Technical Note

In Vivo ^{1}H Magnetic Resonance Spectroscopy of Amniotic Fluid and Fetal Lung at 1.5 T: Technical Challenges

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Purpose: To identify the major technical challenges associated with in utero single-voxel proton spectroscopy of amniotic fluid and fetal lung and to evaluate the feasibility of performing in utero fetal spectroscopy for fetal lung maturity testing.

Materials and Methods: Fetal magnetic resonance (MR) spectroscopy of amniotic fluid and fetal lung were performed at 1.5 T in 8 near-term pregnant women. Presence/absence of lactate and choline peaks was tabulated. Ex vivo spectra were obtained from amniotic fluid samples to investigate and refine sequence parameters.

Results: Spectroscopy failed in 3 of 8 cases due to maternal discomfort ($n = 1$) or fetal gastroschisis ($n = 2$). Both fetal motion and low signal-to-noise ratio were limiting factors for the remaining 5 clinical in vivo studies at 1.5 T. Ex vivo and in vivo studies suggested feasibility for detecting lactate from amniotic fluid within a reasonable clinical scan time (4–5 minutes). Lactate was detected in 3 of 5 patients. Choline detection was limited and was detected in 1 patient.

Conclusion: Minor motion effects can be overcome but continuous fetal motion is problematic. Lactate detection seems clinically feasible, but choline detection requires additional technical development and, potentially, further imaging at a higher field strength because of the low signal-to-noise ratio at 1.5 T.

Key Words: amniotic fluid spectroscopy; fetal lung spectroscopy; lung maturity; respiratory distress syndrome; fetal imaging
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RESPIRATORY DISTRESS SYNDROME is a major cause of neonatal morbidity and mortality and is due to insufficient surfactant production in the lungs at birth. Diagnosis currently requires amniocentesis, an invasive procedure with inherent risks such as infection and pre-term labor (1). Moreover, the biochemical tests, such as surfactant:albumin (SA) ratio, are associated with a high rate of false-positive results in fetuses with intermediate lung maturity (2–4).

The development of a non-invasive and potentially more accurate alternative to amniocentesis for the evaluation of fetal lung maturity would be advantageous. Phosphatidylcholine (lecithin), one of the major components of surfactant, has a characteristic magnetic resonance (MR) spectroscopic peak near 3.2 parts per million (ppm). As such, MR spectroscopy for fetal lung maturity evaluation has the potential to be a more specific test in its theoretical ability to directly measure components of surfactant. One group has presented preliminary in vivo data from three fetuses showing detection of a choline peak in both amniotic fluid pockets and fetal lung using a breath-hold single-voxel spectroscopy technique (5). Preliminary ex vivo MR spectroscopy experiments demonstrating the promise of this technique for the application of evaluating fetal lung maturity have also been presented (6).

Although development of a non-invasive MR test for fetal lung maturity will have clinical benefits, in vivo spectroscopy of the fetus is technically challenging and issues regarding fetal motion and low signal-to-noise ratio have been raised (7,8). Investigators in previous initial studies were aware of these issues but did not carefully address their effects. Therefore, we undertook this study to investigate the feasibility of performing in vivo single-voxel proton spectroscopy at 1.5 T in near-term fetuses, with a specific focus on the issues of

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Table 1
Summary of Patients' Clinical Characteristics and Spectroscopy Exam Results

Patient	GA at scan	Clinical history	Fetal outcome	SNR	Voxel size and imaging time	Spectroscopy result
1	36 wk 1 d	Gastroschisis	Intubated × 7 days secondary to nuchal cord	*	N/A	Voxel could not be placed†
2	35 wk 2d	Placenta previa	Viable male infant, no RDS, refused amniocentesis	15.7	9 cm^3, 9 min (fluid); 11cm^3, 8 min (lung)	Detected lactate in fluid (SNR 6.7), choline in lung (SNR 3.9)
3	37 wk	Diabetes mellitus type 1	Viable male infant, no RDS, amniocentesis at 37 wk, SA 66 mg/g	13.0	8 cm^3, 4 min (fluid); 4.5 cm^3, 12 min (lung)	No choline detected (lactate SNR ~1)
4	37 wk	Diabetes mellitus type 2	Viable male infant, no RDS, amniocentesis at 37 wk 1 d, bloody specimen insufficient for SA analysis	6.7	10 cm^3, 3 min (fluid); 9.5 cm^3, 3 min (lung)	No choline detected; continuous fetal motion
5	37 wk 3 d	Diabetes mellitus type 2	Viable male infant, no RDS, insufficient fluid at amniocentesis for SA analysis	*	22 cm^3, 3 min (lung)	No choline detected (low SNR)
6	35 wk, 1 d	Gastroschisis	Fetal demise before delivery (ischemic bowel)	3.2	N/A	Voxel could not be placed†
7	38 wk 5 d	Suspected airway obstruction	Viable female infant, no RDS, no clinical indication for amniocentesis	20.0	1 min	Exam terminated due to patient discomfort
8	37 2/7	Breech presentation	Viable male infant, amniocentesis 37 wk 2 d, SA 59 mg/g	12.1	25 cm^3, 4 min (fluid); 18 cm^3, 4 min (lung)	No choline detected; continuous fetal motion (lactate SNR ~2)

