



Office: (240) 356-0056
Fax: (866) 929-5455

Alizée Pathology, Inc. Executive Summary Pathology Report

Study Number IEF18-534

Alizée
Project Number IEF18-534

Study Title

NON-GLP EVALUATION OF
DENTAL PULP ALLOGRAFT
INJECTIONS ON AN EQUINE
DEEP DIGITAL FLEXOR
TENDON VS.
CONTRALATERAL NAÏVE
CONTROL

Pathology
Site Alizée Pathology, Inc.
20 Frederick Road
Thurmont, MD 21788

Testing
Facility Valley View Animal Hospital
2167 Progress Street
Dover, OH 44622

Sponsor VetGraft, LLC
1275 Kinnear Road
Columbus, OH 43212

Date 4/19/2019

Purpose

The purpose of this study was to evaluate the tissue regeneration efficacy as well as the collagen alignment, restructuring of normal anatomy, tumorigenicity or any malignant pathological changes of a dental pulp allograft injection into an equine deep digital flexor tendon.

Methods

The portions of Study Number IEF18-534 (Alizée Project Number IEF18-534) conducted at Alizée were neither intended nor required to be in compliance with United States Food and Drug Administration Good Laboratory Practice regulations set forth in Title 21 Code of Federal Regulations (CFR) Part 58. As such, quality assurance inspections and oversight were not performed. However, all study conduct performed at Alizée was completed in accordance with the technical memo, Alizée Standard Operating Procedures (SOPs) and sound scientific judgment. The data and results from analysis provided in this report have undergone a thorough quality control review and accurately reflect the raw data. An electronic copy of this report in portable document format (PDF) will be provided to VetGraft, LLC in addition to the hard copy. The PDF is a representation of the pathology report hard copy. However, only the signed hard copy of the pathology report is considered raw data. Digital images appearing in this report are for illustrative purposes only. All pathologic diagnoses were derived from the original histological preparations.

This pathology executive summary by Alizée presents the results of the histopathological analysis of one (1) horse for VetGraft, LLC, Columbus, OH. All surgical procedures and tissue harvests were performed at the test facility, Valley View Animal Hospital, Dover, OH, under the direction of Curt T. Honecker, DVM. Histological processing was performed at Alizée, Thurmont, MD. Resulting slides were evaluated via light microscopy at Alizée by Joan Wicks, DVM, Ph.D., DACVP.

According to client communication, one horse (Arnold) underwent a series of five (5) ultrasound guided injections to an injured deep digital flexor tendon (DDFT) unilaterally in the left front limb. The injured DDFT was treated with PulpCyte injections (Test Article) and the normal contralateral DDFT was untreated (Control).

Due to pain caused by limb support laminitis on the right front limb, the horse was sacrificed approximately four (4) years after the first injection. The treated DDFT and the contralateral normal DDFT were collected and stored in formalin for about approximately ten (10) months until shipped to Alizée for histopathological processing and evaluation. Upon receipt at Alizée, fixed tissues were trimmed, cut into a longitudinal section and a cross section and embedded in paraffin. Once embedded, the blocks were microtomed at 5 µm to produce three (3) serial sections mounted to glass slides. Slides were then stained, one with hematoxylin and eosin (H&E), one with Alcian Blue pH2.5/Periodic Acid Schiff's (AB/PAS) and one with Herovici's stain (HER) for evaluation.

After initial evaluation determined that the sections were not taken from the area of damage, the left, treated leg was trimmed again and step sectioned (0.5 to 0.8 mm thick) into eight (8) levels. Section level 1 was the most proximal and section level 8 was ~ 1/3 to 1/2 away from the distal end of the received tendon sample. From these sections, levels 1, 4, 7 and a section ~ 2 cm distal to level 8 were embedded in paraffin with the up side embedded down. Once embedded, the blocks were microtomed at 5 µm, mounted to glass slides and stained with H&E.

