PRENATAL COUNSELING

New approaches to the genetic evaluation, risk assessment, and classification of **stillbirth** contribute to understanding and management of this troubling phenomenon

›› **Louise Wilkins-Haug, MD, PhD**
Dr. Wilkins-Haug is Director of the Division of Maternal–Fetal Medicine and Reproductive Genetics at Brigham and Women's Hospital, and Associate Professor of Obstetrics, Gynecology, and Reproductive Biology at Harvard Medical School in Boston, Mass.

The author reports no financial relationships relevant to this article.

Three important areas of research into stillbirth have evolved over the past year, furthering our understanding of the phenomenon and our ability to provide comprehensive, evidence-based care:

- **Genetic studies.** Karyotype analysis is useful in determining the cause of stillbirth, especially when analysis is based on a sample of amniotic fluid that was obtained before delivery. And array-based comparative genomic hybridization, which yields information on the chromosome count as well as microduplications and deletions, can be performed on nondividing cells.
- **Risk factors.** Further investigation implicates advanced maternal age, obesity, and African-American race.
- **Classification.** Paring down the more than three dozen systems that exist for classification of stillbirth was the main challenge addressed by an international consensus group in 2009 and the focus of a separate analysis.

The individual studies that contribute to our knowledge base in these areas are discussed in more detail in the articles that follow.

Stillbirth is broadly defined as fetal demise after 20 weeks' gestation and with a fetal weight exceeding 350 g. In the United States, stillbirth occurs in 1 of every 160 live births (6 stillbirths for every 1,000 live births). Although the rate of neonatal demise has decreased over the past decade, the rate of stillbirth has declined less strikingly.

For an analysis of karyotype, amniotic fluid is best

Korteweg FJ, Bouman K, Erwich JJ, et al. Cytogenetic analysis after evaluation of 750 fetal deaths: proposal for diagnosis workup. Obstet Gynecol. 2008;111:865–874.

ACOG Practice Bulletin #102: Management of stillbirth. Obstet Gynecol. 2009;113:748–760.

When stillbirth occurs, determination of the cause of death fulfills several goals:

- It informs counseling of the parents, who must come to terms with the loss
- It aids in determining the risk of recurrence, which informs family planning

TABLE Genetic components of stillbirth assessment

Type of assessment	Steps
Inspection of fetus and placenta	Measure head circumference and length of fetus
	Weigh fetus and placenta
	Photograph fetus and placenta, including frontal and profile shots of whole body, face, extremities, palms, and any abnormality
	Document findings
Cytologic analysis	Obtain consent from parents
	Obtain acceptable specimens using one of the following sterile techniques:
	• Amniocentesis at the time of prenatal diagnosis of demise
	• Placental block (1 x 1 cm) taken from below the cord-insertion site on the unfixed placenta
	• Umbilical cord segment (1.5 cm)
	• Internal fetal tissue specimen, e.g., costochondral junction or patella (not skin)
	Preserve specimens in a sterile culture medium of lactated Ringer's solution at room temperature during transfer to laboratory
Fetopsy	Obtain parental consent; if no consent is given, send placenta for pathologic analysis
	Perform autopsy and pathologic assessment of the placenta
	Consider whole-body fetal radiographs

Source: ACOG Practice Bulletin #102

- It furthers research into stillbirth and facilitates the comparison of national and international data.

Chromosomal anomaly is one potential cause of stillbirth. Its frequency depends on the presence of structural malformation. For example, Korteweg and colleagues found a rate of chromosomal anomaly of 4.6% among stillbirths involving fetuses without structural abnormality, but the rate rose to 38% when anatomic malformation was present. The distribution of chromosomes among stillbirths mirrored the pattern seen in live births, including 45,X and trisomies of chromosome 21, 13, and 18.

The utility of karyotype assessment when ultrasonography (US) has not identified structural malformation has been debated. Given the 5% incidence of chromosomal anomaly in the absence of structural abnormality, and the limitations of US in detecting subtle dysmorphology, a karyotype seems advisable to assess all stillbirths.

