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In-utero radiofrequency ablation in fetal piglets: Lessons learned

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ABSTRACT

Introduction: Radiofrequency ablation (RFA) is increasingly utilized in minimally invasive fetal intervention. However, the response of different fetal tissues to RFA is poorly characterized. We sought to determine the extent of RFA damage in a fetal environment.
Methods: 90 Day gestation Yorkshire piglets (term 115 days) were subjected to RFA of the chest and abdominal viscera under various temperatures and wattages. The extent of tissue damage was determined by NADPH diaphorase histochemistry.
Results: Tyne temperature was widely variable and displayed varying responses between lung and liver tissue. Tyne exposure to amniotic fluid resulted in an increase in amniotic fluid temperature. Collateral damage, even across the diaphragm, was readily seen, and ultrasonography did not always reflect this injury.
Conclusions: Utilization of extracorporeal tynes heats fluid at a greater rate than solid tissue and reliance on temperature sensitive probes may result in overheating. The extent of injury may extend beyond damage observed by ultrasound examination and varies for different tissues. Additional studies on the use of devices that regulate tyne temperature are needed to define optimal conditions and better define the extent of adjacent tissue injury.

Fetal surgical intervention is reserved for life-threatening conditions or those associated with severe morbidity. To decrease the morbidity associated with these procedures, there is considerable interest in investigating minimal access techniques for fetal therapy. Fetoscopic urethral valve ablation [1], endoscopic tracheal occlusion for treatment of congenital diaphragmatic hernia [2], endoscopic release of amniotic bands [3], and laser ablation of communicating vessels in twin–twin transfusion [4,5] are some examples of minimal access fetal interventions. Radiofrequency ablation (RFA) involves the destruction of biological tissues by transferring electricity from a rapidly alternating current at radiofrequencies. Therapeutic frequencies must be high enough to cause molecular friction heating without stimulating the neuromuscular junction and electrolysis, yet low enough to confine energy transmission to a controllable tissue mass. The first electrocautery devise was developed by Bovie and Cushing in the early 1920s [6]. The therapy was modified to target solid organ lesions and is now generally accepted as a first-line treatment for hepatocellular tumors [7,8]. With the advanced imaging modalities now available for specific and real-time imaging of a lesion, RFA is utilized to treat multiple lesions in organs throughout the body in adults [7,8].

In the arena of fetal therapy, RFA has been used successfully to obliterate flow in umbilical vessels in cases of twin reversed arterial perfusion (TRAP) sequence with a survival rate as high as 85% or discordant twin pregnancies undergoing selective reduction; both involving fetal demise as a result of the intervention [9–12]. RFA has also been proposed for antenatal intervention for the ablation of fetal tumors such as sacrococcygeal teratoma (SCT) and congenital cystic adenomatoid malformation (CCAM). Following preliminary experiments using fetal animal models, several reports of human fetal intervention were published, some with dire consequences [13]. Of the few cases of intrauterine RFA therapy for SCT, one child was left with an obliterated hip joint and a flaccid limb [14]. In the report by Paek et al. two of four fetuses with SCT who underwent in-utero ablation of the tumors died and the other two suffered severe perineal injuries [15]. These clinical outcomes highlight the need for the establishment of a clear set of guidelines for the use of RFA in fetal therapy where the fetus is expected to survive.

A review of the published fetal RFA experience demonstrates that instrument settings were often arbitrarily chosen or extrapolated from experience with adult tissues, while the duration of therapy was, in the best of cases, based on temperature change or ultrasound findings. In the case of cord occlusion, therapy was continued until absence of Doppler flow was demonstrated. Otherwise, extent of administration was entirely discretionary and by definition inconsistent. We embarked upon this study in an attempt to better define the use of RFA in fetal tissues, selecting one of the newer RFA probes to test. We aimed to determine the extent of tissue injury following RFA in the fetus, identify any

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collateral damage that may result from RFA use, determine any difference in the response of different fetal organs to RFA, and determine any evidence of heat dissipation that may affect a cotwin.