GA, gestational age; SNR, signal-to-noise ratio; RDS, respiratory distress syndrome; SA, surfactant-to-albumin ratio.
*A different type of localizer sequence was used for patients 1 and 5, and therefore the SNR calculation would not be comparable to the others presented.
†Externalized bowel in fetus with gastrochisis interfered with spectroscopic voxel localization over amniotic fluid.

motion and signal to noise. Although investigating the choline compound would give us more direct results, in this study we used both lactate and choline in determining their effects.

MATERIALS AND METHODS

This prospective study was approved by our institutional review board. Written informed consent was obtained from all patients. Eight patients in the third trimester of pregnancy, who were willing to undergo fetal MR imaging, were referred for the study. Their clinical characteristics are presented in Table 1.

MR Imaging

Patients underwent fetal MR imaging at 1.5 T (GE Healthcare Technologies). No medications were administered. Patients were imaged in the left lateral decubitus position for patient comfort and to avoid compression of the inferior vena cava. A four-channel torso phased-array coil was placed over the expected location of the fetus and adjusted as necessary after scout images. Imaging was performed using single-shot fast spin-echo (SSFSE) sequences in multiple planes (TR/TE = 4500/90, matrix 256 × 160 to 192, FOV 32 to 40 cm, slice thickness 3 to 4 mm for imaging and 10 to 20 mm for spectroscopy localizers).

MR Spectroscopy Technique

Single-voxel spectroscopy using the point-resolved spectroscopy (PRESS) sequence was performed using SSFSE images as localizers for PRESS box selection. For the spectral acquisition from the amniotic fluid and the fluid-filled fetal lung, the spectroscopy routine was divided into sections of 1-minute acquisitions (TR/TE = 1000/144 or 35 msec) with interleaved SSFSE imaging sequences to check for fetal motion and to reposition the spectroscopic voxel if necessary. To reduce the overall scan time, prescanning was not performed because the overall variations in the settings did not change significantly even when there was fetal motion. The TR of the spectroscopic acquisition was chosen at a relatively low value to reduce the overall scan time and patient discomfort. These 1-minute acquisitions were repeated for a total scan time of up to 12 minutes (based on ex vivo signal-to-noise ratio [SNR] analysis experiments) or terminated earlier if any patient discomfort was detected. For patient 2, these 1-minute acquisitions were further divided into four 15-second continuous segments obtained during maternal breath-holding to minimize motion.