Results and Discussion

At trim, there was a well circumscribed, irregular white discoloration of the accessory ligament of the deep digital flexor tendon (ALDDFT), within the more proximal section of the tendon. In this region the deep digital flexor tendon (DDFT) and superficial flexor tendon (SDFT) demonstrated no abnormal findings (Levels 1 and 2 in Figure 1). Moving distally, as the ALDDFT blended with the DDFT, there was irregular white mottling involving both the DDFT and SDFT. Levels 1, 4 and 7 were evaluated microscopically and results presented in Figures 2, 3 and 4, respectively.

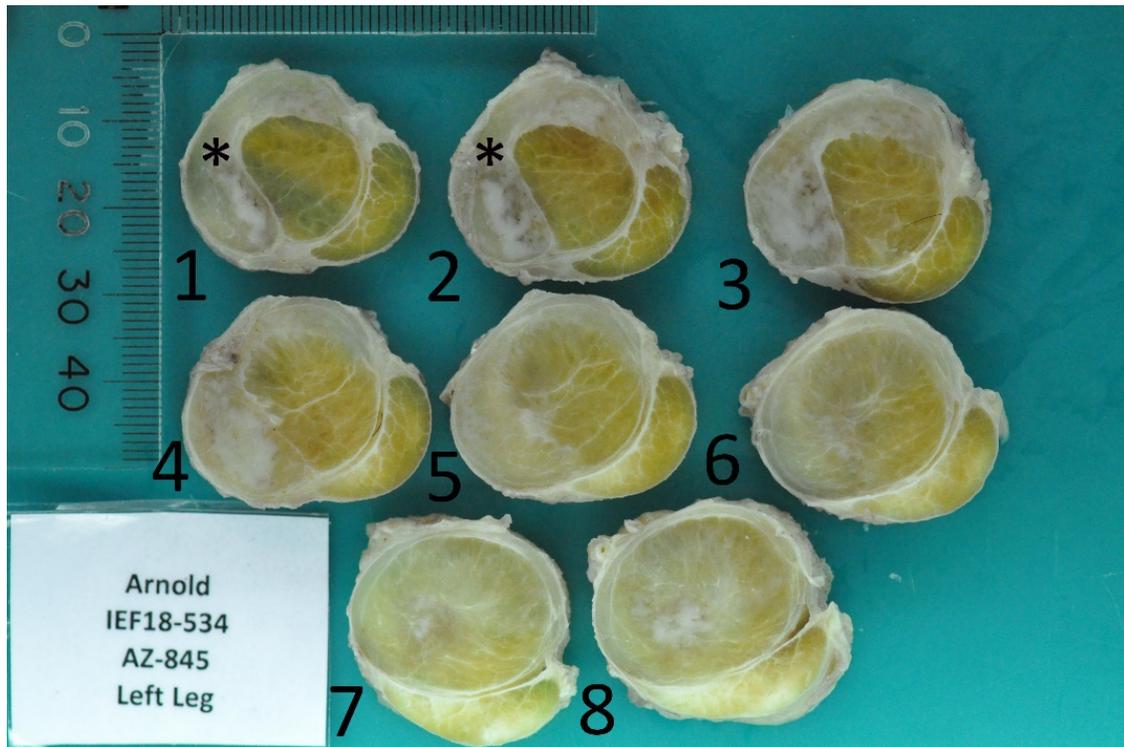


Figure 1. Flexor tendon from treated left leg ('Arnold'). Cross sections of tendon (approximately 1 cm thick) with level 1 being most proximal, and level 8 being distal. For levels 1 and 2, the asterisks (*) demonstrate the clear presence of the accessory ligament of the ALDDFT. Levels 3 and beyond are located in region where the ALDDFT blends with the DDFT. Note the white irregular color of the ALDDFT gradually dissipated distally.

