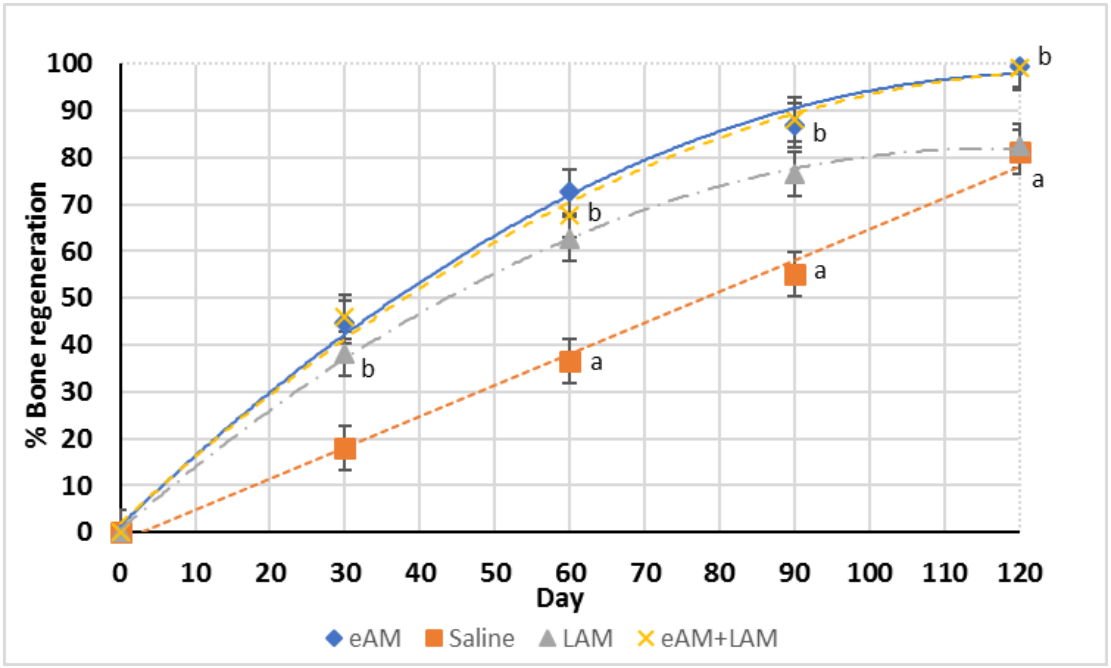

StemWrap for Bone Regeneration studied at Autonomous University of City Juarez

Abstract from American College of Veterinary Surgeons Study Submission

Title: Acellular preserved or morselized liquid amnion accelerated bone regeneration in rams.

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The aim of this study was to evaluate the effectiveness of preserved and/or liquid morselized amnion – StemWrap for bone regeneration in a 120-day study. A 10-mm diameter surgical perforation was created on each tibial crest of 8 rams. Each ram received one treatment in both legs: Control=Saline, eAM=equine preserved amnion (eAM, BioScaff®, AniCell), LAM=liquid morselized amnion (LAM, StemWrap+®, AniCell), or the combination=

(eAM+LAM). Clinical signs (temperature, pain, lameness and inflammation, using a scale: 0=none to 4=highest), blood test and radiological analysis (bone regeneration measured by software analysis) were done between day 1 and 120. Data was analyzed using SAS statistical software. For clinical signs, no differences were found in the treatment slopes or day by treatment interactions ("DxT") ($P>0.1$) for lameness, pain, or body temperature. However, Control had higher inflammation score slopes ($P<0.05$); and higher rate of inflamed surgical lesions compared to eAM, LAM, and eAM+LAM up to 30 days post-surgery (66.7 vs. 16.7, 16.7, 16.7%, respectively; $P<0.05$). For blood analysis, no treatment or "DxT" were found ($P<0.1$) and blood values were inside the normal range for each treatment. For bone regeneration, there was a "DxT" ($P<0.05$), where amniotic treatments had higher regeneration at day 30 (1.1 to 1.6X), 60 (0.7 to 1X), 90 (0.4 to 0.6X) and 120 (0.2X, except LAM; $P>0.1$) than the control group. Also, the slope of the control differed from amniotic treatments ($P<0.05$). These results support the use of amnion to decreased inflammation, and increased bone regeneration, with no adverse clinical effects in the animals.

Long Abstract

Title: Commercially available acellular desiccant and morselized liquid amnion accelerate bone regeneration in rams without adverse clinical effects.

Reasons for performing the study: Amnion is a rich source of extracellular matrix components (collagen, hyaluronic acid, etc.) and growth factors (VEGF, TGF, etc.). Orthopedic application of amnion dates to 1930s, and its bioactivity and positive effects have been previously reported on bone fusion and fracture healing in humans; however, the potential for scaffold

components to modulate the development of bone has not been extensively investigated.

Hypothesis/Objectives: We hypothesize that the use of commercially available amniotic extracellular matrix either as preserved membrane (BioScaff®) and/or liquid morselized form (StemWrap+®), would accelerate and improve bone regeneration compared to control (Saline), and the combination of products would have a synergic effect. The aim of this study was to evaluate the effectiveness of amnion in membrane and/or liquid form for bone regeneration.

