SFM Fetal Therapy Practice Guidelines: Fetal Tissue Biopsy

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Abstract

Prenatal diagnostic invasive procedures are used to determine if a fetus is at risk of a genetic disease. Analysis based on cells obtained from amniocentesis or chorionic villous sampling is the more commonly performed prenatal diagnostic invasive procedure. For certain indications, however, fetal skin biopsy, muscle biopsy, kidney biopsy, and fetal tissue biopsy of a tumor or mediastinal mass have also been performed. These procedures are rarely indicated in the current scenario when molecular tests are available for most of the genetic conditions. However, there are some genetic conditions for which no specific molecular test is available and prenatal diagnosis can only be done by biopsy of the fetal part during ultrasound guidance.

Keywords
- fetal skin biopsy
- fetal tissue biopsy
- fetus
- ultrasound
- prenatal

Introduction

Prenatal diagnostic invasive procedures are used to determine if a fetus is at risk of a genetic disease. Analysis based on cells obtained from amniocentesis or chorionic villous sampling is the more commonly performed prenatal diagnostic invasive procedure. For certain indications, however, fetal skin biopsy, muscle biopsy, kidney biopsy, and fetal tissue biopsy of a tumor or mediastinal mass have also been performed. Historically, there are reports of histologic diagnosis of fetal tumors and fetal mediastinal masses, but such procedures were abandoned due to the risk of uncontrollable bleeding leading to severe fetal damage and even fetal death. Therefore, any fetal tissue biopsy should be performed only when the yield is higher than the risk involved. These procedures are rarely indicated in the current scenario when molecular tests are available for most of the genetic conditions. However, there are some genetic conditions for which no specific molecular test is available and prenatal diagnosis can only be done by biopsy of the fetal part during ultrasound guidance.

1. **Fetal skin biopsy:** Quality of life is affected by the long-term morbidity associated with inheritable skin conditions. Chromosomal abnormalities or enzyme defects can be detected by amniocentesis or chorionic villous sampling in a few of such cases; however, in the majority of such cases with inheritable genodermatoses, prenatal diagnosis could be done by histologic and ultrastructural studies of fetal skin biopsy in utero.[1] Also, ultrasound is not always useful for accurate diagnosis. Thus, actual visualization of the skin and histology is the most reliable and certain way to make such diagnoses. It is best performed between 15 and 22 weeks. The underlying basis is the age-related appearance of structures and expression of antigens, both of which can serve as markers for prenatal diagnosis. In order to complement the ultrastructural analysis, a number of monoclonal and polyclonal antibodies with immunohistochemical tests have been introduced to certain basement membrane components. Knowledge of an affected fetus can allow psychosocial and financial preparation and the option of termination of pregnancy if treatment is unavailable. In today's era, fetal

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skin biopsy has been superseded by DNA-based diagnostic tests using fetal DNA from amniotic fluid or chorionic villi samples. However, an important prerequisite for DNA-based tests is a prior delineation of genetic markers. This may be available for only a few of the diseases of concern where mutations have been identified. With the advancement of DNA-based diagnostic tests, the landscape of prenatal diagnosis has changed. In the modern era, fetal skin biopsy remains relevant in cases where the causative gene or specific mutation has not been elucidated or other genetic testing methods are not available.[2]

2. **Fetal liver biopsy:** The liver is important for many metabolic functions in the body. Most of these enzymatic reactions can be documented in chorionic villi and amniotic fluid; however, enzyme activity is limited to the liver for certain disorders and there can be liver-specific enzyme deficiencies, which are not expressed in amniotic fluid. Thus, prenatal diagnosis of inborn errors of metabolism and glycogen storage diseases that show defects in enzyme activity confined to the liver will require such a biopsy. It is best performed between 15 and 20 weeks period of gestation under ultrasound guidance.
3. **Fetal muscle biopsy**: It is best performed under ultrasound guidance at about 18 weeks period of gestation to analyze the muscle fibers histochemically for prenatal diagnosis. It was commonly used in the past for Duchenne muscular dystrophy and can still be used in some cases when no deletion is identified. It can also be used to test for mitochondrial and other myopathies.

Indications

Skin biopsy: The common indications are blistering diseases, disorders of keratinization, pigment cell diseases, and disorders of epidermally derived skin appendages[3] (►**Table 1**). Occasionally, it may be indicated to confirm true fetal mosaicism.