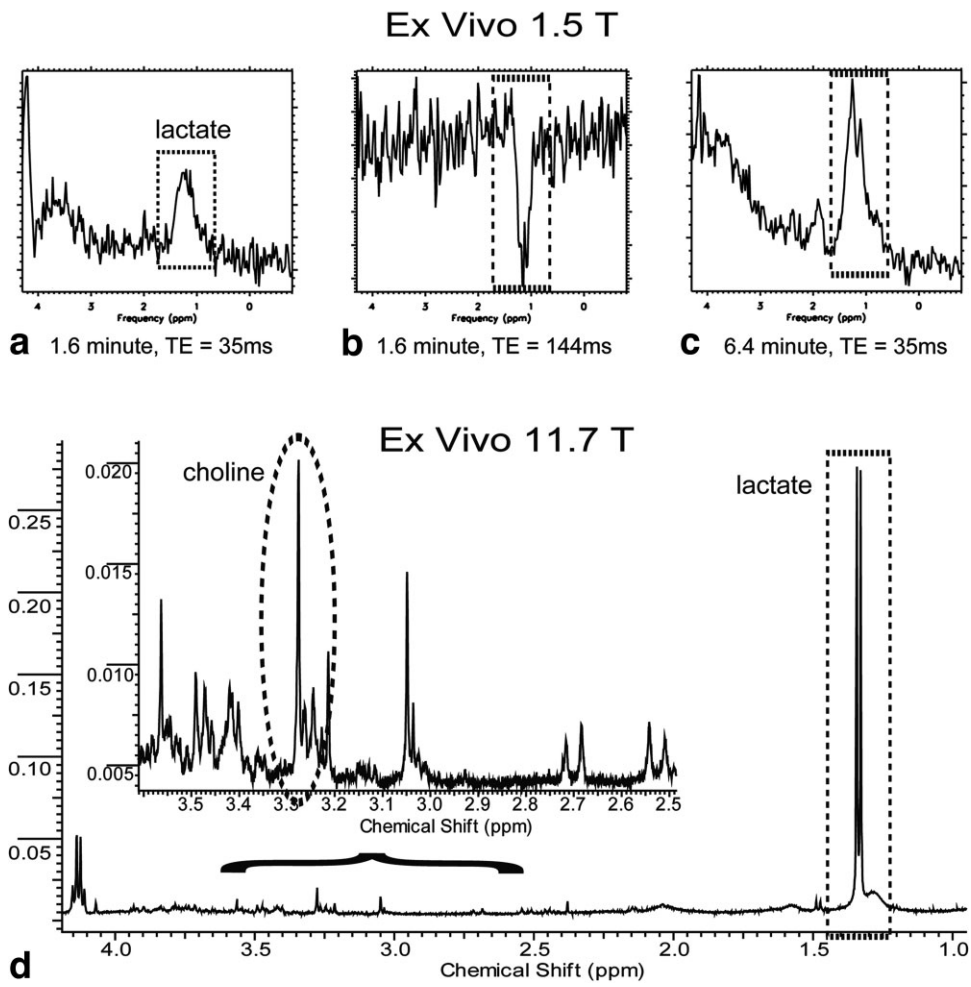

Figure 1. Single-voxel proton spectroscopy was performed on a third trimester amniotic fluid sample ex vivo at 1.5 T **(a–c)** and 11.7 T **(d)**. Although 1.5-T scans demonstrate the ability to identify lactate, other metabolites cannot be seen. The 11.7-T HR-MAS spectra reveal that the 1.3-ppm lactate signal intensity is considerably higher than the choline peak of 3.28 ppm. Using the lactate SNR calculated from **(a)** and **(c)**, the required imaging parameters to detect lactate in vivo can be predicted, as shown in Figure 2.

Spectroscopy Processing—Motion Correction

Spectroscopy data from each TR were stored and processed individually using SAGE/IDL software (GE Healthcare Technologies). Prior to summing the individual spectral acquisitions, the position of the water frequency was aligned to restore any frequency shifts due to motion.

SNR Analysis for Determining Clinical Imaging Parameters

To evaluate the achievable SNR for detecting the lactate peak in amniotic fluid spectra, samples of amniotic fluid in 2 patients with SA testing results indicating lung maturity were tested ex vivo on a 1.5-T clinical scanner using a 3-inch surface coil. The purpose of the ex vivo exam on a clinical scanner was to assess required imaging times vs. voxel size parameters needed to perform in vivo spectroscopy of amniotic fluid to detect lactate. Lactate was initially chosen over choline because it is the more easily detectable metabolite seen in larger quantities based on ex vivo experiments (9,10).

Fluid samples were frozen at −80°C until the ex vivo MR spectroscopy analysis at 1.5 T. Previous degradation studies have revealed minimal changes in metabolites as a result of storage (11). Similar parameters were used for ex vivo spectroscopy acquisition as for the in vivo acquisitions, with the exception of imaging time which was varied. The distance of the sample to the coil was adjusted so that the SNR from the localizer images matched the SNR obtained from the in vivo cases. This approach was used to determine parameters, such as scan time and voxel size, needed to achieve particular SNR values. As a reference to identify the compounds within the collected amniotic fluid, high-resolution magic angle-spinning (HRMAS) spectra were obtained using an 11.7-T spectrometer (INOVA; Varian, Inc.).

