FINAL SCRIPT: APPROVED FOR FILMING

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Title: White and Brown Adipose Grafts: An Approach to Correct Reproductive, Metabolic, and Renal Deficits in Black and Tan Brachyury (BTBR) Obese Mice

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 10
Number of Shots: 22

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Erika Bevilaqua Rangel:** This research investigated whether combined white and brown fat transplantation in leptin-deficient BTBR obese mice can reverse subfertility, obesity, hyperglycemia, and kidney dysfunction, thereby improving reproductive, metabolic, and renal outcomes.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: Figure 1*

What research gap are you addressing with your protocol?

1.2. **Marcella Liciani Franco:** We standardized a combined WAT and BAT transplantation protocol, defining optimal animal age and fat quantity to address metabolic and reproductive dysfunctions in the BTBR obese model.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.6*

What advantage does your protocol offer compared to other techniques?

1.3. **Melise Oliveira Mariano:** Compared to leptin therapy or single-fat transplants, our protocol offers a physiological, sustained hormone release with broader improvements in metabolism, fertility, and kidney function, using a reproducible and scalable approach.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

How will your findings advance research in your field?

1.4. **Maria Clara Soares Klein:** By standardizing this protocol, we enable broader use of the BTBR model to study cardio-renal-metabolic disease and diabetic complications, while aligning with the 3Rs in animal research.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

What research questions will your laboratory focus on in the future?

1.5. **Marcella Liciani Franco:** We aim to systematically refine and replicate this protocol, enhance transplantation efficacy, integrate novel biomedical platforms, and identify additional fat implantation sites to optimize therapeutic outcomes in BTBR obese mice.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Testimonial Questions (OPTIONAL):

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. **Melise Oliveira Mariano:** Publishing with JoVE enhances scientific visibility by democratizing knowledge, increasing educational impact, and enabling researchers worldwide to accurately replicate protocols through clear, accessible, and visually detailed methodological demonstrations.

1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committees of Hospital Israelita Albert Einstein (HIAE) and registered with the Jewish Institute of Research and Education, São Paulo, SP, Brazil

Protocol

2. Surgical Procedure for Adipose Transplantation in Mice

Demonstrators: Marcella Liciani Franco, Bruno Serrano Barbosa, Melise Oliveira Mariano

2.1. To begin, position the euthanized mouse in dorsal recumbency with its belly facing upward **[1-TXT]**. Disinfect the lower abdomen using 0.5 percent alcoholic chlorhexidine digluconate **[2]**. Using surgical scissors, make a midline abdominal incision approximately 2 to 3 centimeters long to expose and harvest the inguinal white adipose tissue **[3]**. Place the excised tissue into a 3-milliliter tube and keep it on ice **[4]**.

2.1.1. WIDE: Talent placing the mouse on its back with limbs secured. **TXT: Euthanasia: 100% v/v isoflurane overdos**e

2.1.2. Talent disinfecting the lower abdomen with a swab soaked in chlorhexidine.

2.1.3. Talent using surgical scissors to create a midline incision and excising the inguinal fat pad.

2.1.4. Talent placing the excised white adipose tissue into a labeled 3 milliliter tube and placing it on ice.

2.2. Next, reposition the mouse in ventral recumbency with its belly facing downward **[1]**. Make a midline dorsal incision of 1 to 2 centimeters between the scapulae using surgical scissors to access and excise the interscapular brown adipose tissue **[2-TXT]**.

2.2.1. Talent flipping the mouse onto its belly and aligning it for dorsal access.

2.2.2. Talent making a dorsal midline incision and removing the brown adipose tissue between the scapulae. **TXT: Pool WAT + BAT from 10–14 donors until 85–90 mg fat/g body weight**

2.3. Using a 3-milliliter syringe fitted with a 16-gauge needle, homogenize the white and brown adipose tissues separately **[1]**.

2.3.1. Talent attaching a 16 gauge needle to a 3 milliliter syringe and homogenizing each fat sample in individual tubes.

2.4. Then, measure the final weight of the fat using an analytical balance **[1]** and keep the

samples on ice until transplantation **[2]**.

2.4.1. Talent placing a weigh boat with the homogenized fat onto an analytical balance and recording the weight.

2.4.2. Talent transferring the tubes containing fat samples to an ice bucket.

2.5. Next, take the anesthetized recipient BTBR *(B-T-B-R)* obese mice **[1-TXT],** once its respiration slows to 1 breath every 3 seconds or less, position the mouse in ventral recumbency **[2]**. Then, disinfect the dorsal area using 2.0 percent non-alcoholic chlorhexidine digluconate **[3]**.

2.5.1. Shot of the anesthetized recipient BTBR obese mice. **TXT: Anesthesia Induction: 100% v/v isoflurane; Maintenance: 1.5-2.5% Isoflurane**

2.5.2. Talent placing the anesthetized mouse belly-down on the surgical platform.

2.5.3. Talent swabbing the dorsal surface of the mouse with 2.0 percent non-alcoholic chlorhexidine digluconate.

2.6. Using a 16-gauge needle, inject 100 to 200 microliters of homogenized fat per site across 6 to 26 locations on the dorsal surface **[1]**. Use forceps to gently assist in evenly distributing the fat at each site **[2]**. Following transplantation, apply 0.5 percent chlorhexidine solution to each injection site **[3]**.

