INTRODUCTION: Tendon injuries are a frequent cause of disability because they often fail to heal or else do so by scar formation. Scar is less organized than normal tendon tissue and exhibits inferior mechanical properties long after the initial injury [1]. Scarring is also responsible for the formation of adhesions, which disrupt the normal gliding mechanism that is critical for function. Previous studies have shown that early gestational fetal skin and articular cartilage heal in a regenerative fashion without scar formation [2,3]. However, whether early gestational fetal tendon is also capable of healing scarlessly, with normal geometry and mechanical properties, is not known. The objective of the current study is to compare the healing of adult tendons with those of fetal tendons during the second and third trimesters of pregnancy. We hypothesized that tendon injured during the second trimester would heal scarlessly through a regenerative pathway examining normal histology, geometry, and mechanical properties while third trimester and adult tendons would heal via scar formation in a reparative pathway.

MATERIALS AND METHODS: Time-dated pregnant ewes at 80-85 (n=5) and 115-120 (n=5) days of gestation (term 145 days) were anesthetized prior to laparotomy and hysterotomy (IACUC approved). Following uterus externalization, each of the fetal limbs was exposed. A longitudinal incision was made mid-diaphysis through the 4th metacarpal or metatarsal. A partial, mid-surface tenotomy (approx. 50% of width) was carefully made on the lateral extensor tendon with a custom scalpel blade. No repair was attempted and wound location was marked with India ink. Analogous 50% tenotomies without repair were created in each of the maternal limbs for comparison. Sheep were sacrificed at 7 days post-surgery.

For histology, fetal and adult tendon wound segments from each animal (n=3-4) were harvested. Adjacent tissue from the medial extensor tendons served as unwounded controls. Specimens were sectioned and stained with H&E or evaluated immunohistochemically using TGF-β antibodies. To quantitate collagen fiber orientations, 3 sections from 2 wounded and 2 unwounded 80-day specimens were viewed under polarized light. Images were taken at 5° increments and digitally analyzed using an automated system [4]. To calculate cross sectional area, tendon wound thickness and width were measured [5].

For elastic and viscoelastic biomechanical studies, specimens (n=4-5) were immersed in a 37°C PBS bath and preconditioned. Either a tensile test to failure (at 0.1%/sec) or a stress relaxation test (to 5% strain at 25%/sec followed by 1200 seconds of relaxation) was performed.

Paired t-tests were used to determine differences within each age group. ANOVAs with Fisher’s post hoc tests (if ANOVA significant) were performed to assess differences between the three age groups.

RESULTS: Histologically, a gap with granulation tissue and inflammatory cells was visible in the site of wound healing both in the adult and the 120-day fetal tendons. In the 80-day fetal tendons, however, no abnormalities were noted near the wound (ink) with complete reconstitution of collagen architecture (Fig 1). Immunohistoassays revealed low levels of TGF-β1 and 3 expression in the wounded and unwounded 80-day specimens, whereas both of these cytokines were upregulated in the 120-day and adult wounds. In the 120-day specimens, TGF-β3 increased more than TGF-β1. In the adult tendons, the greatest increase occurred in TGF-β1, both in the wound and in adjacent areas.

Quantitative polarized light analysis showed that entropy (a measure of fiber orientation randomness) ranged from 2.2-2.6 and the coefficient of variation (a measure of fiber distribution spread) ranged from 11.0-15.1, indicating a high similarity in the wounded and unwounded 80-day specimens. This analysis could not even be performed on the 120-day or adult specimens due to a lack of collagen in the wound space (Fig 2).

Geometrically, the cross-sectional area increased significantly in the wounded adult tendons, but not in either of the fetal groups (Table 1). Biomechanically, significant decreases were found in the ultimate stress and modulus when comparing the wounded to the unwounded tendons in all three groups (Table 1, Fig 3). However, no significant differences were found in the wounded/unwounded ratios for ultimate stress and modulus across the three groups. There were also no differences between the equilibrium/peak stress ratios (from stress relaxation experiments) within the three groups (Table 1), nor were there differences in the wounded/unwounded ratio of this value across groups.

![Figure 1: H&E section of wounded 80-day tendon, 200X.](image1)

![Figure 2: Viewed under polarized light. A) Wounded 80-day fetal tendon, 100X B) Wounded adult tendon (arrow depicts injury location), 50X](image2)

![Figure 3: Material properties of wounded and unwounded tendons. *p = 0.05](image3)

**REFERENCE:**


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