RESULTS

SNR Experiments

Results of the ex vivo amniotic fluid experiments are presented in Figure 1. The upper row (Fig. 1a–c) shows ex vivo spectra collected from the 1.5-T scanner using different imaging parameters. Lactate can be identified but no choline-containing compounds could be seen. Subsequent ex vivo 11.7-T spectra from the same sample using HRMAS are also shown in Figure 1d. The HRMAS data clearly show that there were indeed choline compounds but at a much smaller magnitude than lactate.

The achievable SNR for in vivo detection of lactate was predicted using the results of Figure 1 and is shown in

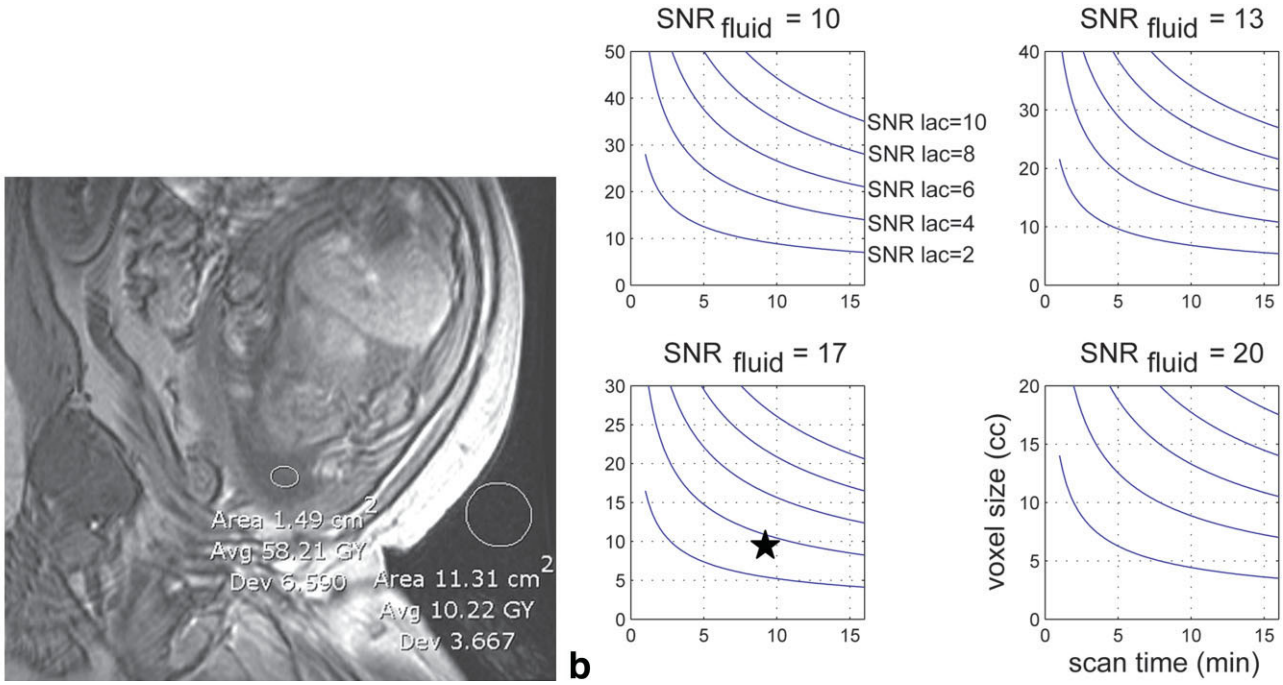

Figure 2. Prediction of scan times and voxel sizes required to detect lactate for different SNR levels. **(a)** Representative gradient echo localizer image from an in vivo exam (TR/TE = 44/1.6 ms, flip angle = 30°, bandwidth = 16 kHz, FOV = 32 cm, 256 × 256, 5-mm thickness). Regions of interest were manually drawn over amniotic fluid and background (white circles) for the SNR calculations. Typical SNR of amniotic fluid in vivo ranged from 5 to 20. **(b)** Plots of voxel size in cubic centimeters (vertical axis) vs. scan time in minutes (horizontal axis) are given for different SNR values of amniotic fluid. The black star indicates the parameters used for patient 2.

Figure 2. Figure 2 shows plots of scan time vs. voxel size to detect lactate at a range of SNR levels. Several plots are given assuming measurements of amniotic fluid SNR to be different for different exams. An example illustrating regions of interest drawn to calculate the SNR from the anatomic image is given (Fig. 2a). Typical SNR of amniotic fluid ranged from 5 to 20 (Table 1).