Comparison of methods points to superiority of amniocentesis

Because fewer than 20% of skin biopsies result in a useful culture, postmortem skin biopsy for karyotype assessment is unreliable. Korteweg and colleagues evaluated other methods of obtaining cells for examination and found that a successful karyotype is most likely with predelivery amniocentesis (85%), followed by umbilical cord culture (32.1%). A karyotype of cells from fascia lata and skin biopsy yielded poor results, especially in the setting of maceration. Placental biopsy is likely to provide an adequate karyotype (71% probability) but findings may be confounded by confined placental mosaicism.[1]

ACOG also advocates predelivery amniocentesis

In its 2009 practice bulletin, ACOG supported inclusion of amniocentesis in the assessment of stillbirth and preparation for delivery. Once an epidural is placed, amniocentesis provides cells for karyotype assessment, polymerase chain reaction (PCR) for viral studies, and any other metabolic or specific genetic studies that may be indicated by fetopsy.

If amniocentesis is not performed, ACOG recommends umbilical cord culture as an alternative. Because nondividing cells can be utilized in fluorescence in situ hybridization (FISH) for chromosome 13, 18, 21, X, and Y, this method should be considered in any case involving culture failure (**TABLE**).[2]

WHAT THIS EVIDENCE MEANS FOR PRACTICE

Perform predelivery amniocentesis whenever possible at the time of diagnosis of demise to obtain a cell sample for karyotype analysis to determine the cause of death.

Array-based comparative genomic hybridization makes assessment of nondividing cells possible

Raca G, Artzer A, Thorson L, et al. Array-based comparative hybridization (aCGH) in the genetic evaluation of stillbirth. Am J Med Genet A. 2009;149A:2437–2443.

Array-based comparative genomic hybridization (aCGH) makes it possible to assess the chromosome count and perform a high-resolution search for microduplications and deletions. With known segments of the genome printed on slides, the clinical scientist can analyze DNA from nondividing cells from a stillbirth. The ability to use nondividing cells is important because no cell culture is required. (Cell culture is often difficult to obtain after stillbirth.) Depending on the array selected, the resolution can be as fine as a single nucleotide polymorphism.

aCGH can inform preconception counseling

Raca and colleagues used a range of arrays to assess 15 stillbirths that involved two or more malformations. Chromosomal abnormalities, including trisomy 21 and an unbalanced translocation, were detected by aCGH in two infants. Identification of these abnormalities helped inform counseling of the parents:

- In the case of trisomy 21, parental karyotypes revealed a nontranslocation event, making it possible to assure the parents that the risk of recurrence is low
- The unbalanced translocation resulted from a balanced chromosome translocation in the mother and was associated with a significant risk of recurrence (in this case, FISH would not have helped because chromosomes 13, 18, 21, X, and Y were not involved).

Limitations of aCGH

One limitation is an inability to detect polyploidy such as triploidy or tetraploidy. This problem can be circumvented through the use of a FISH preparation prior to aCGH.

In most centers, parental blood samples are drawn at the time of aCGH studies. Because aCGH offers greater resolution of chromosome regions, an increasing number of benign variations (i.e., present in one parent) are being identified. As aCGH technology advances, we are accumulating data on copy-number variations.

A large clinical trial is needed to assess the full potential of aCGH in this setting.

WHAT THIS EVIDENCE MEANS FOR PRACTICE

Use of array-basic comparative genomic hybridization to assess cells from a stillborn fetus can help determine the cause of death and inform counseling of the parents about the risk of recurrence.

FAST TRACK

With known segments of the genome printed on slides, the clinical scientist can analyze DNA from nondividing cells from a stillbirth

CONTINUED ON PAGE 24

Risk factors for stillbirth include advanced maternal age, obesity, and black race

ACOG Practice Bulletin #102: Management of stillbirth. Obstet Gynecol. 2009;113:748–760.

Willinger M, Ko CW, Reddy UM. Racial disparities in stillbirth risk across gestation in the United States. Am J Obstet Gynecol. 2009;201:469.e1–469.e8.

Fretts RC. The study of stillbirth. Am J Obstet Gynecol. 2009;201:429–430.