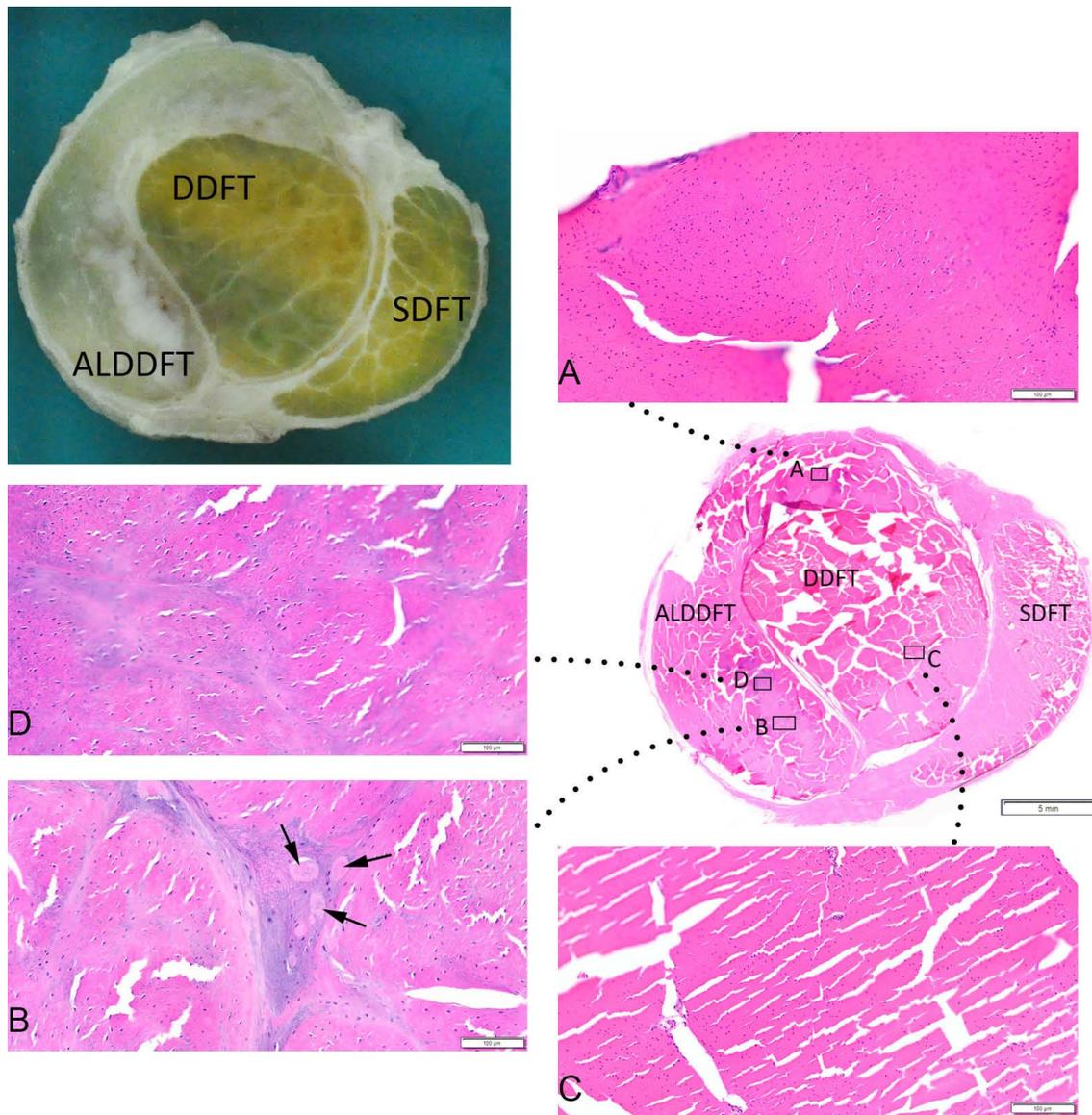


Figure 2. Left leg. Macroscopic and microscopic evaluation of Level 1 (located approximately between the upper one third and one half of the proximal metacarpal region) from Figure 1. The macroscopic image is shown in the upper left, with identification of the ALDDFT, DDFT and SDFT. The H&E stained microscopic section is shown with higher power images of various regions throughout the ALDDFT and DDFT. Box C is taken from the DDFT and is consistent with normal tendon. Fragmentation is artifactual. Box B and D correspond to the regions in the ALDDFT with the most intense white discoloration. Microscopically these areas have disruption of the normal collagen orientation with regions of metachromasia (gray-blue staining). In Box B, in a region of fibrocartilaginous metaplasia, chondroid cells are clearly evident (arrows). Box A demonstrates more normal collagenous stroma, but with more densely packed nuclei, very minor metachromasia, and slightly more irregular orientation of fibers.

