Jennifer M. Hoskovec, MS, CGC
Director, Prenatal Genetic Counseling Services
Department of Obstetrics, Gynecology and Reproductive Sciences

Prenatal Screening for Major Fetal Malformation in Multifetal Pregnancies
Disclosure Statement

- I do not have relevant financial relationships with commercial interests related to the content of this presentation.

- I am a paid member of an advisory committee on non-invasive prenatal testing for the National Coalition for Health Professional Education in Genetics (NCHPEG)
Learning Objectives

1. Describe the current available screening and testing options for aneuploidy in multifetal pregnancies, including the benefits, risk and limitations of each.

2. Interpret the current data on new screening tests such as NIPT for multifetal pregnancies.

3. Determine a desired algorithm for aneuploidy screening in multifetal pregnancies based on available options, published data and professional society recommendations.
Structural Anomalies

- All pregnancies have a background risk of ~3%
- Incidence of fetal anomalies has been reported to be higher in multifetal pregnancies than in singletons
  - specifically CNS and cardiac anomalies
- Monozygotic twins –
  - Rate of structural anomalies is ~3 times higher than in DZ twins
  - Rate of structural anomalies is ~5 times higher than in singletons
- Dizygotic twins –
  - Rate of anomalies not increased per twin; however each baby is at individual risk resulting in ~ double the background risk for the pregnancy
Chromosome Abnormalities

- Maternal Age Effect
- Risk per fetus is not increased in MZ or DZ twins
- Monozygotic twins –
  - Identical genetic information = both fetuses affected
  - Risk for chromosome abnormality is the same as a singleton pregnancy (based on maternal age)
- Dizygotic twins –
  - Share 50% of genetic information
  - Risk for chromosome abnormality per fetus is the same as a singleton
  - Double risk for the pregnancy to account for each fetus’ risk
Incidence of chromosomal abnormalities in at least 1 fetus in a multifetal gestation

<table>
<thead>
<tr>
<th>Maternal Age (Years)</th>
<th>Singleton and MZ twins</th>
<th>DZ twins</th>
<th>Triplets</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>1/526 (0.19%)</td>
<td>1/263 (0.38%)</td>
<td>1/175 (0.57%)</td>
</tr>
<tr>
<td>25</td>
<td>1/476 (0.21%)</td>
<td>1/238 (0.42%)</td>
<td>1/150 (0.67%)</td>
</tr>
<tr>
<td>30</td>
<td>1/385 (0.26%)</td>
<td>1/192 (0.52%)</td>
<td>1/128 (0.78%)</td>
</tr>
<tr>
<td>35</td>
<td>1/192 (0.52%)</td>
<td>1/96 (1.04%)</td>
<td>1/64 (1.56%)</td>
</tr>
<tr>
<td>40</td>
<td>1/66 (1.52%)</td>
<td>1/33 (3.03%)</td>
<td>1/22 (4.55%)</td>
</tr>
</tbody>
</table>

ACOG Recommends...

- Maternal serum screening and invasive diagnostic testing for aneuploidy should be discussed and made available to all women regardless of age

ACOG Practice Bulletin No 77, 2007
ACOG Practice Bulletin No 88, 2007
Standard Screening and Testing Options for Fetal Aneuploidy

- **Screening Options:**
  - First Trimester Screening
  - Quadruple Marker Screen
  - Integrated, Sequential, or Contingency Screens
  - Anatomy Scan

- **Benefit(s):**
  - Non-invasive = no risk
  - Identifies women from low risk pool who are at increased risk

- **Disadvantage(s):**
  - Risk calculation only
  - False positive/negative
  - Limited to trisomy 18, 13, 21
  - Timing, insurance coverage
  - Patient anxiety

- **Testing Options:**
  - Chorionic Villus Sampling (CVS)
  - Amniocentesis

- **Benefit(s):**
  - Diagnostic information on all aneuploidies
  - Additional testing available such as microarray, PCR

- **Disadvantage(s):**
  - Invasive, risk of pregnancy loss (1/300-1/500)
Nuchal Translucency (NT)

- Amount of fluid that has accumulated between the skin and tissue overlying the cervical spine
- Measured between 11-14 weeks
- Operator dependent
- Allows for earlier screening options
- Allows for independent measurement for each fetus
NT cont.