Motion Correction

Post-processing of the individual spectral acquisitions provided a useful tool to minimize motion effects. Figure 3 shows an example in which bulk motion of the fetus was obvious on SSFSE images acquired just before and just after the spectroscopic exam. Motion during the spectroscopic acquisition resulted in a lipid component entering the PRESS box (Fig. 3b), which obscures data interpretation. Post-processing to remove the lipid-contaminated data was performed.

The use of breath-holding in patient 2 during the 1-minute scan (which was divided into 4 breath-holds, each of 15-second duration) resulted in a slight increase in the signal intensity for fetal lung fluid scans (∽10%) and amniotic fluid scans (∽3% to 5%) compared to scans without breath-holding. Therefore, consider-

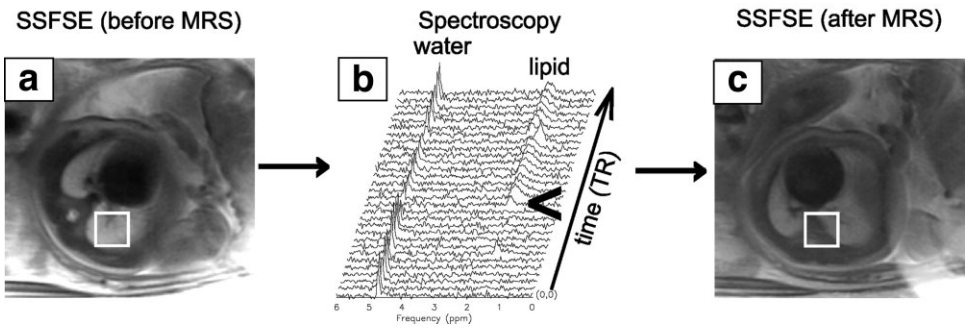

Figure 3. Fetal bulk motion between successive scans and during the scan can be detected and "bad data" can be eliminated from the final analysis. Although the SSFSE acquired before **(a)** and after **(c)** spectroscopy clearly shows bulk movement of the fetal lung, the stack of spectroscopy data **(b)** reveals that the motion occurred roughly midway during the spectroscopy exam where the lipid signal appears (arrowhead). The ability to process each spectroscopy time frame separately allows removal of contaminated data and diminishes effects of motion.