FAST TRACK

The increased risk of stillbirth associated with advanced maternal age is present at all gestational ages—though it becomes most profound at 37 to 42 weeks' gestation

Women who have diseases such as insulin-dependent diabetes and systemic lupus erythematosus have long been recognized as having a six- to 20-fold increase in the risk of stillbirth, compared with the general population. However, each of these disorders accounts for 2% and less than 1% of the pregnant population, respectively, so their overall contribution to stillbirth is small. Larger portions of the population have a lower—but still significant—risk of stillbirth:

- women older than 35 years
- women who have a body mass index (BMI) above 30
- non-Hispanic black women.

Each of these categories represents 15% or more of the typical obstetric population, and each group faces a risk of stillbirth approaching 1%. The ACOG practice bulletin and the study by Willinger and colleagues address these risks in detail.

Advanced maternal age is particularly risky among nulliparous women

Advanced maternal age (>35 years) is associated with increased rates of chromosomal abnormality and maternal morbidity, such as hypertension, that are known to raise the risk of stillbirth. Even when these and other variables associated with advanced maternal age, such as placenta previa, diabetes, and multiple gestation, are controlled, however, the increased risk of stillbirth remains.

Advanced maternal age in a first pregnancy carries a particularly elevated risk. For example, the risk of stillbirth in a 40-year-old nulliparous woman is more than twice the risk in a 40-year-old multiparous woman (1 in every 116 pregnancies vs 1 in every 304).[3]

The increased risk of stillbirth associated with advanced maternal age is present at all gestational ages, though it becomes most profound at 37 to 42 weeks' gestation, notably for:

- women 35 to 39 years old (1 in every 382 pregnancies; relative risk [RR] of 1.32, compared with women <35 years old; 95% confidence interval [CI], 1.22, 1.43)
- women >40 years old (1 in every 267 pregnancies; RR, 1.88; 95% CI, 1.64, 2.16).

These numbers remain significant even after controlling for medical conditions.[3]

The utility of antepartum surveillance and induction of labor for delivery is unclear, given the risk of iatrogenic prematurity.

Risk of stillbirth is doubled among obese and markedly obese women

Although the number of adults who are overweight (BMI 25–30) has remained fairly constant over the past 20 years (30% to 35% of the population), the percentage of women of reproductive age who are obese (BMI >30) has risen markedly. Obesity is now present in 35% of the population, and marked obesity (BMI >40) affects an additional 6%. Both obese and markedly obese women face a twofold relative risk of stillbirth, compared with women of normal weight. The rate of stillbirth in this population is 12 to 18 for every 1,000 births—a 1.2% to 1.8% risk.

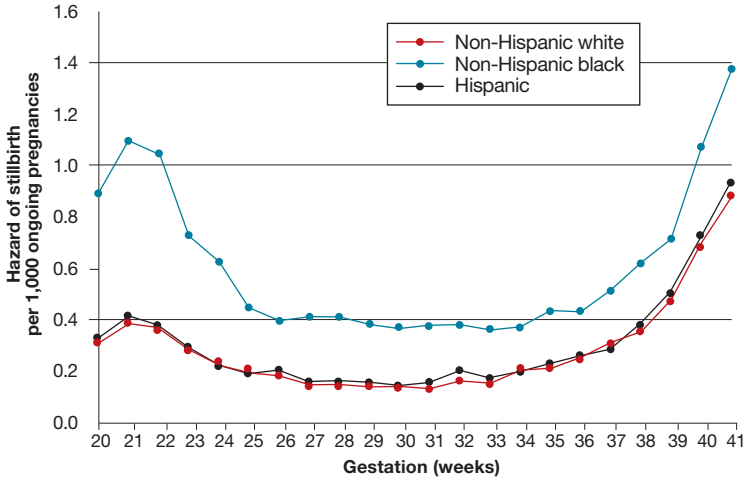
Although obesity-related stillbirth likely has multiple causes, the risk remains elevated even after exclusion of confounding factors such as smoking, gestational diabetes, and preeclampsia.

Race is an independent contributor

Racial differences in the rate of stillbirth remain despite a decrease in the overall stillbirth rate over the past 20 years (**FIGURE**). In 2003, the rate of stillbirth was 5 for every 1,000 births among non-Hispanic whites, 5.5 among Hispanics, and 12 among non-Hispanic blacks. In other words, the risk of stillbirth was 1 in 202, 1 in 183, and 1 in 87 births for white, Hispanic, and black women, respectively.