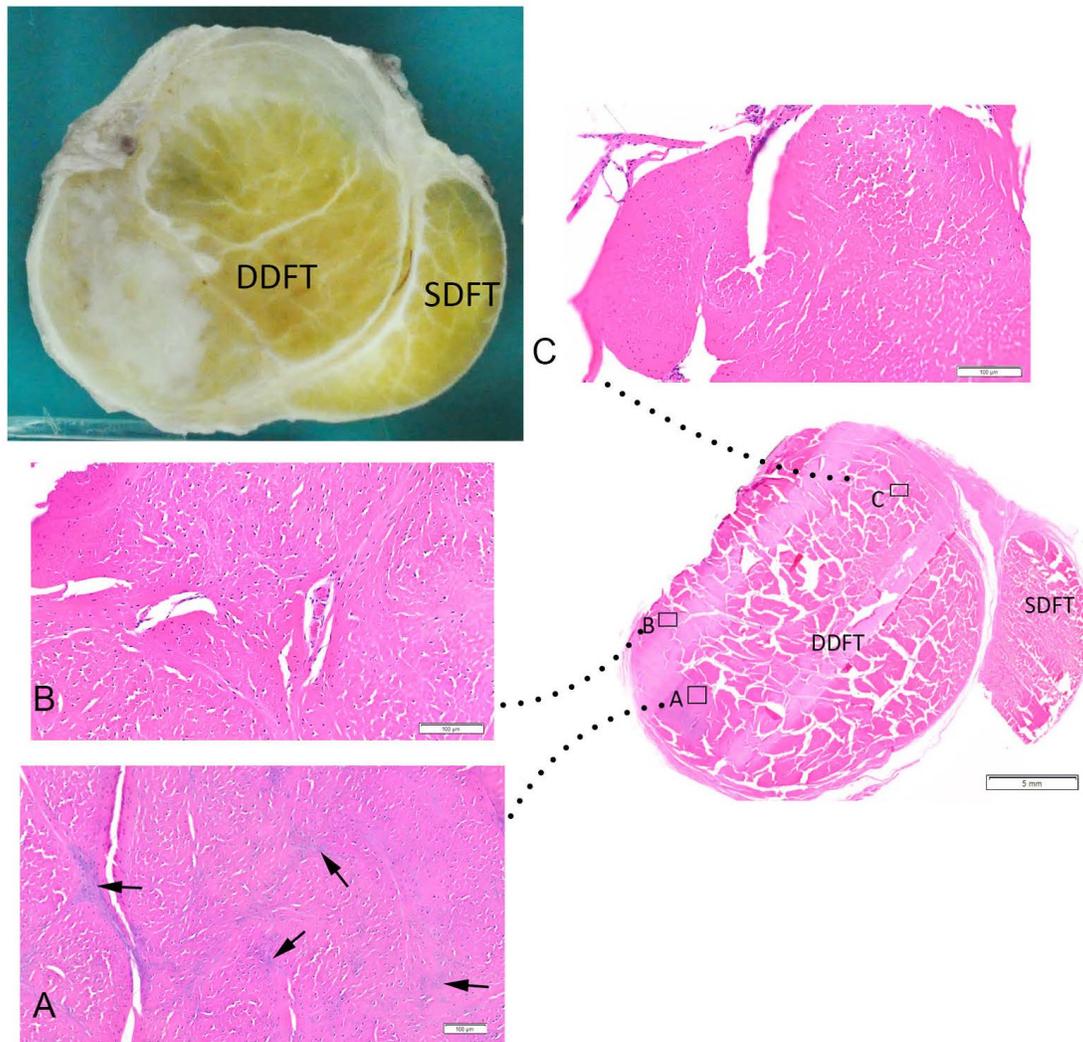


Figure 3. Left leg. Macroscopic and microscopic evaluation of Level 4 (located approximately mid-metacarpal) from Figure 1. The macroscopic image is shown in the upper left, with identification of the DDFT and SDFT. The ALDDFT is no longer a distinct structure with blending of the DDFT. The H&E stained microscopic section (with artifactual loss of a portion of the SDFT and DDFT) is shown with higher power images of various regions throughout the DDFT. Box A corresponds to the regions in the DDFT with the most intense white discoloration. Microscopically this area has disruption of the normal collagen orientation with regions of metachromasia (arrows). Boxes B and C demonstrate regions with less intense change (more irregular orientation of fibers, and more densely packed nuclei than normal, with very minor metachromasia).