Liver biopsy: It is mostly carried out to diagnose inborn errors of metabolism. Common indications include:

a. Ornithine transcarbamylase deficiency
b. Von Gierke's disease
c. Carbamoyl phosphate synthetase deficiency
d. Primary hyperoxaluria type

Muscle biopsy:

a. Duchene muscular dystrophy
b. Mitochondrial myopathy

Contraindications

1. Preterm prelabor rupture of membranes
2. Suspected abruption
3. Chorioamniotic separation
4. Chromosomal or congenital abnormalities in the twin

Maternal Risks

1. Maternal bleeding
2. Injury to the bladder or bowel
3. Infection

Fetal Risks

1. Fetal loss: 3 to 5% (with fetoscopy), 1 to 2% (with ultrasound-guided procedure)
2. Preterm birth in 10% of cases
3. Rupture of membranes and amniotic fluid leakage
4. Infection ($<$ 1: 1000)
5. Fetal scarring (rare)
6. Fetal bleeding in cases of liver or muscle biopsy

Other Risks

Tissue specimens may be accidentally removed from fetal membranes, myometrium or even trophoblast leading to negative report

Information Leaflet for the Patients

What are the indications for fetal tissue biopsy? There are certain genetic conditions for which no specific molecular test is available. In such cases, a prenatal diagnosis can be made only by biopsy of the fetal tissue. Also, ultrasound visualization is not very useful and informative in a majority of serious cutaneous or other organ abnormalities. Actual

Table 1 Common indications for fetal skin biopsy

Keratinization disorders	Blistering diseases	Pigments disorders	Epidermal appendages disorders
Ichthyosiform erythroderma: bullous/nonbullous	Epidermolysis bullosa (EB)	Tyrosinase-negative oculocutaneous albinism	X-linked hypohidrotic ectodermal dysplasia
Harlequin ichthyosis	Junctional EB	Congenital nevus	Autosomal-recessive anhidrotic ectodermal dysplasia
Sjögren-Larsson syndrome	Recessive dystrophic EB	Incontinentia pigmenti	
	Dominant dystrophic EB		
	EB simplex (general)		
	EB simplex Dowling-Meara type		

visualization of fetal tissue such as the skin and its histology such as of the fetal liver, muscle, or the skin is the only way to make a certain diagnosis.

How is fetal tissue biopsy performed? It is performed under local anesthesia. A small amount of light maternal intravenous sedation may be used in some cases. It was performed previously using fetoscopy, but with recent advances in the technique of ultrasound, it is currently performed under ultrasound guidance.

When are fetal tissue biopsy procedures performed? The biopsy is typically performed between 15 and 20 weeks period of gestation.

Is there any maternal or fetal risk associated with the tissue biopsy procedures? The procedure is safe for the mother and the fetus. Fetal loss can be seen in as many as 3 to 5% of cases. Other rare complications include bleeding, rupture of membranes, and infection. The biopsy sites heal with no scar formation.

General Counseling Points

Preprocedure care and counseling are vital to explain the disease pathology, and need for the test, set expectations from the procedure, and counsel about possible outcomes in order to allay the anxiety of a couple. They should be explained about the need for the procedure, and all the steps of the procedure in detail including pre and postprocedure care. It is also important to discuss the possible results that can be obtained and the pitfalls of the procedure. Informed consent for the procedure should be taken after the counseling.

1. Explain about the need and rationale for doing the procedure. Obtain family history. Obtain clinical reports of affected siblings or other family members to be certain of the original diagnosis and to determine how the disease is expressed in affected members of the family.
2. Always explain all the available options for prenatal diagnosis.
3. Describe the procedure in brief and explain what to expect during the procedure.
4. Describe the maternal and fetal risks associated with the procedure.
5. Discuss the success and failure of the procedure and postoperative complication.
6. Describe the follow-up of pregnancy after the procedure.
7. Discuss the neonatal outcome.