Willinger and colleagues utilized data from the National Center for Health Statistics and assessed 2001–2002 birth and infant death datasets for 36 states, examining the stillbirth hazard risk for more than 5 million singleton pregnancies. Stillbirth peaked at 20 to 23 weeks and 39 to 41 weeks' gestation, as expected. However, at 20 to 23 weeks, the risk of stillbirth among non-Hispanic black women was more than twice the rate for non-Hispanic white women (RR, 2.8). Although it then declined as term approached, it remained greater than that of non-Hispanic white women (RR, 1.6).

FIGURE Racial disparities in the risk of stillbirth



Hazard of stillbirth for singleton pregnancies by gestational age and race and ethnicity, 2001–2002. SOURCE: Willinger et al.

Greater acceptance and use of induction of labor at term among whites merits attention

In an editorial accompanying the study by Willinger and colleagues, Fretts pointed out the higher rate of induction of labor at term among white women that has been observed in at least three studies of vital statistics. (Willinger and colleagues also pointed out this difference.) The acceptance and use of

labor induction at term—and the lower stillbirth rate—among white women warrants further investigation.

Education appears to reduce the risk of stillbirth to a greater degree among whites than it does among blacks. Again, nulliparity and advanced maternal age were important contributors to the risk of stillbirth across all three races.

WHAT THIS EVIDENCE MEANS FOR PRACTICE

Counsel African-American gravidas and women older than 35 years that their risk of stillbirth is elevated.

Obese women should be advised to lose weight before conception if at all possible to reduce the risk of stillbirth.

Needed: Standardized analysis and documentation of stillbirth

Reddy UM, Goldberg R, Silver R, et al. Stillbirth classification—developing an international consensus for research: executive summary of a National Institute of Child Health and Human Development workshop. Obstet Gynecol. 2009;114:901–914.

Flenady V, Frøen JF, Pinar H, et al. An evaluation of classification systems for stillbirth. BMC Pregnancy Childbirth. 2009;9:24.

Further guidance for the clinical management of stillbirth will come from investigations of the underlying pathologies and associated risk factors. Key to development of this guidance is the involvement of obstetricians in documenting the antenatal record and delivery information. Also needed is a standardized system for recording this information. More than three dozen systems have now exist.

An international consensus group published guidelines on how to describe the cause of death in research endeavors, recognizing the need to maintain the ability to attach a level of uncertainty. In addition, Flenady and colleagues compared the most widely used systems in clinical practice, assigning the highest score for components such as ease of use, interobserver variability, and proportion of unexplained stillbirths to CODAC [cause of death and two associated causes]. This system assigns a primary cause of death from a specified list of choices and allows inclusion of two possible contributing causes.

Both the international consensus classification and the CODAC scoring system are accessible through links embedded within the articles. Both systems require the establishment of standardized evaluation and review of stillbirth that should include obstetricians, pathologists, and geneticists.

WHAT THIS EVIDENCE MEANS FOR PRACTICE

Because assessment and classification of stillbirth are fundamental to its prevention, as well as a critical part of clinical practice, ObGyns should become familiar with the international consensus classification and CODAC scoring systems and adopt a standardized approach to assessment and documentation.

References

1. Rodgers CS, Creasy MR, Fitchett M, Maliszewska CT, Pratt NR, Waters JJ. Solid tissue culture for cytogenetic analysis: a collaborative survey for the Association of Clinical Cytogeneticists. J Clin Pathol. 1996;49:638–641.
2. Rivasi F, Schirosi L, Bettelli S, et al. FISH analysis in cell touch preparations and cytological specimens from formalin-fixed fetal autopsies. Diagn Cytopathol. 2008;36:633–636.
3. Reddy UM, Ko CW, Willinger M. Maternal age and the risk of stillbirth throughout pregnancy in the United States. Am J Obstet Gynecol. 2006;195:764–770.
4. MacDorman MF, Mathews TJ. NCHS Data Brief #9: Recent trends in infant mortality in the United States. Atlanta, Ga: National Center for Health Statistics; October 2008. Available at: http://www.cdc.gov/nchs/data/databriefs/db09.htm. Accessed Dec. 15, 2009.