1. Materials and methods

1.1. Radiofrequency ablation procedure

Following approval by the Institutional Animal Care and Use Committee at Baylor College of Medicine, one time-dated pregnant Yorkshire sow at 90 days gestation (term = 115 days) was sedated with Ketamine 15 mg/kg and Xylazine 1 mg/kg and anesthetized with isoflurane 0.5–4% in oxygen. A midline abdominal incision was performed to gain access to the uterine horns containing fetuses. The pig fetuses at this gestation are about 550–650 grams and correlate, by size, to a 23–24 week gestation human fetus. Following laparotomy, the uterine horns were sequentially exposed and interrogated with an Acuson Sequoia® ultrasound (Siemens Inc., New York, NY) with a 3.5 or 6 MHz probe to assess the orientation and number of fetuses in utero. A Starburst XL 17 gauge probe (RITA Medical Systems, Fremont, CA) with 4 expandable tynes was connected to a RITA continuous 460 kHz generator (RITA Medical Systems, Fremont, CA) and was inserted into the intact uterus and guided to the fetal organs under sonographic visualization for each fetus. The generator was capable of monitoring the temperature at each tyne of the probe and dispersed energy varied from 18 to 105 watts. Tyne deployment was to a diameter of 2 cm. Target temperature was set to an assigned temperature of either 75 °C or 90 °C and ablation was stopped when this target temperature was reached for 2 to 3 minutes, regardless of the degree of damage to surrounding tissue as visualized on the ultrasound image. A thermometer was inserted into the uterus to measure the temperature of the amniotic fluid subsequent to RFA. A total of thirteen fetal piglets from the sow were evaluated. Baseline amniotic fluid temperature was measured in untreated littermates while each sequential fetus was being treated. Upon completion of therapy, the fetuses were immediately removed from the uterus and euthanized. They then underwent necropsy. The RFA lesion in each piglet was located and photographed, and serial tissue samples were excised at 1, 2, 3, and 4 cm from the center of the lesion.

1.2. Tissue preparation and staining

The extent of gross tissue injury from the probe insertion site was measured and photographed. Tissue samples were either flash frozen in liquid nitrogen or fixed using Histochoice® synthetic fixative (Amresco Inc., Parkway Solon, OH). Frozen specimens were cut into 20 μm sections and processed for NADPH diaphorase enzyme histochemistry, a reliable method for assessing tissue viability post-RFA [16,17]. The procedure was performed as described previously [18]. Fixed samples were dehydrated in alcohol, paraffin embedded, sectioned (5 μm), and stained with hematoxylin and eosin. All sections were observed under a light microscope.

2. Results

A total of thirteen fetal pigs at 90 days of gestation were detected by ultrasonography. Eleven were treated with RFA and two served as controls to monitor amniotic fluid temperature (Table 1). On occasion, upon targeting of the umbilical cord, one of the tynes would be located within the amniotic fluid. Continued monitoring of the tyne temperature allowed for prompt recognition of this issue, as noted in Fig. 1. In an attempt to compensate for this heat sink when located within the amniotic fluid and to attain the set goal temperature, some of the tynes reached a temperature greater than the set limit. Some newer generation instruments allow individual discordant tynes to be shut down and eliminated from treatment.

2.1. Amniotic fluid temperature

Baseline amniotic fluid temperature in fetal pigs was between 38 °C and 39 °C prior to manipulation. RFA treatment did not significantly increase the amniotic fluid temperature (38.1–39.2 °C) unless the tynes were not completely intracorporeal. With a tyne in the amniotic fluid, the fluid temperature increased to as high as 43 °C. The impact this may have on a cotwin is unclear.

2.2. Extent of tissue injury

An assigned temperature of either 75 °C or 90 °C and duration of 2 or 3 minutes was preset per piglet. The effects of tissue ablation were observed via ultrasound, and in some cases "bubbling" of the tissues was noted at the site of treatment. In many instances, no ultrasound changes were noted despite the fact that the preset temperature had been attained. Gross examination of the tissues revealed injury beyond the 2 cm diameter of the probe. With lung ablation, collateral damage to the heart, stomach, liver, and chest wall was noted (Fig. 2A). These appeared to be effects of heat rather than direct necrosis caused at the probe site, as suggested by the fact that these injuries were all intracorporeal in nature. Damage seen at the 75 °C setting was as great as that observed at 90 °C.

Targeted lung ablations were performed in only 5 fetuses, and the time required to reach target temperature from room temperature of the probe (28 °C) was typically 1 minute. It became evident that variations in temperature occurred between the various tynes even after target temperature was reached. Lesions measured at a maximum of 8 cm in diameter on gross examination and varied up to 4 cm when histologically analyzed, regardless of whether the probe temperature was set to 75 °C or 90 °C (Fig. 2B, C). Target temperature was maintained for a

Table 1
Tyne temperature and duration of deployment to target tissue for each piglet are detailed below. The resulting areas of injury are also noted.

Piglet Identification	Tyne Temperature (degree Celsius)	Assigned Duration of Tyne Deployment (minutes)	Target	Injured Areas
1	75	2	Lung	Lung
2	75	2	Lung	Lung
3	75	3	Lung	Lung
4	90	2	Lung	Axilla
5	90	3	Lung	Lung
6	75	2[a]	Liver	Liver
7	75	2[a]	Liver	Lung, diaphragm, liver, stomach
8	90	2[a]	Liver	Lung, diaphragm, liver
9	75	3	Umbilical cord	Cord occlusion
10	90	3	Umbilical cord	Cord occlusion
11	90	3	Umbilical cord	Cord, lung, diaphragm, liver
12 (control)	–	–	–	–
13 (control)	–	–	–	–

[a] Tyne deployment aborted <1 minute because of temperatures exceeding set limit.