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● *Original Contribution*

DEVELOPMENTAL IMPACT AND LESION MATURATION OF HISTOTRIPSY-MEDIATED NON-INVASIVE TISSUE ABLATION IN A FETAL SHEEP MODEL

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Abstract—Non-invasive histotripsy therapy has previously been used to achieve precise fetal tissue ablation in a sheep model. To further assess the clinical viability of the technique, this study investigated potential effects of histotripsy therapy during the remaining gestation and its local impact on fetal development. Five ewes (six lambs) at 95–107 d of gestation were treated and allowed to complete the full gestation period of 150 d. A 1-MHz focused transducer was used to treat the fetal kidney and liver with 5-μs pulses at 500-Hz repetition rates and 10- to 16-MPa peak negative pressures; ultrasound imaging provided real-time treatment guidance. The lambs were euthanized after delivery and treated organs were harvested. Samples were examined by magnetic resonance imaging and histopathologic analysis. These data were compared with results from four other ewes (four lambs) that underwent similar treatments but were sacrificed immediately after the procedure. The sheep tolerated the treatment well, and acute lesion samples displayed well-defined ablated regions characterized by the presence of fractionated tissue and hemorrhage. All fetuses that were allowed to continue gestation survived and were delivered at full term. The lambs were healthy on delivery, with no signs of external injury. A minor indentation was observed in each of the treated kidneys with minimal presence of fibrous tissue, while no discernible signs of lesions were detected in treated livers. In a sheep model, histotripsy-mediated fetal tissue ablation caused no acute or pregnancy-related complications, supporting the potential safety and effectiveness of histotripsy therapy as a tool in fetal intervention procedures. (E-mail: yohankim@umich.edu)

Key Words: **Non-invasive fetal therapy, Pulsed cavitational ultrasound, Histotripsy, Therapeutic ultrasound.**

INTRODUCTION

Current prenatal care technology is capable of detecting and diagnosing congenital diseases, such as fetal tumors, by the second trimester of gestation (Avni et al. 2009). Although the majority of prenatally diagnosed malformations are still managed postpartum, fetal surgery may, in select cases, be a desirable treatment option to prevent malformations such as large lung masses and teratomas from progressing into life-threatening conditions.

In utero fetal intervention is a challenging task, requiring experienced operators, with risk often commensurate with the degree of invasiveness of the procedure. Clinically available options consist primarily of minimally invasive percutaneous or endoscopic procedures and open fetal surgery, which carry risks of infection, preterm labor, and premature rupture of membranes, among other complications (Kunisaki and Jennings 2008). A fully non-invasive therapeutic approach to fetal intervention could minimize such risks, potentially improving maternal recovery and fetal outcomes.

Histotripsy therapy fractionates tissue through the control of cavitation bubble clouds generated by microseconds-long, high-instantaneous-pressure ultrasound pulses, typically delivered at millisecond intervals. Cavitation bubbles have been known to initiate depending on a pressure threshold mechanism for short ultrasound pulses (Fowlkes and Crum 1988), and more recently, shockwave scattering has been identified as an additional and important factor governing the formation

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of the bubble clouds in histotripsy therapy (Maxwell et al. 2011b). In conjunction, these phenomena limit the cloud expansion to a small spatial zone where the shock waves are strongly focused, confining the bubble cloud within the focus of the therapeutic transducer and allowing the ablation accuracy to achieve millimeter-scale precision (Xu et al. 2005).

The short pulse sonication regime used in histotripsy therapy has been shown to be more energy efficient for ablation purposes than the application of long pulses (Wang et al. 2010), and results in low insonation duty cycles (<5%), which minimize the risk of overheating surrounding tissues (Parsons et al. 2006). Real-time monitoring of the treatment can be provided by conventional ultrasound imaging systems, which are able to readily detect and image the highly echogenic cavitation bubbles (Lake et al. 2008; Roberts et al. 2006). Regions ablated by histotripsy treatment may also be imaged with standard ultrasound techniques because of the marked reduction in ultrasound imaging backscatter or elasticity change (Hall et al. 2007a; Wang et al. 2012), lending the therapy a simple and useful feedback mechanism to evaluate treatment efficacy. Previous *in vivo* animal studies have investigated histotripsy therapy for several applications in which non-invasive tissue ablation is desired such as treatments for benign prostatic hyperplasia (Hempel et al. 2011), congenital heart disease (Xu et al. 2010; Owens et al. 2012), thrombosis (Maxwell et al. 2011a), and renal tumors (Styn et al. 2010).

