

Clinical Technique

How to Perform Transabdominal Ultrasound-Guided Fetal Fluid Sampling in Mares



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ARTICLE INFO

Article history:

Received 1 April 2014

Received in revised form 28 May 2014

Accepted 13 June 2014

Available online 21 June 2014

Keywords:

Allantois

Amnion

Pregnancy

Ultrasound

Equine

Centesis

ABSTRACT

Little is known about the composition and physiology of fetal fluids in all domestic mammals in comparison with humans, where the amniotic fluid has been the focus of numerous reports. Previously, in the horse, there have been concerns regarding the safety of fetal fluid sampling and the risks to the well-being of the fetus and techniques limitations that may preclude serial assessment. The objective of this report was to describe a transabdominal ultrasound-guided technique to safely perform multiple fetal fluid collections during the last trimester of gestation in mares. Similar methodology has been described previously; however, here we described step by step how to perform fetal fluid sampling. Several illustrative images have been included to facilitate the understanding of the technique by others lacking experiences with the procedures. In addition, small modifications on the sedation protocols and sampling have been performed in the present study. Six light horse mares carrying normal singleton pregnancies (260–280 days of gestation) were sampled three times at 5- to 7-day intervals (i.e., 0, 5, and 12 days). There were no apparent complications using the protocol described here. All mares delivered normal foals uneventfully. We foresee that the publication of this report may be useful to other research laboratories interested in studying the fetal fluids in mares, as well as in specific clinical evaluations of fetal well-being in research mares.

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1. Introduction

In domestic mammals, particularly in the horse, the physiology of fetal fluids is poorly understood. Few studies have been conducted to examine the physiopathology and chemical composition of the equine fetal fluids [1–8], in comparison with humans, where the amniotic fluid has been the focus of numerous reports [9,10]. Initial attempts to collect equine fetal fluids were carried out before the wide use of ultrasonography and resulted in numerous

adverse outcomes (i.e., abortion and even peritonitis). Later attempts using ultrasonography to guide the collections reduced the risks of abortion [4,5], but some of the protocols using transabdominal ultrasonography to locate pockets of equine fetal fluids are still associated with abortion [1]. As an alternative method, Lyle et al [6] reported the placement of an indwelling catheter in the allantoic compartment by laparoscopy in an attempt to allow continuous fetal fluid sampling. Unfortunately, several complications were reported (e.g., lack of fixation of the catheter, infection around the catheter and chorioallantois, and abortion) and the catheters remained patent for only 5–9 days because of blockage by cell debris.

Transabdominal ultrasound-guided fetal fluid sampling appears to be the safest method [5]. Recently, our group has performed multiple transabdominal ultrasound-guided

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fetal fluid sampling, as part of ongoing research to examine proteomics of the fetal fluids in normal and placentitis mares [11], although we have made some modifications in a successful previously described protocol [5] to obtain fetal fluid samples during the third trimester of pregnancy. Our uneventful and successful outcomes stimulated us to describe here in great detail a protocol for transabdominal ultrasound-guided fetal fluid sampling, which is currently in use at our laboratory to collect repetitive samples of equine fetal fluids.

2. Material and Methods

The experimental protocol (#2010-0769) was approved by the Institutional Animal Care and Use Committee at the University of Kentucky. This project was carried out from April to August of 2012, at the Maine Chance Farm, University of Kentucky, Lexington, Kentucky. Six light horse mares carrying normal singleton pregnancies (260–280 days of gestation) were sampled three times at 5- to 7-day intervals (i.e., 0, 5, and 12 days). To assure that the procedures did not induce any damage in the placentas, and as part of another study, all placentas were submitted for microbiologic and pathologic examination at the University of Kentucky Veterinary Diagnostic Laboratory. None of the mares received any medication throughout gestation other than sedation and local anesthesia (see the following) for the fetal fluid sampling.

Before each fetal fluid sampling, the mare was given xylazine hydrochloride (0.4 mg/kg IV; Anased; Lloyd Laboratory, Shenandoah, IA) and allowed to rest quietly (approximately 5 minutes) to facilitate handling, clipping, and ultrasonography. Once sedated, the mare was moved slowly to the stock to avoid stress and excitement.