Counseling Statement for Medical Records

1. Biopsy sites can be difficult to find and when located they are small and have little or no cosmetic sequence. They tend to heal without scars.
2. Due to the advent of advances leading to DNA-based testing, currently, there are other options of molecular testing for various genetic conditions for which the causative gene is known and the pathogenic mutations and/or informative markers are defined.
3. Sampling error, inadequacy of samples for analysis, and difficulty in interpreting the morphologic and immunohistochemical features can pose problems and may necessitate repeat sampling.
4. The procedure should ideally be performed after the 16^{th} week of gestation.
5. An inadequate number of samples from only one or two regions of the fetal body jeopardizes the chances for an accurate diagnosis, especially in cases where the onset of the disease and expression of the disease may be variable.

Consent

I, ……., & my husband/ family member ……(name and relation) have understood that prenatal diagnosis can only be carried out by sampling of the fetal tissue as no molecular tests are yet available for this condition. The expected progression of the possible problem, its likely consequences and complications thereof as well as the various management options that are available to us at this gestational age were explained. All possible complications to the mother and fetus were also explained in detail and include preterm delivery, premature membrane rupture, bleeding, infection, miscarriage, and even fetal demise. We understand that the procedure will be performed under local/regional anesthesia with or without maternal sedation. Maternal risks and risks to the mother's life are, therefore, minimal but not nil. We accept the risks involved after fully understanding them and agree to go ahead with fetal tissue biopsy on our free will.

Patient's signature Husband/Relative's signature
Date/ time Date/ time

Preoperative Checklist and Preparation of Patient

1. Detailed ultrasound checklist:
   a. Fetal cardiac activity
   b. Mapping of placenta
   c. Identify the site for entry of instruments
2. Case record checklist:
   a. Comprehensive case review and detailed medical, past and family history
   b. Consent
   c. Relevant blood and urine investigations
3. Preanesthetic check-up and relevant investigations checklist: blood testing for complete blood count, type, liver function test, renal function test, coagulation profile, electrolytes
4. Preoperative medication checklist:
   a. Light breakfast can be taken. Nil per oral for 8 hours if light maternal intravenous sedation is planned.
   b. One ringer lactate on the morning of surgery at the rate of 100 mL/hour (slow IV fluid) if nil per orally.

c. Antibiotic prophylaxis—1 g ceftriaxone or 2 g cefazolin 30 minutes before needle insertion.
d. Intramuscular progesterone (17 alpha hydroxyprogesterone caproate) on the day of surgery.
e. Tablet nifedipine 10 mg stat 30 minutes before procedure for tocolysis.

Operating Room Requirements

1. Ultrasound machine.
2. Sterile covers for camera, ultrasound probe
3. Standard universal sets for operative site disinfection and sterile draping
4. 11-blade scalpel for initial incision
5. 14 gauge flexible catheter (Angiocath)
6. Surgical dressing
7. Biopsy forceps (cupped, 20 cm, 20 G)
8. Fixative
9. Tru-cut needle or biopsy gun
10. Coring biopsy gun

Personnel Required

1. Operator: Trained in ultrasound and endoscopic-guided procedures
2. Assistant: Trained in handling the ultrasound probe
3. Nurse: To set tray and provide things
4. Sonographic assistant: To handle ultrasound machine
5. Anesthetist: For sedation/regional anesthesia if required

Procedure Steps

Fetal Skin Biopsy

In the past, the techniques involved fetal skin biopsy sampling through direct visualization of the fetus using a fetoscope. The technical aspects of fetal skin sampling have improved as ultrasound guidance has replaced the use of fetoscopy and sampling instruments have become smaller. The site and number of samples are important as some disorders are expressed in utero with regional variability.[4]

Site: Common sites include the back, thighs, or scalp. It is important to note that the buttock or leg is a good site in fetuses at risk of having epidermolysis bullosa, and the scalp or eyebrow is a good site for the detection of albinism.

Size: A sample should be a minimum of 1.5 mm in length and 0.5 to 1 mm in diameter. It is often mushroom-shaped as the piece of the skin is taken by a forceps that is similar to a pair of spoons.

Number of samples: In all cases, it is desirable to have a minimum of three to four samples, preferably from different sites.

Steps: [5]

It can be fetoscopy-guided or ultrasound-guided.