- NT is increased in DS, T18 and T13*
- 30% detection used alone (60%- 70% if maternal age is also used)
- NT is also increased in
  - Turner syndrome
  - Triploidy
  - Congenital heart defects
  - Skeletal dysplasias
- The measurement considered “increased” depends on the exact gestational age, so CRL measurement is very important
  - NT measurement of 3.0 mm is > than that of 95% of fetuses
    - At an increased risk for aneuploidy
  - NT ≥3.5 offer targeted ultrasound and fetal echo
    - At an increased risk for aneuploidy
    - At an increased risk for cardiac defect
Combined First Trimester Screen

- Performed between 11-14 weeks
- Includes:
  - Maternal age
  - NT measurement
  - Maternal serum analytes
- Screens for:
  - Down syndrome: 78-91% DR
  - Trisomy 18: 91-96% DR
  - Trisomy 13*: ~80% DR
- Does not screen for ONTD
First Trimester Screening in Multifetal Pregnancies

- Benefit: NT measured independently for each twin
- NT + maternal age
  - Detection rate for DS = 60-70%
  - 5% FPR
- Combined FTS (NT + maternal age + serum analytes)
  - Detection rate for DS = 60-80% range (lab dependent)
  - Addition of analytes may lower FPR
- Limitations:
  - Insufficient data regarding detection rate for Trisomy 18/13
  - Cannot determine contribution of each individual fetus to the biochemical marker values
  - Discordant NT measurements in MZ twins
Second Trimester Maternal Serum Quad Screen

- Performed between 15-22 weeks
- Takes into account maternal age
- Measures the level of markers in maternal serum:
  - Alpha-fetoprotein (AFP)
  - Unconjugated estriol (uE3)
  - Human chorionic gonadotropin (hCG)
  - Inhibin A (DIA)
- Modifies a woman’s risk for:
  - Open neural tube defects
  - Down syndrome
  - Trisomy 18
- Results are in the form of risk figures
Second Trimester Screening in Multifetal Pregnancies

- Labs do have adjusted MoMs, but still have reduced detection
  - Cannot determine contribution of each individual fetus to the biochemical marker values
  - Chorionicity affects analytes – not accounted for by labs

- Quad- many labs give a pseudodrisk
  - Divide the amount measured in half
  - ~50% detection of DS; ~58% detection of ONTD
  - Will not give T18 risk
Diagnostic Testing in Multifetal Pregnancies: Chorionic Villus Sampling (CVS)

- Performed between 10-14 weeks
- Transabdominal or Transcervical
- More complicated procedure in multifetal pregnancies
- Documentation of chorionicity is essential
- Fetal loss rate in singletons 0.5-1%
- Fetal loss rate in twins 0.5-2%
  - Operator dependent
  - With experienced operator, CVS in multifetal pregnancy is not associated with an increased risk for fetal loss compared to use in a singleton pregnancy
Diagnostic Testing in Multifetal Pregnancies: Amniocentesis

- Performed ≥16 weeks
- Fetal loss rate in singletons 1/300-1/500 (ACOG)
- Fetal loss rate in twins 0.3-1%
  - Operator dependent
- Documentation of chorionicity is essential
  - MZ twins – identical genetic information
    - Monoamniotic – tap 1 sac
    - Diamniotic – may tap 1 or both sacs (discordant karyotype possible but rare)
  - DZ twins – tap both sacs
Non-Invasive Prenatal Testing
NIPT

- Available clinically since November 2011 in the United States
- Analyzes cell-free fetal DNA circulating in maternal blood: (cffDNA)
  - Placental and fetal-derived cells
  - Possibly through the breakdown of fetal cells in circulation
- ~10-15% of cell-free DNA circulating in maternal blood is from the fetus
- Quantitative differences in chromosome fragments can identify fetuses with Down syndrome, trisomy 18, trisomy 13, and sex chromosome aneuploidy
NIPT WITH MPSS

Each diagrammatic fragment represents many thousands of sequenced fragments from chromosome 21.