Methods/Experimental Design: A total of 12 rams (> 8 months) were randomly allocated into four treatment groups: Control= Saline treatment (1 mL 0.9% NaCl), eAM= equine preserved amniotic membrane (4 cm^2, BioScaff, AniCell), LAM= liquid morselized amnion (1 mL, StemWrap+, AniCell), and combination of treatments (eAM+LAM, 4 cm^2 +1 mL), in a 120-day study. A surgical 1 cm diameter side by side perforation was done in both tibial crests. The perforation site was cranial to the collateral medial ligament and cranial distal to the insertion of the tendon of Sartorius muscle. Once the periosteum was visible, the perforation was done with a 10-mm diameter drill bit (A5226, Arthrex) using a pneumatic drill (Aesculap, Braun) with continuous saline solution irrigation to avoid bone necrosis from friction. The drill bit passed the cortex of the medial then the lateral tibial crests. After the hole was done, saline solution wash was used to remove all bone fragments. The same treatment was randomly placed inside the cortex of the bone in the two legs of each animal, and surgical planes were sutured. Animal clinical signs (temperature, pain, lameness and inflammation, were measure by the same person using a scale: 0=none to 4=highest), hemogram

(red and white blood cells) and radiological analysis (bone regeneration) were done between day 1, 30, 60, 90, & 120. Inflammation of the surgical wound was also analyzed at day 30. For bone regeneration, a digital picture of mediolateral X-ray was taken and the perforated area was measured (~78.5 mm^2 for 10-mm diameter drill bit, Figure 3) using Image J software (NIH). For each leg, the area of the hole was measured in a giving day and subtracted from day 1 measurement to calculate the bone regenerated area (BRA) in mm^2; then, converted to percentage (BRA x 100 divided by day 1 measurement) to determine the bone regeneration rate. Data was analyzed using SAS statistical software by Regression and ANCOVA including two way interactions and post-hoc analysis with LSD test, and Fisher's Exact Test for categorical data.

Results: For the animal clinical signs, no differences were found for lameness, and pain up to day 30 or body temperature up to day 60 for treatment slopes or day by treatment interactions (DxT) ($P>0.1$). However, inflammation score for the control group was higher than eAM, LAM and eAM+LAM ($P<0.05$, Figure 1), when slopes where compare from day 0 up to day 30 post-surgery. Likewise, the control group had the highest rate of inflamed surgical lesions vs. eAM, LAM, and eAM+LAM at 30 days post-surgery (66.7 vs. 16.7, 16.7, 16.7%, respectively; $P<0.05$) (Figure 1 and 2). For blood analysis, no treatment or DxT interactions were found ($P<0.1$) and red and white blood cells values were inside the normal range for each treatment. For bone regeneration, there was a DxT interaction ($P<0.05$), where amniotic treatments had a higher bone growing rate at day 30 (1.1 and 1.6X), 60 (0.7 to 1X), 90 (0.4 to 0.6X) and 120 (0.2X, except LAM; $P>0.1$)

than the control group (drilled bone + saline) (Figure 4). Also, the slope of the control differed from amniotic treatments ($P<0.05$) (Figure 4).

Discussion: These results support the hypothesis that either acellular preserved or liquid morselized amnion accelerated and improved bone regeneration compared to control (Saline). Amnion decreased inflammation score and reduced the rate of inflamed surgical lesions compared to control. This suggests that extracellular matrix components of the amnion helped in the healing process. Also, amniotic products increased bone regeneration rate over the control, with no adverse clinical effects in the animal. Different formats of eAM proved to impact the regeneration at different timelines.

Main Study Limitations: We used the ram bone model to study bone regeneration and might have different regeneration rates in other mammal subjects. Also, surgically created bone wounds (perforation) do not accurately mimic the traumatic bone fractures on which these products are being used clinically.

Scientific or Clinical Relevance: For the last couple of years, acellular and cellular regenerative therapies in both veterinary and human medicine have reemerged to regenerate different musculoskeletal pathologies. In this study, favorable results were achieved using commercially-available acellular amnion products, which are a vast source of extracellular matrix and growth factors and are a more affordable choice than cellular therapies. Nowadays, it is common to use allogenic amniotic therapies in animals and humans with no adverse reactions. In this case, xenogeneic grafts were used without adverse clinical effects in the animal. Commercial acellular products are

sterile, able to be used “off-the-shelf” the same day as diagnosis and are a safe and effective regenerative treatment for bone healing.

Figure 1. Inflammation score, and inflammation rate (±SEM) of the surgical wound at day 30 after surgery for different treatments (^{a,b} $P < 0.05$).

Figure 2. Superficial surgical lesions of ram legs treated with different treatments 30 days after surgery.

Figure 3. Medial-Lateral X-rays picture of perforated tibias (10-mm drill bit) at day 1, 30, 60, 90, and 120 days after surgery of the animals treated with control (saline) and equine membrane (eAM).

Figure 4. Bone regeneration (±SEM) from day 1 to 120 after surgery for Control (saline), equine membrane (eAM), liquid morselized amnion (LAM) and the combination (eAM+LAM) (^{a,b} $P < 0.05$).

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