Ethanol (70%) was spread through the mare's ventral abdomen to facilitate the visualization of the pregnant uterus via transabdominal ultrasonography (curvilinear transducer, 2–6 MHz; C362; Sonoscape Sonosite Bethel, WA). If needed, the mare was clipped before ultrasonography. Ideally, a large area should be clipped before ultrasonography. For the centesis, a combination of sedation and analgesia was provided by additional administration of detomidine hydrochloride (0.008 mg/kg IV; Dormosedan; Pfizer Animal Health, Exton, PA) and butorphanol tartrate (0.008 mg/kg IV; Turbogesic; Pfizer Animal Health, Fort Dodge, IA).

A large area of the mare's ventral abdomen was surgically prepared with chlorhexidine scrub and ethanol 70%. To decide where to perform local anesthesia, a pocket of fetal fluid was located by ultrasound. Once the puncture site was located, lidocaine hydrochloride (i.e., 10–15 mL; Lidocaine hydrochloride; VEDCO, Saint Joseph, MO) was injected (23–25 G 2") in the subcutaneous tissues and body wall (Fig. 1). The transducer was placed inside a sterile sleeve containing sterile lube. Two operators were needed, and strict aseptic technique was used to perform fetal fluid sampling.

The operator sampling the mare held the transducer in one hand and the echotip spinal needle (18 G × 6"; 30° short bevel; Chiba-Type spinal needle, coupled with a stylet, Echo Block PTC Havel's Cincinnati, OH) in the other

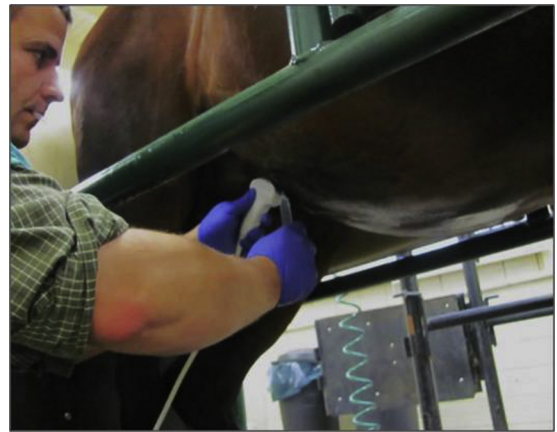


Fig. 1. Local lidocaine block guided by ultrasound. Note that the transducer was held where the operator found a readily accessible pocket of fetal fluid.

hand (Fig. 2A). At this point, the operator had confirmed the location of a pocket of fetal fluid. Thereafter, the needle was inserted through the body wall and uterus into the fetal fluid compartments (Figs. 2A, 3C, and 3D). The amniotic compartment could be located as an echogenic membrane surrounding the fetus; by transabdominal ultrasound, the operator could localize the pocket of amniotic fluid (anechoic appearance) between the fetal parts and the amniotic membrane; whereas, the allantoic fluid is the fluid present between the uterine wall and amniotic membrane, it could be more easily accessed caudally closer to the mare's bladder. Midline or paramedian approaches did not appear to be essential, but rather, how a pocket of fetal fluid was accessible to the operator. After needle placement, the second operator on the opposite side of the mare removed the stylet and connected the extension set with the syringe to the needle (Fig. 2B). Very gentle suction was applied by the operator to obtain fetal fluids (Fig. 2C). Once the amount of fetal fluid desired was collected, the needle was rapidly removed (Fig. 2D). Afterward, the mare was allowed to recover from sedation in a quiet stall or paddock without hay or water. Fig. 3A–D illustrates all the different steps from sedation to sampling.

3. Results

There were no apparent complications using the protocol described here. All mares delivered normal foals uneventfully. Four mares passed their placentas ≤3 hours postpartum. One mare, after retaining her placenta (approximately 6 hours), was treated with IV bolus of oxytocin (100 units, diluted in 1 L of Lactate Ringer solution) delivered her placenta (approximately 45 minutes) after treatment [12]. Another mare retained her placenta for 4 days and was treated with daily high volume uterine flushing, oxytocin, cloprostenol, sulfamethoxazole and trimethoprim, and flunixin meglumine [12]. Postpartum pathologic placental examinations were unremarkable, except one placenta that had advanced autolysis as a consequence of the prolonged retention.

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