An initial feasibility study by our group has demonstrated that histotripsy therapy is capable of non-invasively generating precise lesions in fetal sheep heart, kidney and liver *in utero*, with no or minimal collateral damage to overlying tissue structures (Kim et al. 2011). In that study, fetuses ranging from 102 to 129 d of gestation (full term: 150 d) were targeted. Cavitating bubble clouds were successfully generated in 19 of 31 (61%) treatment attempts; in the remaining 12 cases (39%), cavitation clouds could not be generated. In those 12 cases, it was observed that bone structures, such as fetal limbs, were a substantial source of blockage for the acoustic pathway. It was hypothesized that fetuses at a later stage of gestation were more likely to present obstructions to ultrasound therapy because of the higher degree of fetal bone development. This was a motivation to treat fetuses at an earlier gestation stage in our current study (95–107 d), when fetal bones are less calcified and less likely to cause ultrasound blockage. Furthermore, treatment at an earlier stage could be clinically desirable in cases where intervention has the potential to limit or stop the progression of certain abnormalities, such as lung masses.

Because of potential bioeffects of ultrasound on tissue, especially those near gas bodies, safety parameters such as the mechanical index have been developed and refined for diagnostic ultrasound (Apfel and Holland 1991; Church and O'Brien 2007). In the fetus, however, the lungs and intestines are gas free, presenting a rather unique situation. Several studies investigated pressure thresholds that induced acute hemorrhage in animal fetuses exposed to non-diagnostic, high ultrasonic fields. In one study (Hartman et al. 1990), fluid-filled fetal tissues were observed to be significantly more resilient to lithotripter shock waves up to 20 MPa than gas-filled adult lungs and intestines. A separate investigation showed that when fetal tissue is exposed to high-amplitude shock waves, superficial hemorrhage is more likely to be found at tissue boundaries near more developed bone, whereas soft tissue that is distant from such structures appears relatively free of hemorrhagic events (Dalecki et al. 1997). In our current study, the minimization of these untoward effects by reducing the impact of bone interfaces was also an incentive for treatment at earlier gestation stages, when fetal bones are less developed. We have also attempted to improve the consistency of outcomes by treating fetuses within a narrower gestation window and by using fixed ultrasound exposure parameters during treatments.

Limited literature exists on non-invasive fetal intervention (Ichizuka et al. 2007, Paek et al. 2005), and long-term safety studies on large animal models exposed to this type of procedure have not been published. In this study, the treated fetuses were allowed to continue gestation to full term for the purposes of investigating potential safety issues during the course of pregnancy and delivery, as well as examining the longer-term impact of the therapy in the treated organs after birth.

METHODS

Nine ewes and ten lambs were treated from 95 to 107 d into gestation (full gestation period: 150 d). The sheep were divided into a chronic group and an acute group. The five ewes and six lambs treated in the chronic group were allowed to continue gestation to full term after receiving treatment, and samples were collected postpartum. In one case from the chronic group, twin lambs were treated, with each sheep receiving a single treatment. The four ewes and four lambs treated in the acute group were exposed to treatment parameters identical to those of the chronic group, but tissue samples were harvested immediately after the procedure.

Sheep were initially sedated with intramuscular injections of diazepam (0.5–1 mL/kg), followed by intravenous injections of propofol (4–6 mL/kg) through vessels in the foreleg. Subsequent endotracheal intubation allowed full anesthesia to be achieved with inhalation of 1–3% isoflurane gas. After induction of anesthesia, the