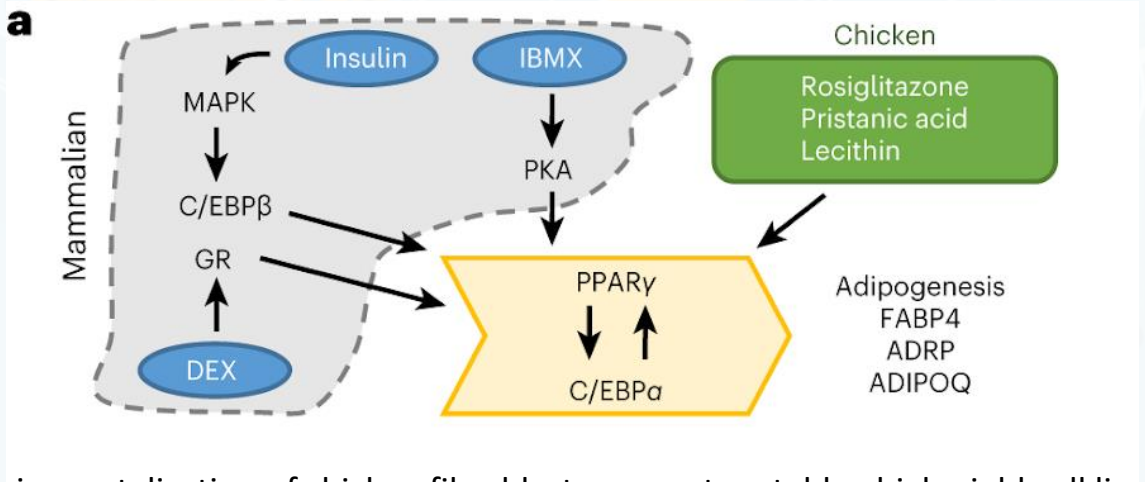


Stout, A. J., Mirliani, A. B., Rittenberg, M. L., Shub, M., White, E. C., Yuen, J. S. K., & Kaplan, D. L. (2022). Simple and effective serum-free medium for sustained expansion of bovine satellite cells for cell cultured meat. *Communications Biology*, *5*(1). https://doi.org/10.1038/s42003-022-03423-8

Adipogenic Differentiation

- Lipid accumulation --> fat droplet
- Bovine (ruminant) adipogenesis is less well understood
- Peroxisome proliferator-activated receptor gamma (PPARG) is master regulator of adipogenesis
- We will test two fat differentiation regimes
  - Conventional
  - Ascorbic-acid containing

