



**ASSESSING THE EFFECT OF AMPUTATION AND BATTLEFIELD-RELEVANT FACTORS ON HETEROTOPIC OSSIFICATION IN AN OVINE MODEL**

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**INTRODUCTION:** Heterotopic ossification (HO) refers to ectopic bone formation, usually in residual limbs and/or peri-articular regions following traumatic injury and subsequent amputation. HO occurs outside of the skeleton and is composed of a hybrid of cortical and cancellous bone. There is general agreement in literature that HO is induced from damage to soft tissue and inflammation. Ectopic bone growth has been most frequently observed after combat-related trauma to service members from blast injuries. Reviews of orthopedic injuries from Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF) have reported that approximately 70% of war wounds have involved the musculoskeletal system, largely from the use of improvised explosive devices (IEDs) and rocket propelled grenades (RPGs). HO has been reported to occur in approximately 65% of wounded service members with limb loss or major extremity injuries. Symptomatic HO may delay rehabilitation regimens as it often requires modifications to prosthetic limb componentry and socket size. Most concerning is that no empirical evidence has indicated a mechanism for quelling or preventing metabolically active HO. Recent studies performed by our team demonstrated that a combination of trauma (see below) can induce HO growth. Surgical/trauma factors included:

- Periosteal disruption
- Holes drilled in the midshaft femur (to allow growth factors from the medullary canal into the surrounding tissue)
- Autograft bone chips placed at the disruption site
- Simulate IED/RPG blast using an air impact device (AID)
- Biofilm inoculation with *Staphylococcus aureus* ATCC 6538
- Tourniquet usage for 45 minutes
- Negative pressure wound therapy (NPWT); NPWT foam placed subdermally and removed after 3-7 days

The observed HO in our previous model appeared localized in the posterior regions/mid-shaft of the femur. The data from that study also indicated that the HO was still actively modeling at a 6-month timepoint. Therefore, the question arose: *Would the localized HO become more florid with additional time?* Another limitation of the previous model was it lacked amputation of the limb, which has been prevalent in wounded soldiers who were injured in OIF/OEF.

Thus, we set out to compare the structure and amount of ectopic bone in non-amputated and amputated ovine limbs following the combination of surgical/trauma factors (listed above). This comparison will determine if the addition of amputation increases the amount of ectopic bone thus more closely resembling HO observed in wounded soldiers 1-year post-injury.

**METHODS:** Animal work was performed at Utah State University following local institutional animal care and use committee (IACUC) and external animal care and use review office (ACURO) approvals. As mentioned above, to simulate an IED blast, an AID that is used in the special effects industry was optimized and safety tested. The AID discharged high-powered bursts of air to the lateral, mid shaft region of ovine femurs to inflict deep tissue trauma. Both Group 1 and Group 2 sheep obtained all of the above-mentioned trauma factors; the single difference was that the operated limb (mid-shaft of the femur) of the sheep in Group 2 were amputated following the AID blast as opposed to having holes drilled in the femur. Following surgery, all sheep in both groups resumed weight bearing activities and were monitored. One-year post-op the sheep were euthanized. The femurs were dissected, radiographed, a micro computed tomography (CT) performed, and samples processed in polymethyl methacrylate (PMMA) for scanning electron microscopy (SEM) and histological analysis.

**RESULTS:** Radiography and micro-CT analyses demonstrated that the addition of the limb amputation in Group 2 resulted in more ectopic bone growth 1-year post-op. Analyses also revealed that the amputated limbs more closely resembled the growth pattern of ectopic bone observed in wounded soldiers by having circumferential growth around the resected bone advancing into the tissue/muscle. This is in stark contrast to the sheep from Group 1, which only showed bone stemming from the medial-posterior side where the trauma occurred. The analysis also demonstrated that the Group 2 (amputated) limbs contained thinner cortical walls near the amputation site that more closely resembles clinical findings, while the Group 1 limbs (non-amputated) revealed a thicker cortex due to a periosteal response. While radiography analyses clearly demonstrated a higher amount of ectopic bone in Group 2, volume analysis will be performed on both groups to obtain a quantitative comparison. Additionally, advanced histological analysis is currently underway to determine if the ectopic bone demonstrates similar characterization as human HO bone growth (e.g. trabecular bone structure, osteon remodeling and hyper-mineralized regions) as observed in the Group 1 (non-amputated) limbs.

**DISCUSSION:** Greater ectopic bone growth and similar growth patterns as observed in human HO were shown to be more prevalent in amputated limbs. This study suggests that amputation may simulate HO growth to an even greater degree than the sheep model we established previously. Successful demonstration of this translatable animal model will help clinicians understand the etiology of this pathological condition, address a major clinical gap that has been sought after by the Department of Defense, and improve care for combat injured service members.

**SIGNIFICANCE:** A significant portion of wounded soldiers who suffer from limb loss and combat-trauma also have accompanying HO. This pathology reduces quality of life, decreases function, and delays rehabilitation. By elucidating the development of HO, other studies may be performed to investigate therapies for prevention and/or treatment.

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