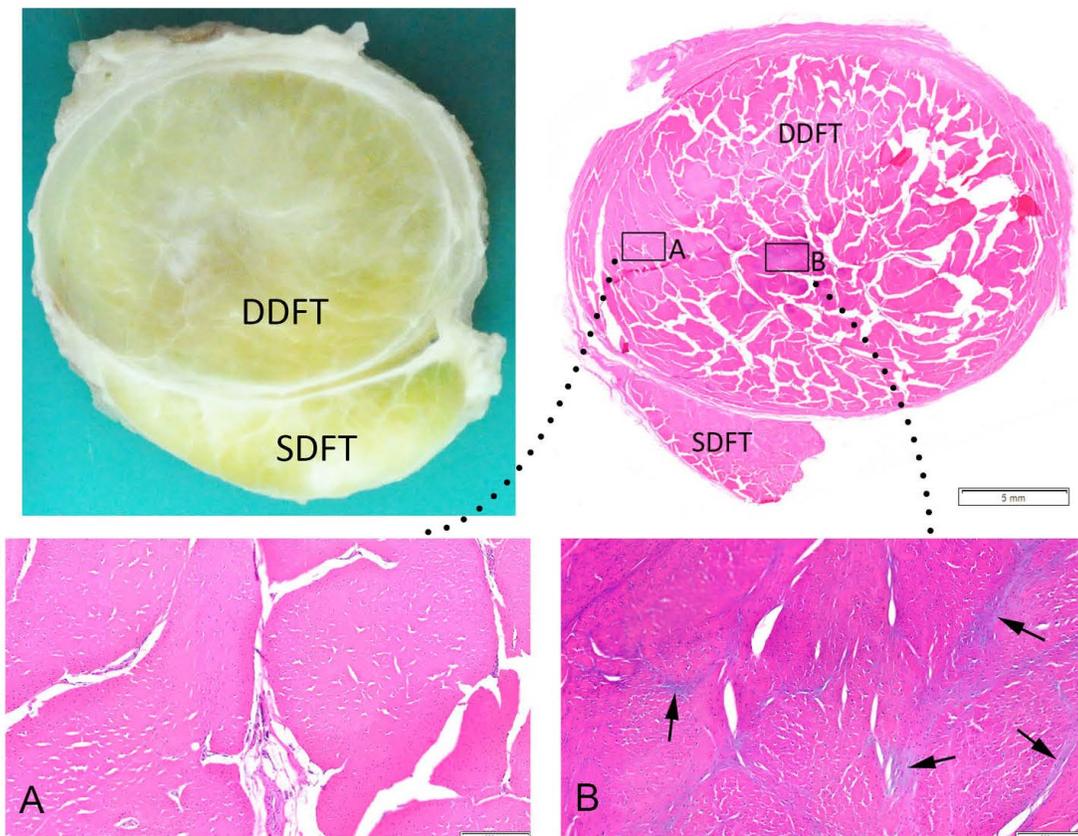


Figure 4. Left leg. Macroscopic and microscopic evaluation of Level 7 from Figure 1. Section is located approximately in the distal one third aspect of the metacarpal region. The macroscopic image is shown in the upper left, with identification of the DDFT and SDFT. The H&E stained microscopic section is shown with higher power images of two regions of the DDFT. There is artifactual loss of a large portion of the SDFT. On the macroscopic image, there are areas of more intense white staining located in the central aspect of the DDFT, which is shown at higher power in Box B. This region has areas of metachromasia (arrows) with irregular orientation of fibers. In Box A, the collagen fibers are more regular in orientation and staining characteristics.

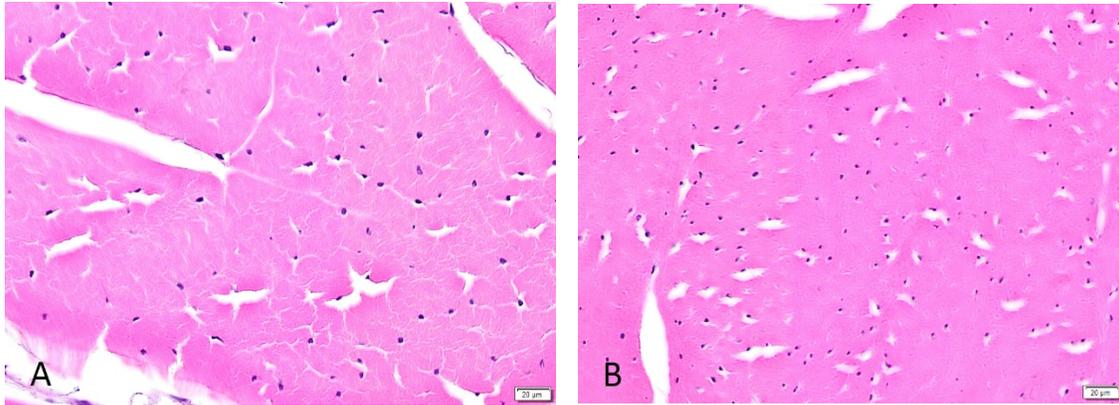
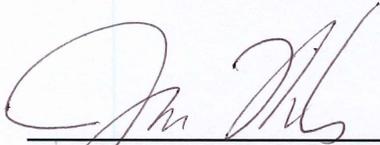


Figure 5. Comparison of the accessory ligament of the deep digital flexor tendon from the right (untreated) and left (treated) legs (H&E). A = Right leg, demonstrating a dense and regular collagen pattern with low density of regularly spaced fibrocyte nuclei. B = Left leg, image taken from a region with less scarring (magnification is similar to that of A) demonstrating similar collagen density, with increased and slightly irregular cellularity when compared to the right leg. This region would be expected to have nearly normal functionality. While many of the nuclei appear to be consistent with fibrocytes, definitive identity could not be confirmed.

Conclusion

Macroscopic and microscopic evaluation was performed on a tendon taken from a horse receiving a series of five (5) ultrasound guided injections of PulpCyte to an injured digital flexor tendon unilaterally. The tendon was collected 4 years following treatment. The most severe evidence of damage both macroscopically and microscopically was evident in the ALDDFT in the more proximal region with irregular extension into the DDFT distally. Microscopically, there was minimal to marked increase in fiber irregularity and cellular density (when compared to uninjured tendon), multifocal areas of metachromasia, and rare areas of fibrocartilage metaplasia. Some areas of the damaged ALDDFT had regions with morphology similar to that of the untreated (uninjured) contralateral ALDDFT. There was no evidence of neoplastic transformation, and all changes were consistent with a normal healing response.



Joan Wicks, DVM, PhD, DACVP
Study Pathologist

4/19/19

Date