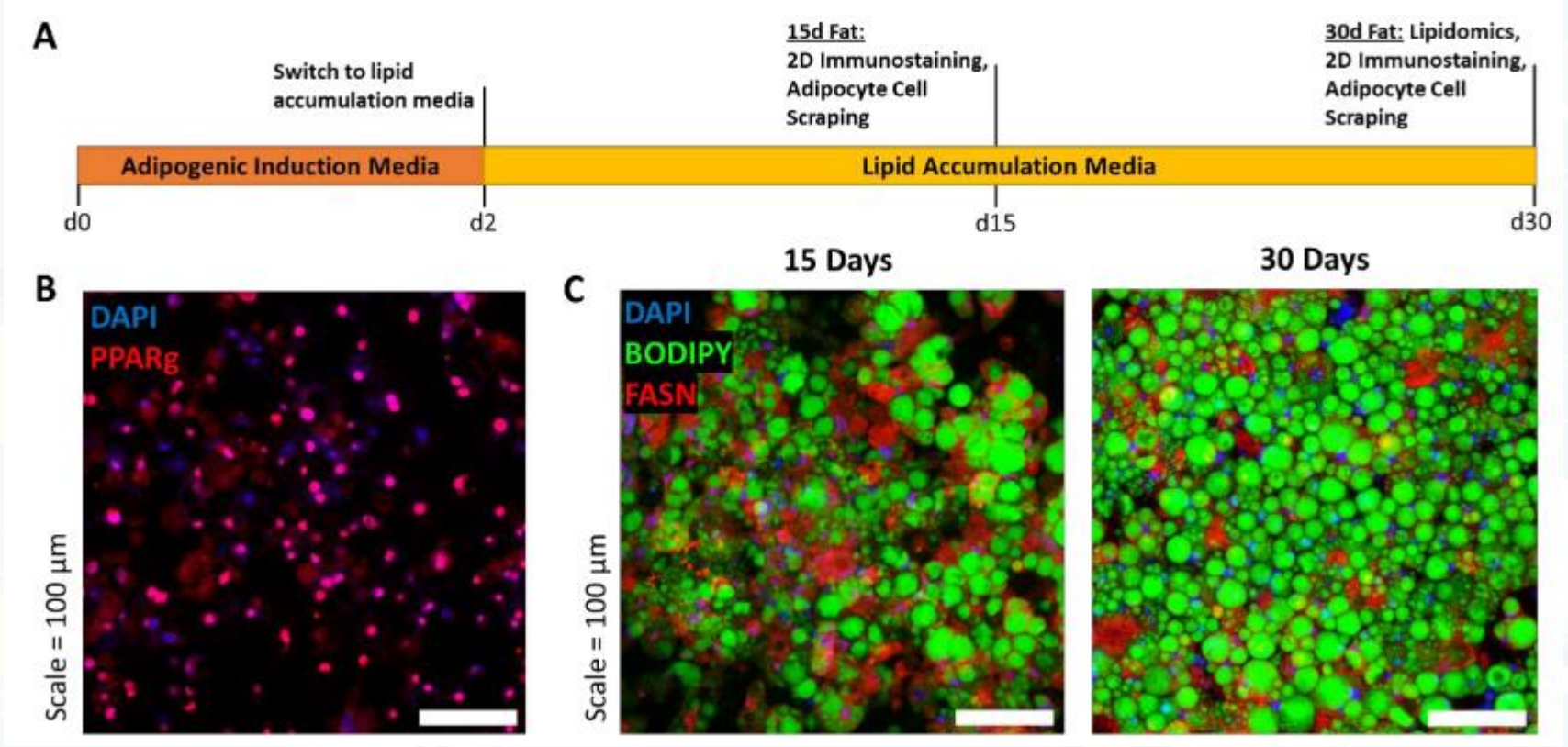


Pasitka, L.,et al. (2022). Spontaneous immortalization of chicken fibroblasts generates stable , high-yield cell lines for serum-free production of cultured meat. *Nature Food*. https://doi.org/10.1038/s43016-022-00658-w

Conventional Fat Differentiation

- Yuen *et al.* demonstrate conventional lipid accumulation in mouse and pig cells
- Two phases
  - Induction: high concentration of PPARG agonists to kickstart
  - Accumulation: pared down PPARG agonists to maintain

Conventional Fat Differentiation



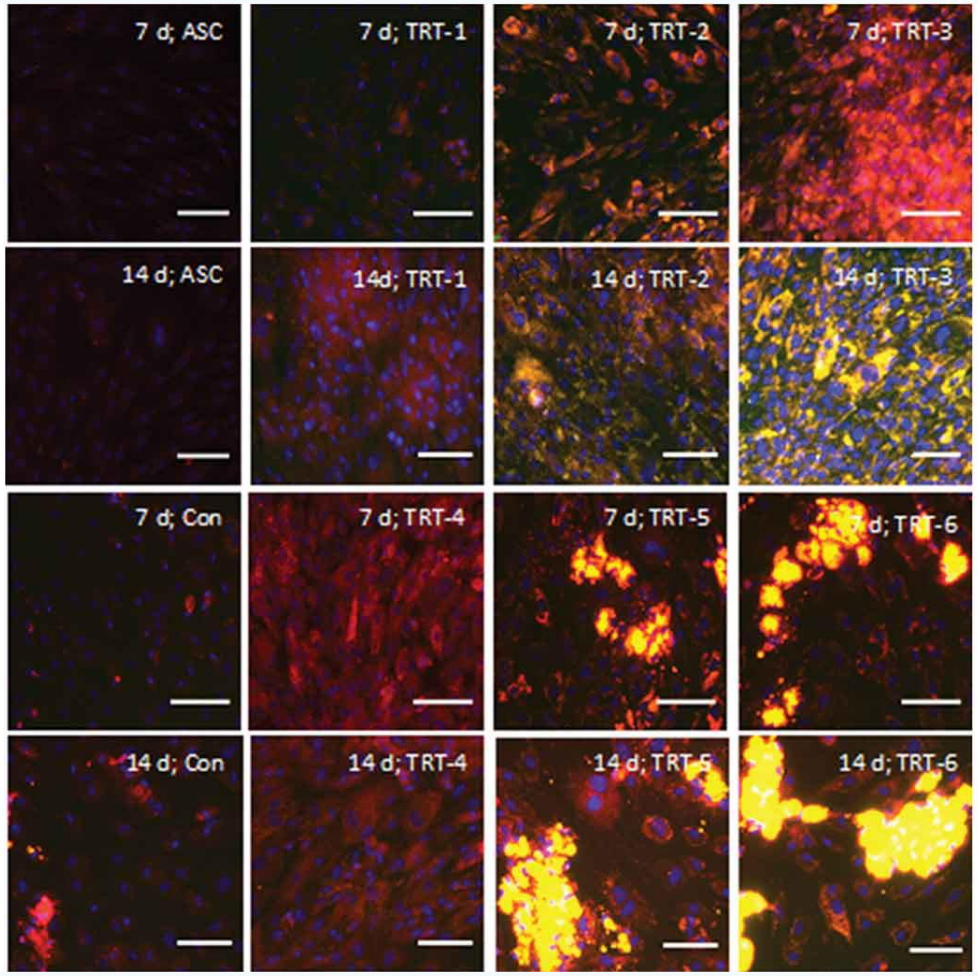
Yuen Jr., J. S., Saad, M. K., Xiang, N., Barrick, B. M., Dicindio, H., & Li, C. (2022). Macroscale Adipose Tissue from Cellular Aggregates : A Simplified Method of Mass Producing Cell-Cultured Fat for Food Applications. *BioRxiv*.