A. Fetoscopy-guided fetal skin biopsy: (historical interest)
a. Prepare the skin as for any invasive fetal procedure.
b. Administer local anesthesia: Inject lidocaine (1%) subcutaneously into the maternal skin for anesthesia.
c. Choose the site of entry of the fetoscope (1.7 mm in diameter) to allow easy access to biopsy sites. The new fiberoptic scopes have lowered the risks of miscarriage.
d. Nick the skin using an a no. 11 scalpel blade.
e. Then under ultrasound guidance, insert the trocar of the fetoscope through the maternal abdomen, through the anterior wall of the uterus and into the amniotic sac.
f. Introduce the fetoscope via a cannula inserted through the abdominal wall into the amniotic cavity.
g. Obtain a clear view of the fetus.
h. Place the fetoscope on the fetal chest, then withdraw it, with the cannula held firmly against the fetal surface.
i. Pass a cupped biopsy forceps (20 cm, 20 G) down the cannula. Alternatively, this can be performed using biopsy forceps inserted through a side channel of the fetoscope.
j. Take the sample under direct visualization with the forceps. (Note: The two blades come together to scoop out the sample, pinching it in the dermis or subcutaneous tissue as it is severed from the underlying tissue.)

B. Ultrasound directed fetal biopsy[5,6]:
a. Prepare the skin for any invasive fetal procedure.
b. Administer local anesthesia: Inject Lidocaine (1%) subcutaneously into the maternal skin for anesthesia.
c. Choose the site of entry into the maternal abdomen for easy access to the fetal biopsy sites
d. Make a stab wound in the skin at the entry site using a no. 11 scalpel blade.
e. Under continuous ultrasound guidance, insert a 14-gauge catheter (Angiocath) past the skin, subcutaneous tissue, uterine smooth muscle and into the amniotic cavity. This catheter is small in caliber and flexible, in contrast to the larger diameter, rigid cannula used previously and, thus appears to guard against the rupture of fetal membranes and laceration of the myometrium.
f. Withdraw the stylet and send an amniotic fluid sample if needed to be sent for any analysis.
g. Introduce a biopsy forceps through the catheter into the amniotic cavity and against the fetal skin.
h. Take adequate samples as per the requirement of the condition being investigated.

Processing of the Specimen

It is very important to achieve tissue fixation and correct orientation of the blocks for subsequent microscopic examination. The biopsy specimens are flushed from the forceps using sterile phosphate buffered saline, pH 7.4, and immediately immersed in primary fixative. The obtained skin specimen is stored in formaldehyde for light microscopy and in glutaraldehyde for electron microscopy.

Liver Biopsy

The technique is the same as skin biopsy and can be performed both under fetoscopic or ultrasound guidance. The

difference is that a Tru-cut biopsy needle or core biopsy instrument is used, which is inserted into the right fetal hypochondrium just below the costal margin. The site is identified as being halfway between the umbilicus and the right nipple.[5] If a needle is used, a syringe is attached to create suction, and the needle is then removed, taking a careful specimen with it. A coring biopsy gun can also be used.

Muscle Biopsy

The technique is the same as a skin biopsy. Under ultrasound guidance, the tip of the Tru-cut biopsy gun is advanced into the fetal buttock or fetal thigh in a down-and-out direction. The coring guide is extended and the trigger is then pulled to cut the biopsy specimen.

Postoperative Checklist and Monitoring of Mother and Fetus

1. Document fetal heart activity and also show it to the patient at the end of the procedure.
2. The patient is observed in the operation theater recovery area for 2 hours after which she can be shifted to the ward.
3. If the woman is Rh negative (nonisoimmunized), anti-D 300 ug to be given.
4. Ultrasound scan after 24 hours is done to assess possible complications such as fetal demise
5. If no complaints from the patient, she is allowed to go home 24 hours postprocedure, with the following advice:
   a. Avoid overstraining/lifting heavy weights for a week
   b. Report to the hospital immediately if there is substantial leaking, bleeding, pain, generalized feeling of unwellness, or fever
6. A detailed procedure report and follow-up plan are generated and one copy is handed over to the patient at the time of discharge. The patient is followed on an outpatient department basis for fetal growth and well-being.

Invasive Report Template

Patient name
Age
Hospital ID
Contact number
Obstetric history: G P A L; Type of conception:;
Consanguineous:
Family history (including pedigree analysis)
Gestational age at diagnosis
Indication
Procedure name
Maternal anesthesia
Uterine entry: Midline, right/left, upper/lower quadrant
Number of attempts: Single/double/multiple
Number of samples obtained
Intraoperative complications
Postprocedure cardiac activity (immediately)
Postoperative advice

Conflict of Interest
None declared.

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