2.6.1. Talent injecting homogenized fat into multiple dorsal sites using a 16-gauge needle.

2.6.2. Talent using forceps to help distribute the fat beneath the skin at each site.

2.6.3. Talent dabbing 0.5 percent chlorhexidine onto each injection site post-transplantation.

2.7. Administer tramadol intramuscularly for analgesia **[1]** and house each animal individually to prevent injury during recovery **[2]**.

2.7.1. Talent injecting tramadol into the hind limb muscle of the mouse using a syringe. NOTE: Since 2.7.1 and 2.8.2 are similar. Three takes were filmed for 2.7.1. Otavio used two of them to compose both items, which allowed us to avoid repeating mouse handling.

2.7.2. Talent placing the mouse into an individual recovery cage.

2.8. On the following day, clean each injection site with 0.5 percent chlorhexidine solution **[1]** and administer a second intramuscular dose of tramadol for continued analgesia

[2].

2.8.1. Talent swabbing each injection site with 0.5 percent chlorhexidine.

2.8.2. Talent administering a second dose of tramadol via intramuscular injection.

2.9. Two weeks after transplantation, co-house the female BTBR obese mice with male BTBR heterozygous mice for mating **[1]**.

2.9.1. Talent introducing female and male BTBR mice into the same cage and monitoring for interaction.

2.10. Measure the body weight of the mice weekly using an analytical balance **[1]**. Assess fasting blood glucose levels using a glucometer **[2]**.

2.10.1. Talent placing a mouse onto an analytical balance and recording the displayed weight.

2.10.2. Talent collects a small drop of blood from the tail tip and using a glucometer to measure fasting glucose.

Results

3. Results

3.1. The transplantation protocol using 10–15% donor fat resulted in a 2.2-fold higher effectiveness in achieving viable offspring compared to the 5–9.9% fat group **[1].**

3.1.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the right bar representing 10–15% fat.*

3.2. A progressive increase in protocol effectiveness was associated with higher proportions of brown adipose tissue in the injected fat, reaching 100% effectiveness when brown adipose tissue made up 25–30% of the total fat **[1].**

3.2.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the rightmost bar labelled "100%" and emphasize the top brown section labelled "26–30%".*

3.3. Crosses involving transplanted BTBR obese females and heterozygous males yielded 1.6 times more obese offspring compared to heterozygous-only crosses **[1].**

3.3.1. LAB MEDIA: Figure 3.

3.4. Ovaries from transplanted mice showed restored folliculogenesis, evidenced by increased corpora albicantia and balanced follicle populations compared to obese controls **[1].**

3.4.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the Tx group middle panel*

3.5. Quantification confirmed higher numbers of pre-antral and antral follicles in obese mice, with partial normalization in the transplanted group **[1].**

3.5.1. LAB MEDIA: Figure 4B. *Video editor: Highlight the bars for "Pre-antral follicle" and "Antral follicle" across BTBR obese and Tx groups*.

3.6. Cleaved caspase-3 staining showed reduced ovarian apoptosis in transplanted mice to levels similar to lean controls**,** suggesting a protective effect of transplantation **[1].**

3.6.1. LAB MEDIA: Figure 4C and D. *Video editor: Highlight the Tx panel and Tx bar in figures 4C and D respectively*

3.7. Transplanted mice had consistently lower body weight from 10 to 24 weeks compared to obese controls **[1],** with significantly reduced percentage weight gain over the same

period **[2].**

3.7.1. LAB MEDIA: Figure 5A. *Video editor: Trace the yellow Tx line and compare it with the upper orange obese group line*.

3.7.2. LAB MEDIA: Figure 5B. *Video editor: Trace the yellow Tx line showing body weight gain*

3.8. Fasting blood glucose levels were significantly lower in transplanted mice than in obese controls between weeks 8 and 20 **[1].**

3.8.1. LAB MEDIA: Figure 5C. *Video editor: Highlight the lower yellow Tx line with the higher orange obese line.*

1. Isoflurane

- **Pronunciation link:** https://www.howtopronounce.com/isoflurane (YouTube, How To Pronounce)
- **IPA (American):** /ˌaɪsoʊˈflʊreɪn/
- **Phonetic Spelling:** eye-soh-FLUR-ayn

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2. Chlorhexidine

- **Pronunciation link:** https://dictionary.cambridge.org/us/pronunciation/english/chlorhexidine (How To Pronounce, Cambridge Dictionary)
- **IPA (American):** /klɔːrˈhɛk.sə.diːn/
- **Phonetic Spelling:** klawr-HEK-suh-deen

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3. Dorsal Recumbency

- **Pronunciation link:** No confirmed link found for the full term “dorsal recumbency.”
- **IPA (American):** /ˈdɔːr.səl rɪˈkʌm.bən.si/
- **Phonetic Spelling:** DOR-suhl ri-KUM-buhn-see

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4. Adipose Tissue

- **Pronunciation link:** No single source page found for “adipose tissue.”
- **IPA (American):** /ˈæd.ɪˌpoʊs ˈtɪʃ.uː/
- **Phonetic Spelling:** AD-ih-pohs TISH-oo

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5. Inguinal

- **Pronunciation link:** No confirmed link found (the Cambridge Dictionary doesn't list the standalone term “inguinal” separately).
- **IPA (American):** /ˈɪŋ.gwɪ.nəl/
- **Phonetic Spelling:** ING-gwih-nuhl