The quantitative over-abundance of Trisomy 21 fragments in an affected pregnancy is significant and can be measured with high precision.

Unaffected Fetus

Fetus with Trisomy 21

Extra Chromosome Fragments = Affected

Slide adapted from Sequenom
NIPT in Multifetal Pregnancies

- 25 twin pregnancies
  - 17 normal pairs
  - 5 with Down syndrome in one fetus of twin pair
  - 2 with Down syndrome in both fetuses of twin pair
  - 1 with trisomy 13 in one fetus of twin pair
- All pregnancies were correctly classified
  - 25/25 confidence interval [59-100]
- Two triplet pregnancies studied
  - Unaffected; correctly classified

*DNA sequencing of maternal plasma to identify Down syndrome and other trisomies in multiple gestations*†

Jacob A. Canick1, Edward M. Kloza1, Geralyn M. Lambert-Messerlian1, James E. Haddow1, Mathias Ehrich2, Dirk van den Boom2, Allan T. Bombard2,3,4, Cosmin Deciu3 and Glenn E. Palomaki1
Unique Issues with NIPT in Multifetal Pregnancies

- Potential benefit: Authors note twin pregnancies have higher placental mass and therefore might have higher fetal fraction and thus better separation of affected and unaffected fetuses despite the presence of multiples.

- Potential drawback: Effective fetal fraction in twins discordant for Down syndrome is potentially lower than that of euploid singleton pregnancies due to the ratio of trisomic to disomic material, which could be problematic when fetal fraction is approaching the lower limit of acceptability for testing.
Limitations of NIPT in Multifetal Pregnancies

- For DZ twins; CVS or amniocentesis is required to determine which twin is affected
- Limited available data
  - Small sample size
  - No data on other chromosome abnormalities
Professional Society Recommendations

- Maternal serum screening and invasive diagnostic testing for aneuploidy should be discussed and made available to all women regardless of age
  - ACOG Practice Bulletins No 77 and No 88, 2007

- Cell free fetal DNA testing should not be offered to low-risk women or women with multiple gestations because it has not been sufficiently evaluated in these groups.
  - ACOG/SMFM Committee Opinion No 545, 2012
Summary

- Multifetal pregnancies are at increased risk (7-10%) for fetal anomalies compared to singleton pregnancies.
- All women should be offered screening and testing for aneuploidy regardless of maternal age.
- Serum screening in twin gestations is generally associated with detection rates at least 10-15% less than in singletons and NT measurements can provide fetus specific information.
- Diagnostic testing can provide accurate information for couples who desire and are comfortable with the procedure related risks which are generally <1-2%.
- NIPT is a promising technology; however, data are limited regarding use in multifetal pregnancies. Until further data is available, standard screening and testing should be utilized.
The Importance of Counseling

- The choice of screening or diagnostic testing in multifetal pregnancies should be a patient centered decision with guidance from her healthcare team (OB, MFM, GC, RN).
- How patients process this information involves many factors:
  - Spontaneous pregnancy vs. IVF
  - The couple’s desire to know if one of the fetuses is affected with a chromosome abnormality.
  - The couple’s willingness to put the pregnancy at risk.
ANEUPLOIDY SCREENING IN MULTIFETAL PREGNANCY - TWINS

1\textsuperscript{st} trimester  2\textsuperscript{nd} trimester

- FTS
  - Positive
  - Negative

- QUAD SCREEN
  - Negative
  - Positive

Genetic Counseling

Anatomy Scan 20 weeks

Genetic Counseling

Genetic Counseling
ANEUPLOIDY SCREENING IN MULTIFETAL PREGNANCY – HIGHER ORDER MULTIPLES

1st trimester                               2nd trimester

NT
<3.0mm                  ≥3.0mm

Anatomy Scan
20 weeks

Genetic Counseling
THANK YOU!