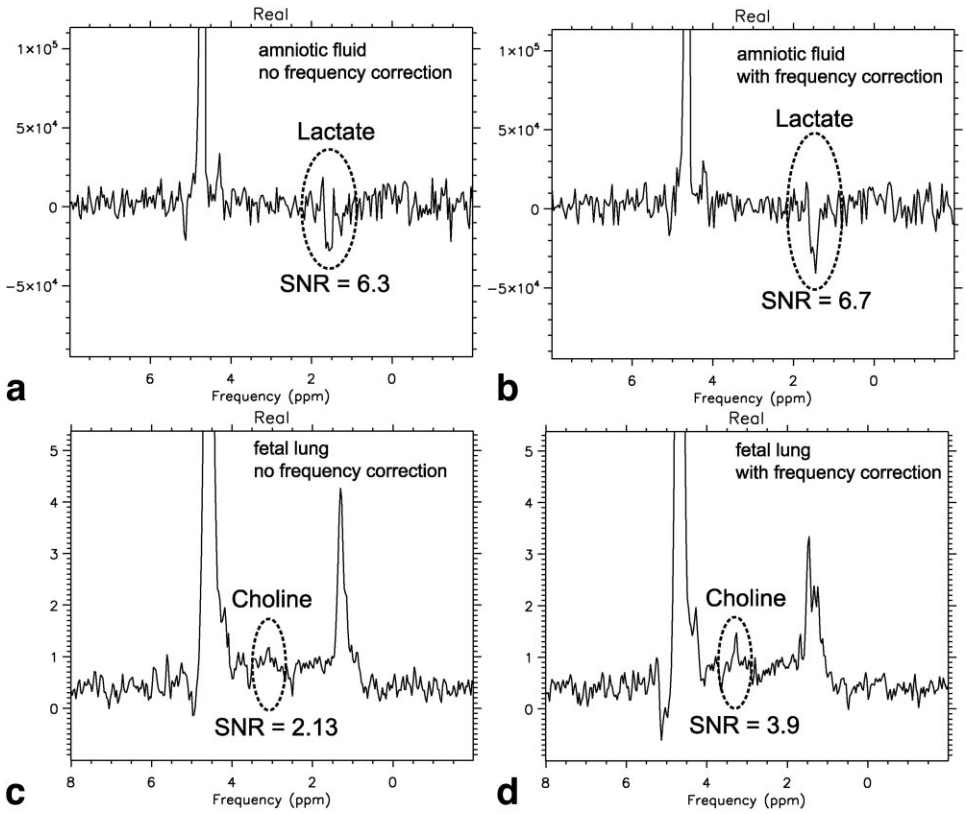

Figure 4. In vivo spectra acquired from amniotic fluid (top row) **(a,b)** and fetal lung fluid (bottom row) **(c,d)** in a fetus at gestational age 35 weeks 2 days (patient 2). For each spectrum acquired, additional post-processing to remove corrupted spectra and realign the water peaks are shown on the right **(b,d)**. This step seems to be valuable for increasing SNR. Lactate SNR increases from 6.3 **(a)** to 6.7 **(b)** in amniotic fluid. Note that lactate is inverted in **(a)** and **(b)** because TE was 144 msec. In the fetal lung, the use of post-processing schemes clearly improves the SNR, and visibility of the choline peak with choline SNR increased from 2.1 **(c)** to 3.9 **(d)**.

ing the effort of the patient, this procedure seemed impractical.

Despite the SNR challenges as illustrated in Figure 2, successful detection of lactate in amniotic fluid, and also presumably choline in fetal lung fluid, is shown in Figure 4 (patient 2). Figure 4 also illustrates how SNR may be increased by applying a frequency-alignment step prior to averaging. Without this step, the detection can be limited as illustrated by the small choline peak obtained from the fetal lung fluid in Figure 4c. The SNRs of lactate and choline were increased after motion correction (Fig. 4c and d). Note that the lipid signal component decreased because several acquisitions were eliminated during the realignment procedure. These lipid components probably originated from the lipids formed in the fetal heart wall.

DISCUSSION

We have presented our experience performing in vivo spectroscopy of fetal amniotic fluid and fetal lung and reported on the major technical challenges encountered and the limitations to detecting choline in vivo at 1.5 T. The issue of motion was managed by dividing the spectroscopy acquisition scheme into smaller time segments and by using post-processing methods to remove lipid-contaminated spectral data related to bulk fetal motion. In the 1 patient in whom choline was detected in the fetal lung fluid, we also employed maternal breath-holding to minimize maternal respiratory motion, although this was more time-consuming and required good communication and cooperation on the part of the mother.

More important than motion is the limitation of available SNR at 1.5 T using existing coil and spectroscopy technology. Previous ex vivo NMR analyses of amniotic fluid estimated choline species levels to be in the micromolar range, which is largely beyond the detectable region for in vivo studies at 1.5 T (9,10,12). Based on our imaging parameter estimates (Fig. 2), the required voxel sizes and scan times for reliably detecting choline in amniotic fluid at 1.5 T are too large to be practical. Previous case reports of in vivo fetal spectroscopy using much shorter scan times (<1 minute) or smaller voxel sizes (<8 cm^3) to detect choline in amniotic fluid will likely be difficult to reproduce.

Our study is limited by the small number of patients, although this is the largest reported series to date on in vivo fetal body spectroscopy. Further technical development is required to address the major limiting factor of low SNR for detecting amniotic fluid metabolites. One approach involves adjusting the placement of the receive coils so that the coil reception profile is greatest where the PRESS box is located, as suggested by Brugger et al (13). This can certainly add SNR but it is time-consuming and can potentially cause patient discomfort. Alternatively, using modern coils with more channels can add to the signal strength. A more fundamental approach would be to image at a higher field strength. No published studies have yet been performed with a fetus at 3 T or higher, mainly due to issues related to SAR (specific absorption rate).

In conclusion, in vivo amniotic fluid spectroscopy at 1.5 T presents some significant technical challenges, particularly with respect to SNR, and results need to be interpreted carefully to avoid pitfalls. Although lactate

may be detected in amniotic fluid at 1.5 T, choline detection is probably impractical. Further technical development will be necessary before these studies can be performed at higher field strengths such as 3 T.

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