Ascorbic Acid Induction of *FABP4*

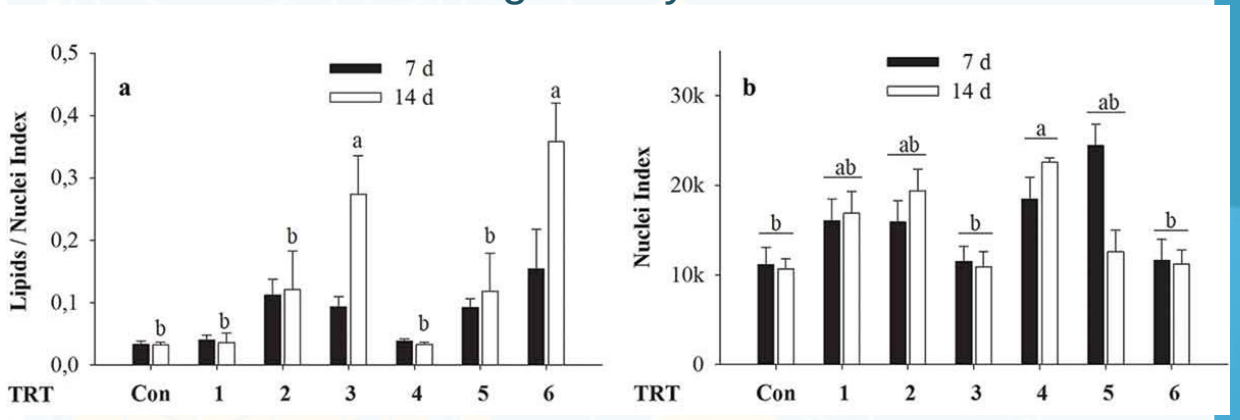
- Jurek et al. demonstrated better lipid accumulation in the absence of FBS but with supplemented ascorbic acid and lipids (BSL = bovine serum lipids)
- We will use intralipid, a soybean oil emulsion, because BSL is hard to source

Ascorbic Acid Induction of *FABP4*

Nile Red Non-Polar Lipid Stain



Nile Red / Nuclei Image Analysis



Treatments	AsA (µg/mL)	FBS (%)	BSL (µL/mL)
TRT-Con	0	0	0
TRT-1	0	10	0
TRT-2	0	10	10
TRT-3	0	0	10
TRT-4	40	10	0
TRT-5	40	10	10
TRT-6	40	0	10

Jurek, S., Sandhu et al.(2020). Optimizing adipogenic transdifferentiation of bovine mesenchymal stem cells: a prominent role of ascorbic acid in FABP4 induction. *Adipocyte*, *9*(1), 35–50. https://doi.org/10.1080/21623945.2020.1720480

Today's Plan

- Make freezing media (10% DMSO)
- Cryopreserve T flasks
- Initiate differentiation of muscle cells and fat cells
  - Muscle differentiation: serum-reduction (2% FBS), serum starvation (cessation of feeding at 90-100% confluence)
  - Fat differentiation:
    - Conventional: DM1-induction (TODAY), DM1-accumulation (SUNDAY)
    - Ascorbic acid-containing: DM2-induction (TODAY), DM2-accumulation (SUNDAY)

Next week

- Confirmation of lipid accumulation and muscle differentiation